Molecular profiling of bacterial species in the caecum of geese

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ABSTRACT: The purpose of this study was to analyse the microbial diversity in the caecum of geese using a 16S ribosomal RNA gene (rRNA) clone library approach. A total of 160 clones and 124 clones were sequenced and phylogenetically analysed from the contents and mucosa of the caecum of Yang Zhou geese, respectively. The result indicated that there was a rich variety of bacteria in the caecum contents. Forty-six operational taxonomic units (OTUs) based on a 98% similarity criterion were classified in the contents of goose caecum, as compared to 29 OTUs based on a 97% similarity criterion in the mucosa of goose caecum. The sequences were assigned to 7 and 5 groups in the contents and mucosa of goose caecum, respectively. Contents of goose caecum were dominantly occupied by *Clostridia*-related species (58.7%) with other abundant sequences being related to *Bacteroidetes* (26.9%) and *Erysipelotrichi* (11.2%). *Gammaproteobacteria* (59.6%) and *Clostridia* (20.1%) were predominant in the mucosa of goose caecum.

Keywords: geese; caecum; microbiota; phylogenetic analysis

Unlike other avian species, the goose is a kind of waterfowl with the relatively developed paired caecum, which can take advantage of fibrous plant materials partly. And birds can digest fibre only through fermentation, mainly in the caecum (McNab, 1973). The caecum is also known as the site for fibre digestion, as reported by Yang et al. (2009), the metabolic rates of NDF, ADF and hemicellulose were decreased significantly after the caecum was removed from geese fed basal diet. Moreover, the microbiota in the caecum is known to actively ferment carbohydrates that have escaped digestion in the upper part of the gastrointestinal (GI) tract. However, the microbiota in the GI tracts of non-ruminant species is a diverse population of organisms composed primarily of bacteria (Mackie et al., 1999). Bacterial populations may also be classified into contents and mucosal populations, and the mucosal microbiota may further be divided into epithelial or cryptal (Ewing and Cole, 1994). Although the microbiota in the GI tracts of animals is considered to be beneficial not only to their nutrition, but also to the health of animals. Reports on the microbial ecology in the caecum of geese are scarce. The dominant bacteria in the caecum of geese, detected from Denaturing Gradient Gel Electrophoresis (DGGE) fingerprints, as reported by Wang et al. (2009), were related to *Pseudomonas* sp. and *Bifidobacterium* sp. However, the complete description of microbiota in the caecal or mucosal contents of geese is missing.

The earlier identification methods rarely allowed definitive determinations of bacterial culture, species and they did not often allow the identification even on the genus level. The diversity and complexity of the community structure of caecal bacteria were much higher than it had been reported previously by culture-based studies (Gong et al., 2007). Since culture-based studies can provide only a limited picture of natural microbial communities, it is necessary to rely on alternate methods like the sequence analysis of 16S rRNA gene clone

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libraries. So far, there have been few reports on the molecular diversity of microbiota in the caecum of geese. However, the analysis of the PCR-derived 16S rDNA clone libraries has shown that microbial communities are highly diverse and complex in ruminants (Whitford et al., 1998; Tajima et al., 1999) and in the GI tracts of other animals, including pigs (Leser et al., 2002), chickens (Gong et al., 2007) and turkeys (Scupham, 2007).

In order to reveal the fibre digestion and health of geese, it is essential to analyse the complex microbial communities in the caecum of geese. The purpose of the present study was to provide a description of the microbial community composition in caecal contents and mucosa of geese using the 16S rRNA gene sequence analysis.

MATERIAL AND METHODS

Animals and sampling

All procedures were approved by the Institutional Animal Care and Use Committee of Yangzhou University. Yangzhou goose is a medium-sized goose species in China, with characteristics of stable genetic performance, high reproduction rate, rapid early growth, good meat quality, strong tolerance and adaptability to coarse feed and so on. Ten Yangzhou geese were raised in concrete pens with straw litter (2–3 cm thickness). The birds were reared in the indoor house with environmental conditions (temperature: 26.0 ± 3.0°C, relative humidity: 65.5 ± 5.0%) from 5–10 W. Geese had free access to diets and water. Geese were fed the mash diet (Table 1).

At 10 weeks of age, ten geese were selected randomly and killed by cutting the carotid arteries. The caecum was removed aseptically, clamped with forceps, and placed into sterile plastic bags on ice. After the caecum was opened longitudinally, caecal contents were immediately sampled and stored at –70°C (Apajalahti et al., 1998). Mucosa samples were collected after digesta had been removed by washing with saline containing 0.1% Tween 80. The mucous layer attached to the caecal wall was gently scraped off with a small sterile spatula (Zhu et al., 2002). All contents or mucosa samples from the ten geese were mixed separately, frozen in liquid nitrogen and stored at –70°C.

DNA extraction

Genomic DNA was isolated from frozen samples using a QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions. DNA extracts were stored at –70°C.

PCR procedures

16S rRNA genes were amplified by PCR from the genomic DNA samples of contents-associated and

Table 1. Ingredient and nutrient composition of the experimental diets

Ingredient	Ingredient content (%)	Nutrient composition	Nutrient level
Corn	61.18	AME (MJ/kg)	11.16
Soybean meal	16.22	crude protein (%)	16.53
Fish meal	4	crude fiber (%)	6.75
Alfalfa meal	5	calcium (%)	0.90
Cellulose	4.5	available phosphorus (%)	0.42
Soybean oil	1.7		
Dicalcium phosphate	1.05		
Limestone	1.00		
Salt	0.35		
Vitamin and trace mineral[1]	5.0		

[1]Supplied per kilogram of total diet: vitamin A 20 000 IU; vitamin D_3 4500 IU; vitamin E 300 IU; vitamin K_3 20 mg; vitamin B_1 10 mg; vitamin B_2 120 mg; vitamin B_6 20 mg; vitamin B_{12} 0.2 mg; nicotinic acid 600 mg; pantothenic acid 180 mg; folic acid 10 mg; folate 10 mg; biotin 0.8 mg; choline, 7 g; Fe 1.2 g; Cu 0.2 g; Mn 1.9 g; Zn 1.8 g; I 10 mg, Se 6 mg

mucosa-associated bacteria using bacterial primers F8 (5'-AGAGTTTGATCCTGGCTCAG-3') and R1492 (5'-GGTTACCTTGTTACGACTT-3') (Eden et al., 1991). The complete gene of 16S rRNA from bacteria was obtained (about 1500 bp).

Thermocycling reactions contained 1000nM of each primer, 2 μg of purified template DNA, 5 μl of 10 × Ex Taq reaction buffer (Mg^{2+} free), 200mM of dNTP, 75μM of $MgCl_2$ and 1.25 U of Ex Taq DNA-polymerase (TaKaRa, Dalian, China), per 50-μl reaction. Reaction parameters included 4-min initial denaturation at 94°C. Cycling consisted of 50 s of 94°C denaturation, 50 s of 56°C annealing and 2 min of 72°C elongation. Reactions were finished with 10-min elongation at 72°C. Genes were amplified from caecal DNAs using the fewest number of cycles possible to generate a visible product, generally 15 cycles.

Cloning of the PCR amplified products and sequence analysis

PCR products were purified using a PCR product purification kit (Invitrogen, Beijing, China) and subcloned [pGEM-T-Easy] (Promega), using a Topo TA cloning kit (Invitrogen). Cloned amplicons were sequenced using vector-specific primers and an ABI PRISM 377 sequencer (Perkin-Elmer) in Invitrogen company (Invitrogen, China).

Our sequences were analysed by the CHECK-CHIMERA programme to remove chimeric rDNA clones. The aligned sequences were also used for analysis in DOTUR (Schloss and Handelsman, 2005) to determine operational taxonomic units (OTUs). One representative clone was chosen for each OUT, and then submitted to BLAST programme and Ribosomal Database Project programme online to obtain the closest published relatives (Cole et al., 2003). The phylogenetic positions of these clusters were tested in neighbour-joining trees with 1000 times resampling to determine the significance of the clusters from bootstrap values with the MEGA3.1 software.

Nucleotide sequence accession numbers

Nucleotide sequences have been deposited in the GenBank database under the accession numbers GQ864251-GQ864254 and GQ871708-GQ871740.

RESULTS

Bacteria associated with caecum contents

160 cloned sequences were distributed in 46 distinct OTUs at the 2% difference level by DOTUR to define an OUT. The presumptive relationships of these sequences were obtained from a database comparison. According to assigning to the closest genus, as shown in Table 2, the highest similarity of cloned sequences was 100%. However, the lowest was 90%. The BLAST data indicated that among the 46 OTUs, 25 OTUs did not correspond to any recorded entries in the NCBI database. These sequences can be considered as novel sequences with an identity of < 97% with the sequences of the database. The other 21 sequences had 97% or higher identity with an already characterized sequence. Ten clones had a high identity (99%) with the cultured species, *Bacteroides coprocola*. Four clones also had a high identity (97%) with *Clostridiales lactatifermentans*. Except for *T. sanguinis*, all the sequences related to ours with a high similarity and had digestive origins from different areas of the gastrointestinal tract of ruminant or monogastric animals. In the contents of caecum, *Clostridia* were the most abundant (94 of 160 clones), representing 58.7% of the clones. *Bacteroidetes* were the second group (representing 26.9% of the clones) followed by *Erysipelotrichi* (11.2%). There were 80 cloned sequences (50%) with less than 97% of relatedness to database sequences and which may thus represent novel species previously unidentified in the contents of goose caecum. The results indicated that there was a rich variety of bacteria in the contents of caecum.

The phylogenetic relationship of the reference and cloned 16S rRNA gene sequences from the contents of caecum is shown in Figure 1. The sequences generated from this study cluster into seven groups, as indicated in the tree.

Cluster I contained 10 OTUs (L4, L30, L212, L90, L59, L137, L230, L154, L11 and L77) together with a number of sequences from non-cultivated organisms. Sequences isolated from chicken caecum, human faeces, chimpanzee faeces were all represented in this area of the tree, demonstrating that at least some of the novel isolates have been extracted from organisms which are similar to those in other digestive systems. This cluster was supported with strong bootstrap values, suggesting that these bacteria were probably related to *Ruminococcaceae*.

Table 2. Distribution of the 160 clones within the 46 OTUs in the content of geese cecum according to the similarity of sequence with the NCBI database*

OUT name	Closest group	Closest species/clone	GenBank accession No.	Origin of the nearest sequence	Similarity (%)	Number of clone
L144	*Bacterium*	*Bacterium* ic1391	DQ0574480	broiler cecum	99	8
L38		New Zealand B	AY721625	duck faeces	97	7
L168	*Bacteroides*	*B. eggerthii*	AB510700		90	1
L43		*B. coprocola*	AB200225	human feces	99	10
L13		*B. uniformis*	EU722741	human feces	91	11
L52		*B. plebeius*	AB200221	human feces	92	2
L161	*Clostridiales*	*C. lactatifermentans*	NR_025651	chicken gut	97	4
L53	*Desulfomonas*	*D. pigra*	AF192152	pig gut	98	1
L76	*Alistipes*	*A. finegoldii*	AB554230		91	2
L95	*Turicibacter*	*T. sanguinis*	NR028816		99	3
L212	*Oscillibacter*	*O. valericigenes*	AB238598		95	2
L3	Uncultured bacteria	CFT114B12	DQ456082	turkey cecum	95	4
L4		cc_17	GQ175377	chicken caeca	99	10
L8		CE3_aai05e10	EU773858	cheetah feces	94	1
L11		cc_181	GQ175477	chicken caeca	98	7
L15		RL303_aal70g10	DQ800284	human feces	91	3
L17		EMP_M36	EU794160	cattle fecal	96	1
L19		cc_115	GQ175443	chicken caeca	96	1
L20		TS25_a02b02	FJ366048	human feces	98	3
L26		R-6426	FJ879997	rat feces	98	1
L30		R-9218	FJ879015	rat feces	97	1
L34		WTB_P48	EU009836	turkey cecum	100	2
L44		SJTU_G_10_50	EF405469	human fecal	96	5
L51		F2	AM500810	composting sample	96	5
L54		RL199_aaj41d08	DQ793259	human feces	92	1
L59		CFT212G3	DQ456384	turkey cecum	97	2
L60		CFT114A7	DQ456069	turkey cecum	93	1
L61		R-8278	FJ881281	rat feces	99	5
L64		CFT19C1	DQ455843	turkey cecum	93	5
L77		RL386_aao87f06	DQ797154	human feces	96	3
L79		SR3	DQ394638	reindeer gut	97	1
L84		8-1K9	FJ682081	beef cattle feces	97	1
L90		P5_D15	EU382017	rumen	94	9
L96		TuCc28	DQ071521	capercaillies cecum	96	3
L110		RL243_aai88b10	DQ809013	human feces	97	8
L137		BY13	DQ342336	chicken intestine	99	2
L154		CFT214C12	DQ456450	turkey cecum	98	1
L165		RL184_aao65c05	DQ809864	human feces	94	1
L172		SMR16	AM930352	composting sample	92	1
L174		RL306aal92g03	DQ805926	human feces	91	1
L201		cc186	DQ057383	chickens cecum	95	10
L207		SJTU_D_13_42	EF401626	human fecal	99	1
L226		TAK_aaa03e08	EU474735	takin feces	90	2
L228		AP10U.233	AM278590	human faecal	99	2
L230		RL183_aao04g09	DQ800889	human feces	92	4
L256		R-8208	FJ881216	rat feces	93	1
Total number						160

*Determined by GenBank BLASTN queries with additional analysis through RDP Sequence Match. Bacterial names were assigned to rRNA gene sequences with closest BLASTN matches to named organisms located in GenBank

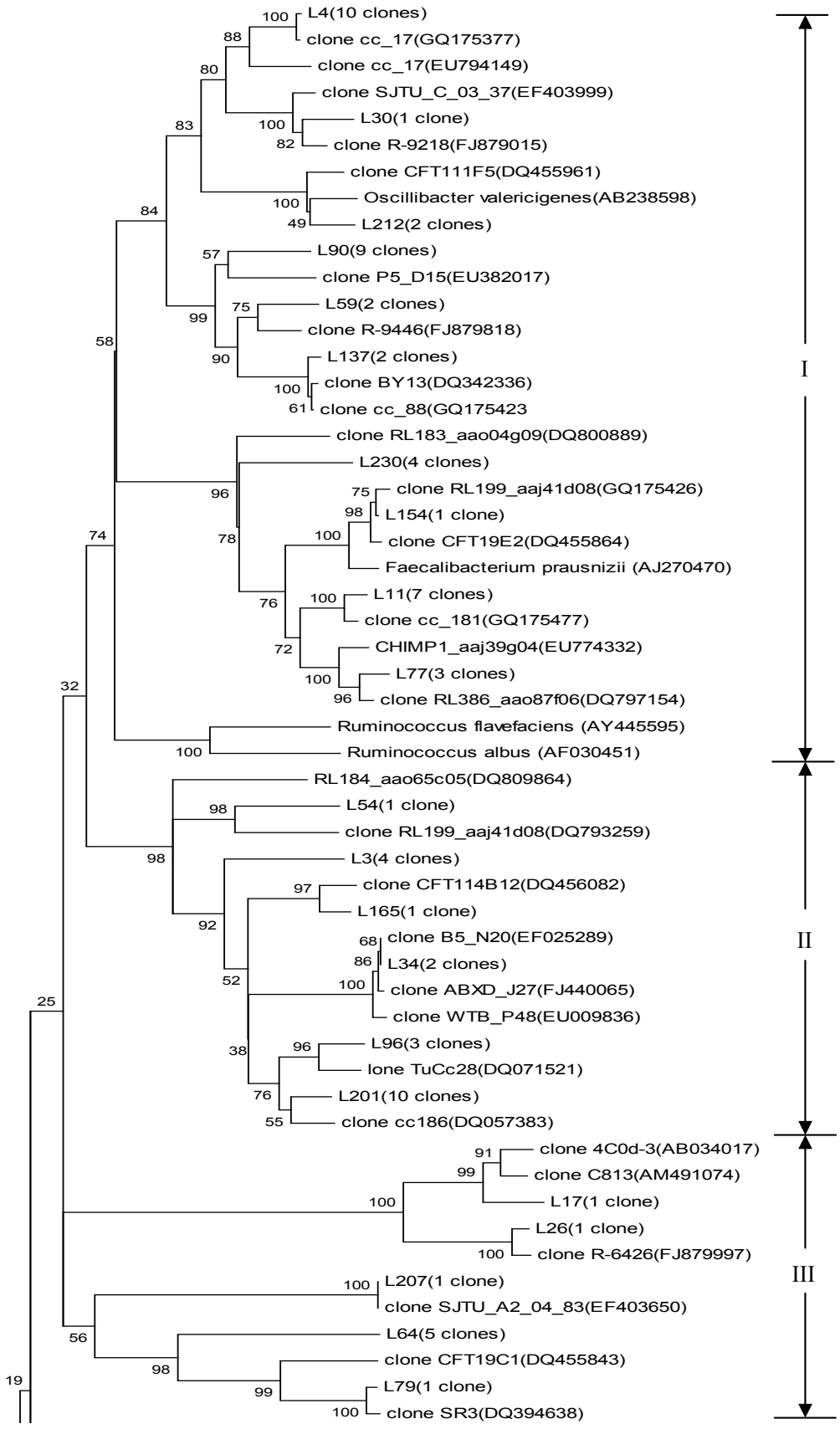

Figure.1. Unrooted phylogenetic tree of contents-associated bacteria in the caecum of geese constructed by a neighbour-joining method

Cluster II contained 6 OTUs (L54, L3, L165, L34, L96 and L201). This cluster was supported by strong bootstrap values, suggesting that these bacteria were probably related to *Lachnospiraceae*.

Cluster III contained 5 OTUs (L17, L26, L207, L64 and L79).

Cluster IV was included in the *Peptostretococcaceae* family. Seven OTUs (L20, L256, L161, L228, L8,

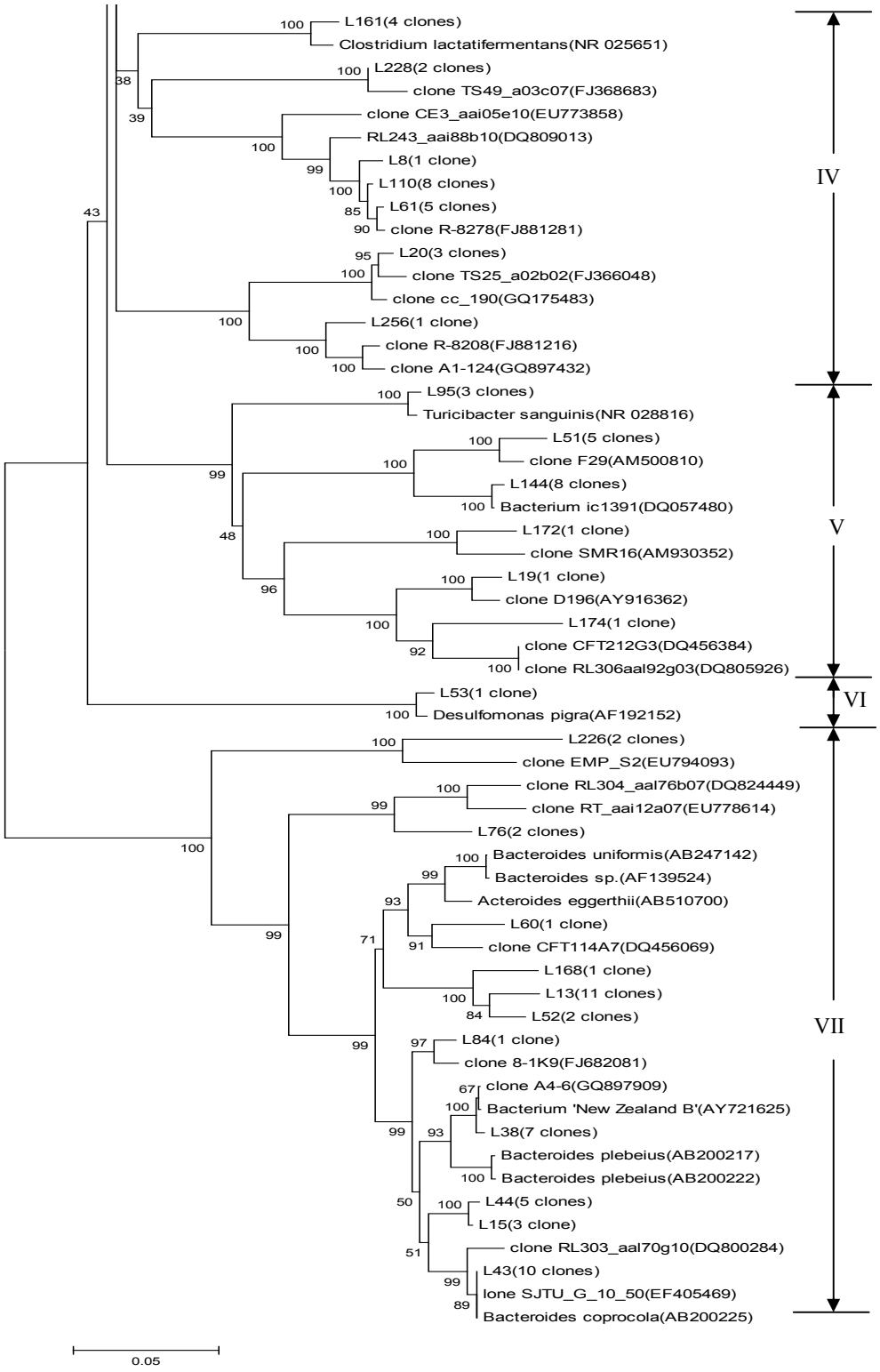

Figure 1 to be continued

L110 and L61) were related to cluster IV, with two novel OTUs and five OTUs characterized previously (L20, L161, L228, L110, and L61, Table 1). Cluster IV included 24 clones in this library. All sequences could be considered as close because of the very short branches of the tree.

Cluster V contained 6 OTUs (L95, L51, L144, L172, L19 and L174).This group is *Erysipeloteichaceae* and its relations.

Cluster VI contained a single OUT (L53) within the *Deltaproteobacteria* class. This sequence was the one with high identity (98%) with *Desulfomonas pigra*. The branches were very short, and the bootstrap values were strong (100). These data indicated a high identity in this group.

Cluster VII was a relatively independent group. It contained 11 OTUs (L226, L76, L60, L168, L13, L52, L84, L38, L44, L15, L43) together with sequences

Table 3. Distribution of the 124 clones within the 29 OTUs in the mucosa of geese cecum according to the similarity of sequence with the NCBI database*

OUT name	Closest group	Closest species/ clone	GenBank accession No.	Origin of the nearest sequence	Similarity (%)	Number of clone
N99	*Aeromonas*	*A. punctata*	EU770300	fish pond	99	4
N195	*Barnesiella*	*B. viscericola*	AB267809	chicken cecum	91	2
N251	*Brevibacterium*	*Brevibacterium* sp.	AM981204	drinking water	97	3
N176	*Desulfovibrio*	*Desulfovibrio* sp.	U07570		99	4
N20	*Ralstonia*	*Ralstonia* sp.	AY864081		100	7
N21	*Stenotrophomonas*	*S. rhizophila*	GU391467	travertine roots	99	14
N78	*Pantoea*	*P. agglomerans*	EU598802	cotton boll rot	99	6
N93	*Lawsonia*	*L. intracellularis*	U30147		98	4
N71	*Pseudomonas*	*P. fluorescens*	DQ207731		97	3
N235		*P. trivialis*	GU391473	forest area	95	2
N74		*Pseudomonas* sp.	EF028122		97	4
N213			EU686687	chicken gut	98	2
N276			AB098591		99	33
N14	Uncultured bacterium	Phe67	AF534216	soil	97	4
N54		J66	GQ389018	drinking water	96	3
N85		M0015_044	EF071175	human colonic mucosal	97	2
N87		SQ_aah80g08	EU779034	squirrel feces	97	2
N129		nbt05h08	EU535895	antecubital fossa	96	1
N143		SJTU_D_11_30	EF401479	human feces	96	3
N156		A3-168	GQ897860	human feces	99	1
N168		CA94	EF434372	human feces	96	1
N172		RL246_aai75a08	DQ793648	human feces	94	1
N175		myd2_aaa04d08	EU504931	mouse cecum	92	2
N187		cc_144	GQ175458	chicken cecum	93	1
N211		B2_040	EU765110	human gut	98	7
N212		EMP_A25	EU794180	cattle fecal	95	4
N214		SedNCA42	FJ849429	environmental sample	97	2
N215		WSp79	GQ867334	seals colon	95	1
N226		oc10	DQ057412	chicken gut	98	1
Total number						124

*Determined by GenBank BLASTN queries with additional analysis through RDP Sequence Match. Bacterial names were assigned to rRNA gene sequences with closest BLASTN matches to named organisms located in GenBank

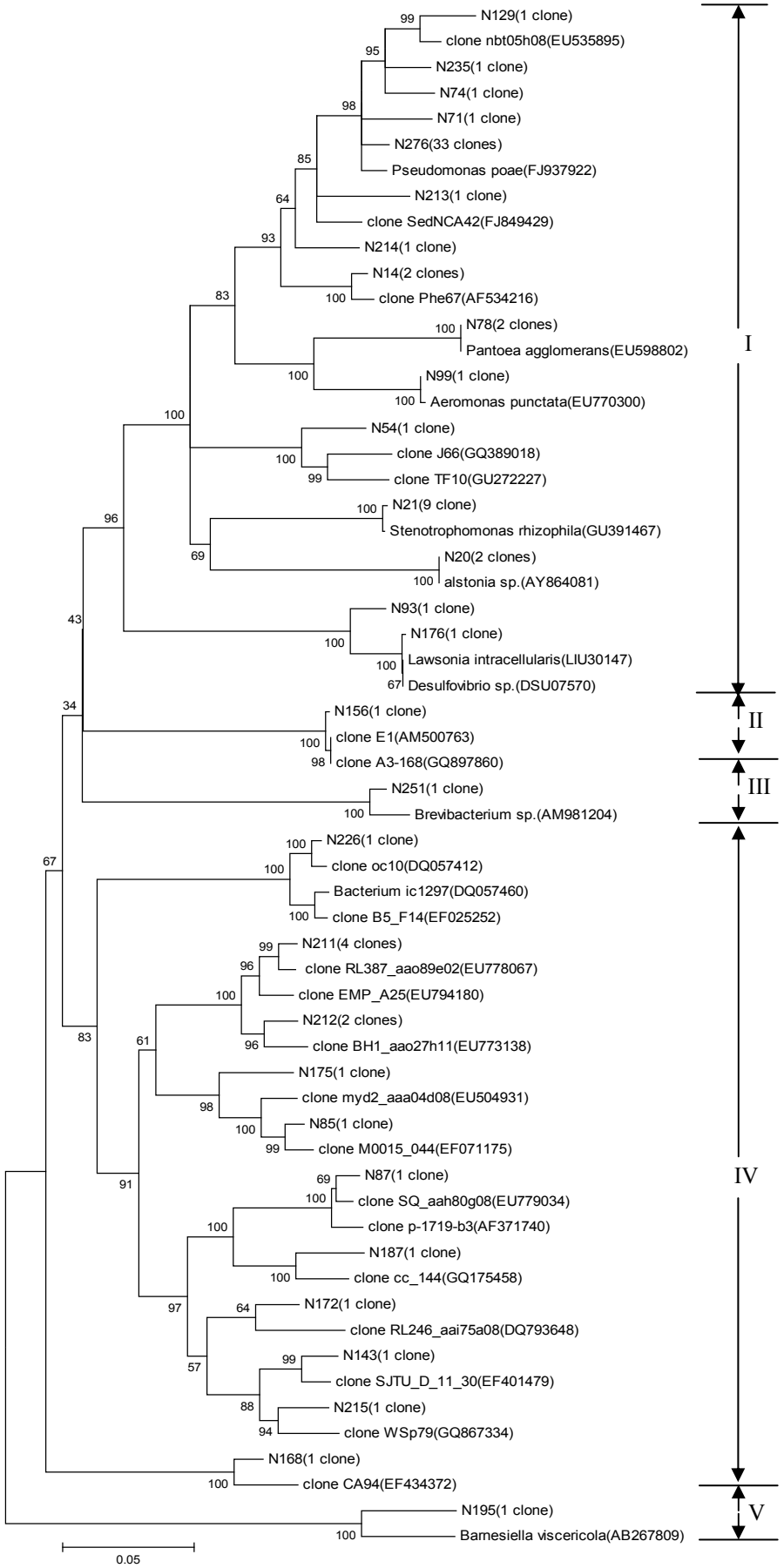

Figure.2. Unrooted phylogenetic tree of mucosa-associated bacteria in the caecum of geese constructed by a neighbour-joining method

from a number of other gut environments, such as cattle faeces, human faeces, rumen. The cluster was supported by strong bootstrap values. This group was mostly *Bacteroides*.

Bacteria associated with caecum mucosa

124 cloned sequences were distributed in 29 distinct OTUs at the 3% difference level by DOTUR to define an OUT. The presumptive relationships of these sequences were obtained from a database comparison. According to assigning to the closest genus, as shown in Table 3, the highest similarity of cloned sequences was 100%. However, the lowest was 91%. The BLAST data indicated that among the 29 OTUs, 11 OTUs did not correspond to any recorded entries in the NCBI database. These sequences can be considered as novel sequences with an identity of < 97% with the sequences of the database. The 18 other sequences had 97% or higher identity with an already characterized sequence. Thirty-three clones had a high identity (99%) with *Pseudomonas* sp. (AB098591). Nine clones also had a high identity (99%) with *Stenotrophomonas rhizophila*. In the mucosa of caecum, *Gammaproteobacteria* were the most abundant (74 of 124 clones), representing 59.6% of the clones. 25 of these clones were assigned to *Clostridia*, representing 20.1% of the clones.

The phylogenetic relationship of the reference and cloned 16S rRNA gene sequences from the caecum mucosa is shown in Figure 2. The sequences generated from this study cluster into 5 groups, as indicated in the tree.

The cluster I was included in the *Proteobacteria* class. Fifteen OTUs (N129, N235, N74, N71, N276, N213, N214, N14, N78, N99, N54, N21, N20, N93 and N176) were affiliated to cluster I, of which twelve OTUs were already characterized and three were novel sequences. Cluster I represented the first main cluster, with 93 clones in this library.

Cluster II contained a single OUT (N156) within the *Erysipelotrichi* class, which clusters together with the sequence from non-cultivated organisms from human faeces material. This area was supported by strong bootstrap values, and the branches in this area of the tree were short.

Cluster III contained a single OTU (N251) within the *Actinobacteria* class. This sequence was highly identified with *Brevibacterium* sp.

Cluster IV contained 11 OTUs (N226, N85, N175, N211, N212, N87, N187, N172, N143, N215, N168). The species were closely related to *Clostridia*.

Cluster V contained 1 OTU (N195), which clusters together with the sequence of *Barnesiella viscericola*. This area was supported by strong bootstrap values, and the branches in this area of the tree were short.

DISCUSSION

The present study was conducted to investigate the diversity and phylogenetic relationships of mucosa-associated and contents-associated caecal bacteria by molecular analysis of 16S rRNA genes. Culture-independent methods such as molecular analysis of 16S rRNA genes have shown greater diversity of the bacterial population than had been achieved by culture-dependent methods (Gong et al., 2002b; Zhu et al., 2002). The composition of animal caecum microbiota can be significantly influenced by diet (Rehman et al., 2008) and other factors, such as the age of animal (Bennegadi et al., 2003). According to Apajalahti et al. (1998) and Gong et al. (2007), since the samples were from the uniform birds, the diversity of bacterial populations can represent the microbiota in the caecum of these birds as a whole, regardless of differences in individual geese. In the study, contents and mucosa samples were collected from ten 10-weeks-old Yang Zhou geese.

In this library, the main part of the 46 OTUs corresponded to new sequences with 25 novel sequences and 21 sequences having high identity with clones sequenced previously (identity cut-off 97%) in caecal contents. A large majority of OTUs in the contents of goose caecum were also observed in human faeces (Ley et al., 2008; Turnbaugh et al., 2009), chicken caecum (Gong et al., 2002a), rumen (Tajima et al., 2000; Brulc et al., 2009), turkeys (Scupham et al., 2008), ducks (Murphy et al., 2005).

In the contents of goose caecum, *Clostridia* were the most abundant (94 of 160 clones), representing 58.7% of the clones. *Bacteroidetes* were the second group (representing 26.9% of the clones). It should be noted that *Clostridia* have been found to be abundant in the chicken caecum with the dominant sequences similar to *Clostridium* and *Ruminococcus* sp. (Lu et al., 2003). Zhu et al. (2002) also described similar results on *Clostridia* in the caecum from chickens fed a maize-soy diet that contained animal proteins and an anticoccidial

compound. A large portion of cloned sequences in caecum contents belonged to the *Ruminococcus* group. A possible reason for this might be a diet containing 6.75% crude fibre. As reported by Matsui et al. (2010a), using the *R. flavefaciens*-specific primer set, an OUT having 97% similarity with *R. flavefaciens* was recovered, and many other OTUs were involved in *Ruminococcus*. In addition, *R. flavefaciens* has been isolated from fresh ostrich faeces (van Gylswyk et al., 1998), suggesting it is one of the major fibrolytic bacterium in the large intestine of the ostrich. However, as regards the *Ruminococcus* group, it is not expected that the microbiota in the goose caecum was similar to that of the rumen. In the rumen, Whitford et al. (1998) found out that the majority of sequences were related to the *Prevotella-Bacteroides* group. However, the *Prevotella-Bacteroides* group was not detected in this clone library in this study, suggesting that the population density of *Prevotella-Bacteroides* was below the detectable limit or *Prevotella-Bacteroides* was absent. Other presence of a large, dominant cluster was the *Bacteroides*. Previous research found out that *Bacteroides* play an important role in helping decompose polyose and raise the utilization rate (Bäckhed et al., 2004) to speed up development of the intestinal mucosa (Stappenbeck et al., 2002) and immune system, then raise the immunity of the host (Hooper, 2004), maintain the balance of intestinal microecology (Sears, 2005). In our study, *Bacteroides* represented the second main cluster (26.8%). As reported by Matsui et al. (2010b), 39.4% of sequences were affiliated with *Bacteroidetes* in the ostrich caecum, and the abundance of *Bacteroidetes* species is an important factor for the fibrolytic and/or actively fermenting microbial ecosystem in the GI tracts. However, in chicken caecum, only a small number of the sequences are affiliated with *Bacteroidetes* (1.9–4.7%) (Gong et al., 2002a; Lan et al., 2002; Zhu et al., 2002; Lu et al., 2003). In contrast, the number of clones affiliated with *Bacteroidetes* is high in the caecum of wild (61.9%) and domestic (55.5%) turkeys, which have a non-fibrolytic ecosystem (Scupham et al., 2007). In the study, we found that there were 80 cloned sequences (50%) with less than 97% of relatedness to database sequences unidentified in the goose gut previously. Sundset et al. (2007) suggested that less than 97% of relatedness to database sequences may represent a new species. Moreover, the physiological role of many more bacteria than newly sequenced bacteria should be investigated.

It is noteworthy that *Pseudomonas* was a major group of bacteria found in the caecum mucosa in our studies. It is perhaps surprising to find evidence of generally aerobic bacteria in the caecal environment, but it is known that some *Pseudomonas* species are capable of anaerobic respiration with nitrate or nitrite (Van-Hartingsveldt and Stouthamer, 1973) and of slow growth in a rich medium containing arginine under anaerobic conditions (Vander-Wauven et al., 1984). However, Gong et al. (2007) found out that the mucosa of the chicken caecum was dominantly occupied by *clostridia*-related sequence (40%) with other abundant sequences being related to *Faecalibacterium prausnitzii* (14%), *Escherichia coli* (11%), *Lactobacilli* (7%) and *Ruminococcus* (6%). This would be explained by differences in breed.

In the present study, we suggest that the diversity of bacteria in the caecal mucosa was lower than in the caecum contents. Remarkably, some sequence clones from the caecum contents were also found in the mucosa. It is possible that the caecal crypts might harbour contents bacteria that cannot be washed off, and these bacteria were also capable of adhesion to or even penetration of the mucosa. Alternatively, it was related to the technology of sampling.

The caecal microbiota of geese is very complex and the majority of the bacterial species have not been cultivated. Despite the limited number of analysed sequences, our results provide a valuable insight into a poorly understood microbial ecosystem and form the basis for further studies into microbial functions affecting the nutrition or health of geese.

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