

# ブタ腸管由来細菌 16S リボソーム DNA 配列のコンピュータ・シミュレーションによる末端制限酵素断片長多型(T-RFLP)解析およびブタとヒトの解析結果の比較

長島浩二

Terminal Restriction Fragment Length Polymorphism Analysis of 16S rDNA Sequences from the Pig Gastrointestinal Tract by Computer Simulation and Comparison of the Results of Porcine and Human Analysis

Koji Nagashima

To evaluate whether a modified method of terminal restriction fragment length polymorphism (T-RFLP), which is named "nagashima method", is applicable to the analysis of pig intestinal microflora, similar to that human intestinal microflora, we performed the *in silico* T-RFLP analysis and a homology search using the bacterial 16S rDNA sequences derived from pig gastrointestinal tract that were deposited in the GenBank database. As a result, 34 putative operational taxonomic units (OTUs representing those T-RFs of approximate size) were detected, 11 OTUs of which were not detected in humans. The characteristics of the other OTUs are considered to be roughly similar to those of the corresponding human OTUs, except for three that seem to vary somewhat the two species with respect to correspondence of phylogenetic bacterial groups.

ヒトと同様にブタなどの家畜においても、腸内細菌叢(フローラ)が健康と密接に関係していることは以前から知られており、培養法によってフローラ解析が行われてきたが、近年では16S rDNAをターゲットとした各種分子手法による解析がなされてきている。

ブタは臓器移植、循環器、消化管、代謝、栄養、新生子、皮膚、神経系、免疫システムなどでヒトと類似した特徴を持っていることなどから、広範囲の分野で実験動物として用いられている。将来的には、食品素材による整腸効果や腸内フローラを介した免疫系や神経系への影響といった機能性評価にブタが使用されることも考えられる。

著者は細菌16SリボソームRNA遺伝子(rDNA)をターゲットとした末端制限酵素断片長多型(T-RFLP)解

析<sup>1)</sup>の一変法(nagashima法)<sup>2)3)</sup>を開発し、ヒト糞便フローラ解析を行って来た<sup>4)</sup>。本法は従来法に比べてデータ解析の煩雑さが改善されていると共に、末端制限酵素断片(T-RF)のサイズから由来した細菌種を推定できるという特長を有している。また著者は、推定した細菌種を確認するための「T-RFのクローニング&シーケンシング法<sup>5)</sup>」や試料安定性に優れた簡便な糞便採取技術<sup>4)</sup>を考案している。

本報告では、著者の開発したT-RFLP(nagashima法)がヒトと同様にブタの腸内フローラ解析にも適用可能かどうかを検証するために、Leserら<sup>6)</sup>が決定したブタ腸管由来細菌16S rDNA配列を用いて行ったコンピュータ・シミュレーションによるT-RFLP解析の結果について述べる。

## 1. 実験方法

### (1) 解析に用いたrDNA配列とホモロジー検索

コンピュータ・シミュレーションによるT-RFLP解析(*in silico* T-RFLP)には、Leserら<sup>6)</sup>によって決定され、GenBankに登録されたブタ腸管由来細菌16S rDNA配列(Accession No. AF371468からAF371949)の内の391配列を用いた(Table 1参照)。これらはほぼ全長の配列を含んでいる。当該配列はまた、それらの配列が由来した細菌種を推定するために、DDBJウェブサイト(<http://www.ddbj.nig.ac.jp/>)でのBLASTホモロジー検索に供した。

### (2) *in silico* T-RFLP

前記16S rDNA配列はAutoAssemblerソフトウェア(Applied Biosystems社)による*in silico* T-RFLPに供した。当該配列は、T-RFLP(nagashima法)で用いられるフォワード・プライマー配列(5'-TGCCAGCAGCCGCGGTA-3'; *E. coli* 16S rDNAの516から532番目までの配列に相当)の5'末端から上流の配列が削除された後、制限酵素*Bs*IIによって*in silico*消化され、得られた5'末端DNA断片(T-RF)のプラス鎖のサイズが測定された。

## 2. 実験結果及び考察

Leserら<sup>6)</sup>は、食餌、週齢、健康状態の異なるブタ(24匹)の回腸、盲腸、結腸の内容物から調製した52試料(DNA)をPCRに供し、4,270の16S rDNA配列からなるライブラリーを構築した。さらに、このライブラリーを97%ホモロジー基準によって検定すると、375の系統型(phyloptype)が認められるとしている。我々はこの内の371系統型の配列を用いて、nagashima法による*in silico* T-RFLPとホモロジー検索を行った。その結果を

Table 1 にまとめた。Leser ら<sup>6)</sup>によると、375 の系統型の内の 83% はデータベースに存在する既知の配列と 97% 未満のホモロジーを示し、未知の菌属・菌種の可能性があることを述べている。また、81% の系統型が low-G+C グラム陽性菌に、11.2% の系統型が *Bacteroides/Prevotella* グループに含まれるとしている。我々のホモロジー検索においても、同様の結果であった (Table 1)。

ブタ腸管由来細菌 16S rDNA 配列の *in silico* T-RFLP によって検出された仮想の OTU (operational taxonomic unit の略; 便宜的な細菌の分類単位で、T-RF あるいは同じ様なサイズの T-RF の集まりを表す) と分類群 (phylogenetic groups) の対応関係をヒトの場合と比較した。Table 2 に示されているように、ブタでは 34 の仮想 OTU が検出され、その内 11 の OTU (p 1, p 9, p 17, p 18, p 19, p 23, p 24, p 27, p 28, p 29, p 31) はヒトでは検出されていないものであった。一方、ヒトでの実際の T-RFLP で検出された 3 つ OTU (OTU 106, 423, 853) と *in silico* T-RFLP で検出された 1 つの OTU (T-RF の 141) はブタでは検出されなかった。これら以外の仮想 OTU の特徴は、おおよそヒトの OTU と共通しているものと考えられたが、3 つの OTU (OTUp 12, p 16, p 21) は、分類群との対応関係に関して、対応するヒトの OTU (OTU 469, 517, 654) とは幾分異なっているように考えられた。すなわち、OTU 469 に対応する主要な分類群は *Bacteroides* であるが、OTUp 12 では *Clostridiales* あり、同様に OTU 517 では *Lactobacillales* であるが、OTUp 16 では *Clostridiales* であった。さらに、OTU 654 では *Clostridiales* であるが、OTUp 21 では *Betaproteobacteria* であった。OTU と分類群の対応関係はあくまでも推定の域を出ないものであることから、腸内フローラの T-RFLP 解析において特徴的な T-RF が見い出された場合、その T-RF をクローニングして塩基配列を決定し、それが由来した菌種を確認する必要がある。

以上の結果から、T-RFLP (nagashima 法) はブタの腸内フローラ解析に対しても適用が可能であると結論づけられた。

### 3. 要約

T-RFLP (nagashima 法) がヒトと同様にブタの腸内フローラ解析に対しても適用可能かどうかを検証するために、GenBank に登録されているブタ腸管由来細菌 16S rDNA 配列 (391 配列) を使用して *in silico* T-RFLP 解析とホモロジー検索を行った。その結果、ブタでは 34 の仮想 OTU が検出され、その内 11 の OTU はヒトでは検

出されていないものであった。一方、ヒトでの実際の T-RFLP で検出された 3 つの OTU と *in silico* T-RFLP で検出された 1 つの OTU はブタでは検出されなかった。これら以外のブタ OTU の特徴は、おおよそヒトの OTU と共通しているものと考えられたが、その内の 3 つの OTU では、分類群と OTU の対応関係に関して、対応するヒトの OTU とは幾分異なっているように考えられた。

*in silico* T-RFLP 解析に関して適切な助言を頂いた株式会社テクノスルガ・ラボの望月 淳 氏に感謝いたします。

### 文 献

- 1) Liu, W.-T., Marsh, T.L., Cheng, H. and Forney, L. J., Characterization of Microbial Diversity by determining terminal restriction fragment length polymorphism of genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **63**, 4516-4522 (1997).
- 2) Nagashima, K., Hisada, T., Sato, M. and Mochizuki, J., Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. *Appl. Environ. Microbiol.*, **69**, 1251-1262 (2003).
- 3) Nagashima, K., Mochizuki, J., Hisada, T., Suzuki, S. and Shimomura, K., Phylogenetic analysis of 16S rRNA gene sequences from human fecal microbiota and improved utility of T-RFLP profiling. *Bioscience and Microflora*, **25**, 99-107 (2006).
- 4) 長島浩二, 福土宗光, 本橋智枝子, 八十川大輔, 神林 勲, 日下部未来, 橋本重子, 武田秀勝, ヒト腸内細菌叢に対する野菜抽出酵素液 (F & E) 摂取の影響, 北海道立食品加工研究センター研究報告, **7**, 27-31 (2007).
- 5) 波岡茂郎, 実験動物としてのブタ・I. *All About Swine*, **22, 23**, 8-10 (2003).
- 6) Leser, T.D., Amenuvor, J.Z., Jensen, T.K., Lindcorna, R.H., Boye, M. and Moller. K., Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl. Environ. Microbiol.*, **68**, 673-690 (2002).

**Table 1 Results of *in silico* BstII-digestion and homology search of 16S rDNA sequences from pig gastrointestinal tract**

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371681	83	<i>Clostridium lactatifermentans</i>	1,275/1,356 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371680	83	<i>Clostridium lactatifermentans</i>	1,275/1,356 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371661	114	<i>Ruminococcus productus</i>	1,245/1,347 (92.4%)	<i>Clostridium</i> subcluster XIVa
AF371662	114	<i>Ruminococcus productus</i>	1,259/1,365 (92.2%)	<i>Clostridium</i> subcluster XIVa
AF371574	114	<i>Roseburia intestinalis</i>	1,232/1,333 (92.4%)	<i>Clostridium</i> subcluster XIVa
AF371691	114	<i>Clostridium lituseburense</i>	1,389/1,425 (97.5%)	<i>Clostridium</i> cluster XI
AF371702	114	<i>Mitsuokella jalaludinii</i>	954/1,004 (95.0%)	<i>Clostridium</i> cluster IX
AF371703	114	<i>Mitsuokella jalaludinii</i>	949/1,004 (94.5%)	<i>Clostridium</i> cluster IX
AF371697	114	<i>Mitsuokella multacida</i>	1,061/1,074 (98.8%)	<i>Clostridium</i> cluster IX
AF371700	114	<i>Mitsuokella jalaludinii</i>	970/1,004 (96.6%)	<i>Clostridium</i> cluster IX
AF371693	114	<i>Dialister invisus</i>	922/959 (96.1%)	<i>Clostridium</i> cluster IX
AF371694	114	<i>Dialister invisus</i>	977/1,014 (96.4%)	<i>Clostridium</i> cluster IX
AF371695	114	<i>Dialister propionicifaciens</i>	1,214/1,264 (96.0%)	<i>Clostridium</i> cluster IX
AF371696	114	<i>Megasphaera elsdenii</i>	1,404/1,417 (99.1%)	<i>Clostridium</i> cluster IX
AF371939	114	<i>Veillonella caviae</i>	1,394/1,402 (99.4%)	<i>Clostridiales</i>
AF371860	114	<i>Halomonas meridiana</i>	1,434/1,449 (99.0%)	<i>Gammaproteobacteria</i>
AF371660	114	<i>Eubacterium hallii</i>	1,307/1,360 (96.1%)	<i>Clostridium</i> subcluster XIVa
AF371704	114	<i>Anaerovibrio lipolyticus</i>	1,207/1,277 (94.5%)	<i>Clostridium</i> cluster IX
AF371701	114	<i>Mitsuokella jalaludinii</i>	1,389/1,488 (93.3%)	<i>Clostridium</i> cluster IX
AF371698	114	<i>Mitsuokella multacida</i>	962/1,006 (95.6%)	<i>Clostridium</i> cluster IX
AF371692	114	<i>Pseudoramibacter alactolyticus</i>	1,201/1,253 (95.8%)	<i>Clostridium</i> cluster XV
AF371699	114	<i>Mitsuokella jalaludinii</i>	973/1,007 (96.6%)	<i>Clostridium</i> cluster IX
AF371663	115	<i>Ruminococcus productus</i>	1,248/1,348 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371712	126	<i>Olsenella profusa</i>	1,370/1,421 (96.4%)	<i>Actinobacteria</i>
AF371711	127	<i>Bifidobacterium boum</i>	1,334/1,416 (94.2%)	<i>Bifidobacterium</i>
AF371870	149	<i>Desulfovibrio piger</i>	1,457/1,471 (99.0%)	<i>Deltaproteobacteria</i>
AF371871	149	<i>Desulfovibrio piger</i>	1,410/1,479 (95.3%)	<i>Deltaproteobacteria</i>
AF371771	172	<i>Clostridium methylpentosum</i>	1,237/1,351 (91.6%)	<i>Clostridium</i> cluster IV
AF371897	172	<i>Prevotella multisaccharivorax</i>	1,314/1,448 (90.7%)	<i>Prevotella</i>
AF371772	172	<i>Clostridium leptum</i>	1,143/1,226 (93.2%)	<i>Clostridium</i> cluster IV
AF371890	317	<i>Prevotella veroralis</i>	1,359/1,467 (92.6%)	<i>Prevotella</i>
AF371891	317	<i>Prevotella veroralis</i>	1,339/1,441 (92.9%)	<i>Prevotella</i>
AF371892	317	<i>Prevotella oulorum</i>	923/985 (93.7%)	<i>Prevotella</i>
AF371893	317	<i>Prevotella oulorum</i>	1,369/1,474 (92.9%)	<i>Prevotella</i>
AF371898	317	<i>Prevotella oulorum</i>	1,056/1,154 (91.5%)	<i>Prevotella</i>
AF371935	317	<i>Blastobacter denitrificans</i>	1,378/1,380 (99.9%)	<i>Alphaproteobacteria</i>
AF371878	318	<i>Prevotella dentalis</i>	1,129/1,231 (91.7%)	<i>Prevotella</i>
AF371895	318	<i>Prevotella oulorum</i>	965/1,037 (93.1%)	<i>Prevotella</i>
AF371504	328	<i>Streptococcus alactolyticus</i>	1,428/1,430 (99.9%)	<i>Lactobacillales</i>
AF371506	328	<i>Streptococcus alactolyticus</i>	1,429/1,430 (99.9%)	<i>Lactobacillales</i>
AF371507	328	<i>Streptococcus equinus</i>	1,414/1,434 (98.6%)	<i>Lactobacillales</i>
AF371508	328	<i>Streptococcus pyogenes</i>	1,412/1,462 (96.6%)	<i>Lactobacillales</i>
AF371509	328	<i>Streptococcus dysgalactiae</i>	1,438/1,454 (98.9%)	<i>Lactobacillales</i>
AF371505	330	<i>Streptococcus alactolyticus</i>	1,412/1,432 (98.6%)	<i>Lactobacillales</i>
AF371689	335	<i>Clostridium glycolicum</i>	1,391/1,421 (97.9%)	<i>Clostridium</i> cluster XI
AF371927	361	<i>Mucispirillum schaedleri</i>	1,371/1,447 (94.7%)	<i>Deferribacteres</i>
AF371889	364	<i>Prevotella bivia</i>	1,148/1,246 (92.1%)	<i>Prevotella</i>
AF371879	364	<i>Prevotella oulorum</i>	929/1,010 (92.0%)	<i>Prevotella</i>
AF371880	365	<i>Prevotella oulorum</i>	1,136/1,229 (92.4%)	<i>Prevotella</i>
AF371881	365	<i>Prevotella oulorum</i>	1,147/1,247 (92.0%)	<i>Prevotella</i>
AF371882	365	<i>Prevotella bivia</i>	922/1,015 (90.8%)	<i>Prevotella</i>
AF371894	365	<i>Prevotella corporis</i>	1,300/1,432 (90.8%)	<i>Prevotella</i>
AF371875	366	<i>Prevotella brevis</i>	1,143/1,247 (91.7%)	<i>Prevotella</i>
AF371883	366	<i>Prevotella bryantii</i>	719/768 (93.6%)	<i>Prevotella</i>
AF371884	366	<i>Prevotella shahii</i>	937/1,019 (92.0%)	<i>Prevotella</i>
AF371876	366	<i>Prevotella oulorum</i>	1,139/1,246 (91.4%)	<i>Prevotella</i>

Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371887	366	<i>Prevotella bivia</i>	930/1,009 (92.2%)	<i>Prevotella</i>
AF371888	366	<i>Prevotella bivia</i>	930/1,009 (92.2%)	<i>Prevotella</i>
AF371908	366	<i>Bacteroides plebeius</i>	1,148/1,244 (92.3%)	<i>Bacteroides</i>
AF371909	366	<i>Bacteroides caccae</i>	1,159/1,235 (93.8%)	<i>Bacteroides</i>
AF371713	366	<i>Eggerthella lenta</i>	1,099/1,194 (92.0%)	<i>Actinobacteria</i>
AF371872	366	<i>Prevotella marshii</i>	1,096/1,214 (90.3%)	<i>Prevotella</i>
AF371885	366	<i>Prevotella melaninogenica</i>	1,130/1,237 (91.4%)	<i>Prevotella</i>
AF371900	366	<i>Bacteroides coprosuis</i>	783/878 (89.2%)	<i>Bacteroides</i>
AF371874	367	<i>Prevotella buccalis</i>	941/1,025 (91.8%)	<i>Prevotella</i>
AF371877	367	<i>Prevotella dentalis</i>	1,127/1,229 (91.7%)	<i>Prevotella</i>
AF371899	367	<i>Prevotella brevis</i>	1,153/1,244 (92.7%)	<i>Prevotella</i>
AF371841	368	<i>Clostridium sardiniense</i>	1,376/1,455 (94.6%)	<i>Clostridium</i> cluster I
AF371707	368	<i>Phascolarctobacterium faecium</i>	1,211/1,273 (95.1%)	<i>Clostridium</i> cluster IX
AF371705	368	<i>Phascolarctobacterium faecium</i>	1,386/1,478 (93.8%)	<i>Clostridium</i> cluster IX
AF371706	368	<i>Phascolarctobacterium faecium</i>	1,227/1,294 (94.8%)	<i>Clostridium</i> cluster IX
AF371780	368	<i>Papillibacter cinnamivorans</i>	1,083/1,190 (91.0%)	<i>Clostridium</i> cluster IV
AF371803	369	<i>Clostridium orbiscindens</i>	921/973 (94.7%)	<i>Clostridium</i> cluster IV
AF371708	369	<i>Acidaminococcus fermentans</i>	1,438/1,480 (97.2%)	<i>Clostridium</i> cluster IX
AF371811	369	<i>Clostridium thermosuccinogenes</i>	1,064/1,194 (89.1%)	<i>Clostridium</i> cluster III
AF371820	369	<i>Clostridium cellobioparum</i>	1,049/1,192 (88.0%)	<i>Clostridium</i> cluster III
AF371947	369	<i>Subdoligranulum variabile</i>	1,163/1,240 (93.8%)	<i>Clostridium</i> cluster IV
AF371948	369	<i>Clostridium orbiscindens</i>	1,291/1,351 (95.6%)	<i>Clostridium</i> cluster IV
AF371763	370	<i>Clostridium methylpentosum</i>	1,110/1,238 (89.7%)	<i>Clostridium</i> cluster IV
AF371805	370	<i>Clostridium straminisolvens</i>	1,119/1,247 (89.7%)	<i>Clostridium</i> cluster III
AF371807	370	<i>Clostridium viride</i>	1,107/1,215 (91.1%)	<i>Clostridium</i> cluster IV
AF371824	370	<i>Clostridium thermocellum</i>	1,058/1,180 (89.7%)	<i>Clostridium</i> cluster III
AF371806	370	<i>Clostridium viride</i>	1,228/1,353 (90.8%)	<i>Clostridium</i> cluster IV
AF371710	370	<i>Sanguibacter inulinus</i>	1,434/1,437 (99.8%)	<i>Sanguibacter</i>
AF371918	467	<i>Bacteroides distasonis</i>	1,271/1,371 (92.7%)	<i>Bacteroides</i>
AF371846	468	<i>Sarcina maxima</i>	1,139/1,155 (98.6%)	<i>Clostridiales</i>
AF371682	468	<i>Eubacterium brachy</i>	1,099/1,212 (90.7%)	<i>Clostridium</i> subcluster XI
AF371678	468	<i>Ruminococcus gnavus</i>	1,332/1,436 (92.8%)	<i>Clostridium</i> subcluster XIVa
AF371683	468	<i>Anaerovorax odorimutans</i>	1,119/1,203 (93.0%)	<i>Clostridium</i> subcluster XI
AF371800	470	<i>Bacteroides capillosus</i>	1,162/1,241 (93.6%)	<i>Bacteroides</i>
AF371796	470	<i>Sporobacter termitidis</i>	1,136/1,203 (94.4%)	<i>Clostridium</i> cluster IV
AF371794	470	<i>Papillibacter cinnamivorans</i>	1,144/1,225 (93.4%)	<i>Clostridium</i> cluster IV
AF371795	470	<i>Sporobacter termitidis</i>	1,260/1,352 (93.2%)	<i>Clostridium</i> cluster IV
AF371804	470	<i>Sporobacter termitidis</i>	734/768 (95.6%)	<i>Clostridium</i> cluster IV
AF371867	470	<i>Campylobacter lanienae</i>	1,383/1,404 (98.5%)	<i>Epsilonproteobacteria</i>
AF371868	470	<i>Campylobacter lanienae</i>	1,424/1,444 (98.6%)	<i>Epsilonproteobacteria</i>
AF371799	470	<i>Bacteroides capillosus</i>	1,267/1,371 (92.4%)	<i>Bacteroides</i>
AF371797	470	<i>Sporobacter termitidis</i>	1,180/1,257 (93.9%)	<i>Clostridium</i> cluster IV
AF371802	470	<i>Bacteroides capillosus</i>	1,149/1,238 (92.8%)	<i>Bacteroides</i>
AF371869	470	<i>Helicobacter rodentium</i>	1,408/1,415 (99.5%)	<i>Epsilonproteobacteria</i>
AF371801	470	<i>Clostridium orbiscindens</i>	1,179/1,259 (93.6%)	<i>Clostridium</i> cluster IV
AF371937	470	<i>Clostridium orbiscindens</i>	1,343/1,419 (94.6%)	<i>Clostridium</i> cluster IV
AF371798	471	<i>Bacteroides capillosus</i>	1,250/1,351 (92.5%)	<i>Bacteroides</i>
AF371949	471	<i>Clostridium orbiscindens</i>	1,291/1,351 (95.6%)	<i>Clostridium</i> cluster IV
AF371512	486	<i>Mycoplasma capricolum</i>	662/750 (88.3%)	<i>Firmicutes</i>
AF371679	486	<i>Clostridium lactatifermentans</i>	1,202/1,244 (96.6%)	<i>Clostridium</i> subcluster XIVa
AF371652	489	<i>Ruminococcus gnavus</i>	1,272/1,360 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371547	489	<i>Ruminococcus schinkii</i>	1,284/1,358 (94.6%)	<i>Clostridium</i> subcluster XIVa
AF371658	489	<i>Eubacterium hallii</i>	1,425/1,453 (98.1%)	<i>Clostridium</i> subcluster XIVa
AF371635	490	<i>Ruminococcus gnavus</i>	1,293/1,364 (94.8%)	<i>Clostridium</i> subcluster XIVa
AF371599	490	<i>Ruminococcus gnavus</i>	1,149/1,222 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371576	490	<i>Ruminococcus productus</i>	1,249/1,349 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371556	490	<i>Clostridium saccharolyticum</i>	1,340/1,441 (93.0%)	<i>Clostridium</i> subcluster XIVa

Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371557	490	<i>Clostridium saccharolyticum</i>	1,331/1,437 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371539	490	<i>Ruminococcus schinkii</i>	1,278/1,340 (95.4%)	<i>Clostridium</i> subcluster XIVa
AF371639	490	<i>Ruminococcus gnavus</i>	1,340/1,446 (92.7%)	<i>Clostridium</i> subcluster XIVa
AF371594	490	<i>Ruminococcus gnavus</i>	1,282/1,333 (96.2%)	<i>Clostridium</i> subcluster XIVa
AF371545	490	<i>Ruminococcus schinkii</i>	1,301/1,350 (96.4%)	<i>Clostridium</i> subcluster XIVa
AF371541	490	<i>Ruminococcus schinkii</i>	1,279/1,350 (94.7%)	<i>Clostridium</i> subcluster XIVa
AF371558	490	<i>Ruminococcus gnavus</i>	1,283/1,353 (94.8%)	<i>Clostridium</i> subcluster XIVa
AF371540	490	<i>Ruminococcus schinkii</i>	1,275/1,363 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371636	490	<i>Ruminococcus gnavus</i>	1,266/1,351 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371941	490	<i>Clostridium bolteae</i>	1,214/1,327 (91.5%)	<i>Clostridium</i> subcluster XIVa
AF371645	491	<i>Eubacterium eligens</i>	1,306/1,360 (96.0%)	<i>Clostridium</i> subcluster XIVa
AF371646	491	<i>Eubacterium eligens</i>	1,305/1,353 (96.5%)	<i>Clostridium</i> subcluster XIVa
AF371648	491	<i>Eubacterium eligens</i>	1,371/1,456 (94.2%)	<i>Clostridium</i> subcluster XIVa
AF371649	491	<i>Ruminococcus gnavus</i>	1,260/1,357 (92.9%)	<i>Clostridium</i> subcluster XIVa
AF371666	491	<i>Clostridium polysaccharolyticum</i>	1,159/1,232 (94.1%)	<i>Clostridium</i> subcluster XIVa
AF371566	491	<i>Clostridium symbiosum</i>	1,264/1,351 (93.6%)	<i>Clostridium</i> subcluster XIVa
AF371583	491	<i>Ruminococcus gnavus</i>	1,235/1,330 (92.9%)	<i>Clostridium</i> subcluster XIVa
AF371647	492	<i>Eubacterium eligens</i>	1,078/1,116 (96.6%)	<i>Clostridium</i> subcluster XIVa
AF371665	492	<i>Clostridium populeti</i>	1,096/1,172 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371684	499	<i>Anaerovorax odorimitans</i>	1,154/1,235 (93.4%)	<i>Clostridium</i> subcluster XI
AF371840	499	<i>Clostridium baratii</i>	1,406/1,434 (98.0%)	<i>Clostridium</i> cluster I
AF371686	499	<i>Anaerovorax odorimitans</i>	1,157/1,244 (93.0%)	<i>Clostridium</i> subcluster XI
AF371843	500	<i>Clostridium chartatabidum</i>	1,408/1,454 (96.8%)	<i>Clostridium</i> cluster I
AF371837	500	<i>Clostridium disporicum</i>	1,419/1,443 (98.3%)	<i>Clostridium</i> cluster I
AF371932	500	<i>Clostridium disporicum</i>	1,389/1,407 (98.7%)	<i>Clostridium</i> cluster I
AF371602	501	<i>Ruminococcus gnavus</i>	1,280/1,374 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371638	501	<i>Ruminococcus gnavus</i>	1,284/1,367 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371836	501	<i>Clostridium disporicum</i>	1,421/1,443 (98.5%)	<i>Clostridium</i> cluster I
AF371564	501	<i>Clostridium bolteae</i>	1,366/1,458 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371676	501	<i>Clostridium populeti</i>	1,158/1,232 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371671	501	<i>Clostridium populeti</i>	1,143/1,230 (92.9%)	<i>Clostridium</i> subcluster XIVa
AF371598	501	<i>Ruminococcus gnavus</i>	1,316/1,365 (96.4%)	<i>Clostridium</i> subcluster XIVa
AF371620	502	<i>Ruminococcus productus</i>	1,214/1,349 (90.0%)	<i>Clostridium</i> subcluster XIVa
AF371552	502	<i>Syntrophococcus sucromutans</i>	1,290/1,365 (94.5%)	<i>Clostridium</i> subcluster XIVa
AF371550	504	<i>Ruminococcus productus</i>	1,320/1,376 (95.9%)	<i>Clostridium</i> subcluster XIVa
AF371668	513	<i>Clostridium oroticum</i>	1,342/1,461 (91.9%)	<i>Clostridium</i> subcluster XIVa
AF371609	514	<i>Roseburia intestinalis</i>	1,400/1,452 (96.4%)	<i>Clostridium</i> subcluster XIVa
AF371610	514	<i>Roseburia intestinalis</i>	1,393/1,447 (96.3%)	<i>Clostridium</i> subcluster XIVa
AF371626	514	<i>Clostridium oroticum</i>	1,330/1,449 (91.8%)	<i>Clostridium</i> subcluster XIVa
AF371633	514	<i>Roseburia intestinalis</i>	1,287/1,351 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371619	514	<i>Clostridium amygdalinum</i>	1,305/1,417 (92.1%)	<i>Clostridium</i> subcluster XIVa
AF371548	514	<i>Ruminococcus schinkii</i>	1,382/1,450 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371634	514	<i>Roseburia intestinalis</i>	1,281/1,351 (94.8%)	<i>Clostridium</i> subcluster XIVa
AF371604	514	<i>Roseburia intestinalis</i>	1,366/1,451 (94.1%)	<i>Clostridium</i> subcluster XIVa
AF371553	514	<i>Eubacterium cellulosolvens</i>	1,082/1,194 (90.6%)	<i>Clostridium</i> subcluster XIVa
AF371588	514	<i>Clostridium bolteae</i>	1,240/1,324 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371632	515	<i>Roseburia intestinalis</i>	1,276/1,351 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371577	515	<i>Clostridium indolis</i>	1,267/1,349 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371584	515	<i>Ruminococcus gnavus</i>	1,251/1,348 (92.8%)	<i>Clostridium</i> subcluster XIVa
AF371587	515	<i>Clostridium nexile</i>	1,260/1,349 (93.4%)	<i>Clostridium</i> subcluster XIVa
AF371531	517	<i>Enterococcus durans</i>	1,465/1,473 (99.5%)	<i>Lactobacillales</i>
AF371709	517	<i>Desulfotomaculum acetoxidans</i>	461/506 (91.1%)	<i>Clostridium</i> cluster IX
AF371571	517	<i>Ruminococcus torques</i>	1,103/1,201 (91.8%)	<i>Clostridium</i> subcluster XIVa
AF371532	517	<i>Enterococcus durans</i>	1,315/1,317 (99.8%)	<i>Lactobacillales</i>
AF371792	518	<i>Bacteroides capillosus</i>	1,086/1,186 (91.6%)	<i>Clostridium</i> cluster IV
AF371538	518	<i>Staphylococcus epidermidis</i>	1,376/1,378 (99.9%)	<i>Staphylococcus</i>
AF371793	519	<i>Sporobacter termitidis</i>	1,169/1,260 (92.8%)	<i>Clostridium</i> cluster IV

Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371611	529	<i>Roseburia intestinalis</i>	1,399/1,452 (96.3%)	<i>Clostridium</i> subcluster XIVa
AF371612	529	<i>Roseburia intestinalis</i>	1,389/1,442 (96.3%)	<i>Clostridium</i> subcluster XIVa
AF371613	529	<i>Roseburia intestinalis</i>	1,417/1,469 (96.5%)	<i>Clostridium</i> subcluster XIVa
AF371618	530	<i>Roseburia intestinalis</i>	1,354/1,418 (95.5%)	<i>Clostridium</i> subcluster XIVa
AF371774	591	<i>Clostridium leptum</i>	929/1,014 (91.6%)	<i>Clostridium</i> cluster IV
AF371938	624	<i>Clostridium lentocellum</i>	1,114/1,190 (93.6%)	<i>Clostridiales</i>
AF371520	644	<i>Catenibacterium mitsuokai</i>	1,411/1,447 (97.5%)	<i>Catenibacterium</i>
AF371864	656	<i>Alcaligenes faecalis</i>	1,116/1,234 (90.4%)	<i>Betaproteobacteria</i>
AF371863	657	<i>Caldimonas taiwanensis</i>	1,206/1,318 (91.5%)	<i>Betaproteobacteria</i>
AF371862	658	<i>Oxalobacter formigenes</i>	1,425/1,467 (97.1%)	<i>Betaproteobacteria</i>
AF371861	658	<i>Pelomonas saccharophila</i>	1,431/1,447 (98.9%)	<i>Betaproteobacteria</i>
AF371856	661	<i>Actinobacillus porcinus</i>	1,430/1,453 (98.4%)	<i>Gammaproteobacteria</i>
AF371624	662	<i>Pseudobutyrvibrio ruminis</i>	1,296/1,403 (92.4%)	<i>Clostridium</i> subcluster XIVa
AF371578	662	<i>Roseburia intestinalis</i>	1,252/1,343 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371565	662	<i>Butyrvibrio fibrisolvens</i>	1,227/1,338 (91.7%)	<i>Clostridium</i> subcluster XIVa
AF371482	662	<i>Lactobacillus reuteri</i>	1,487/1,496 (99.4%)	<i>Lactobacillales</i>
AF371510	662	<i>Streptococcus australis</i>	1,391/1,446 (96.2%)	<i>Lactobacillales</i>
AF371579	662	<i>Roseburia intestinalis</i>	1,246/1,351 (92.2%)	<i>Clostridium</i> subcluster XIVa
AF371511	662	<i>Streptococcus sanguinis</i>	1,401/1,460 (96.0%)	<i>Lactobacillales</i>
AF371858	663	<i>Alishewanella fetalis</i>	1,421/1,448 (98.1%)	<i>Gammaproteobacteria</i>
AF371859	663	<i>Halomonas meridiana</i>	1,434/1,449 (99.0%)	<i>Gammaproteobacteria</i>
AF371575	663	<i>Ruminococcus schinkii</i>	1,113/1,184 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371483	663	<i>Lactobacillus vaginalis</i>	1,395/1,409 (99.0%)	<i>Lactobacillales</i>
AF371484	663	<i>Lactobacillus vaginalis</i>	1,388/1,399 (99.2%)	<i>Lactobacillales</i>
AF371473	663	<i>Lactobacillus delbrueckii</i>	1,414/1,469 (96.3%)	<i>Lactobacillales</i>
AF371480	663	<i>Weissella confusa</i>	1,477/1,477 (100.0%)	<i>Lactobacillales</i>
AF371481	663	<i>Weissella confusa</i>	1,315/1,315 (100.0%)	<i>Lactobacillales</i>
AF371568	663	<i>Clostridium polysaccharolyticum</i>	1,116/1,225 (91.1%)	<i>Clostridium</i> subcluster XIVa
AF371670	663	<i>Clostridium populeti</i>	1,139/1,222 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371476	663	<i>Lactobacillus gasserii</i>	1,489/1,498 (99.4%)	<i>Lactobacillales</i>
AF371477	663	<i>Lactobacillus johnsonii</i>	1,447/1,454 (99.5%)	<i>Lactobacillales</i>
AF371479	663	<i>Lactobacillus gasserii</i>	1,448/1,456 (99.5%)	<i>Lactobacillales</i>
AF371468	663	<i>Lactobacillus kitasatonis</i>	1,487/1,495 (99.5%)	<i>Lactobacillales</i>
AF371469	663	<i>Lactobacillus kitasatonis</i>	1,488/1,495 (99.5%)	<i>Lactobacillales</i>
AF371470	663	<i>Lactobacillus kitasatonis</i>	1,485/1,492 (99.5%)	<i>Lactobacillales</i>
AF371471	663	<i>Lactobacillus kitasatonis</i>	1,484/1,491 (99.5%)	<i>Lactobacillales</i>
AF371472	663	<i>Lactobacillus kitasatonis</i>	1,441/1,461 (98.6%)	<i>Lactobacillales</i>
AF371474	663	<i>Lactobacillus delbrueckii</i>	1,468/1,486 (98.8%)	<i>Lactobacillales</i>
AF371488	663	<i>Lactobacillus mucosae</i>	1,517/1,518 (99.9%)	<i>Lactobacillales</i>
AF371500	663	<i>Lactobacillus murinus</i>	1,411/1,418 (99.5%)	<i>Lactobacillales</i>
AF371533	663	<i>Atopobacter phocae</i>	1,183/1,273 (92.9%)	<i>Firmicutes</i>
AF371491	663	<i>Lactobacillus farciminis</i>	1,465/1,492 (98.2%)	<i>Lactobacillales</i>
AF371501	663	<i>Lactobacillus sharpeae</i>	1,383/1,463 (94.5%)	<i>Lactobacillales</i>
AF371502	663	<i>Lactobacillus sharpeae</i>	1,393/1,470 (94.8%)	<i>Lactobacillales</i>
AF371503	663	<i>Lactobacillus sharpeae</i>	1,437/1,496 (96.1%)	<i>Lactobacillales</i>
AF371675	663	<i>Clostridium populeti</i>	1,164/1,229 (94.7%)	<i>Clostridium</i> subcluster XIVa
AF371677	663	<i>Clostridium populeti</i>	1,144/1,212 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371669	663	<i>Ruminococcus gnavus</i>	1,301/1,434 (90.7%)	<i>Clostridium</i> subcluster XIVa
AF371493	663	<i>Lactobacillus paraplantarum</i>	1,454/1,481 (98.2%)	<i>Lactobacillales</i>
AF371494	663	<i>Lactobacillus paraplantarum</i>	1,428/1,483 (96.3%)	<i>Lactobacillales</i>
AF371492	663	<i>Lactobacillus spicheri</i>	1,279/1,341 (95.4%)	<i>Lactobacillales</i>
AF371572	663	<i>Butyrvibrio crossotus</i>	1,256/1,374 (91.4%)	<i>Clostridium</i> subcluster XIVa
AF371496	663	<i>Lactobacillus hammesii</i>	1,405/1,447 (97.1%)	<i>Lactobacillales</i>
AF371489	663	<i>Pediococcus parvulus</i>	1,441/1,444 (99.8%)	<i>Lactobacillales</i>
AF371657	663	<i>Ruminococcus gnavus</i>	1,321/1,435 (92.1%)	<i>Clostridium</i> subcluster XIVa
AF371673	663	<i>Roseburia intestinalis</i>	1,344/1,455 (92.4%)	<i>Clostridium</i> subcluster XIVa
AF371487	663	<i>Lactobacillus pontis</i>	1,518/1,530 (99.2%)	<i>Lactobacillales</i>

Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371495	663	<i>Lactobacillus paracollinoides</i>	1,450/1,492 (97.2%)	<i>Lactobacillales</i>
AF371499	663	<i>Lactobacillus ruminis</i>	1,483/1,486 (99.8%)	<i>Lactobacillales</i>
AF371485	663	<i>Lactobacillus panis</i>	1,439/1,452 (99.1%)	<i>Lactobacillales</i>
AF371498	663	<i>Lactobacillus agilis</i>	1,462/1,467 (99.7%)	<i>Lactobacillales</i>
AF371475	663	<i>Lactobacillus fornicalis</i>	1,251/1,331 (94.0%)	<i>Lactobacillales</i>
AF371486	663	<i>Lactobacillus coleohominis</i>	1,469/1,483 (99.1%)	<i>Lactobacillales</i>
AF371497	663	<i>Lactobacillus salivarius</i>	1,479/1,483 (99.7%)	<i>Lactobacillales</i>
AF371857	663	<i>Pasteurella aerogenes</i>	1,452/1,480 (98.1%)	<i>Gammaproteobacteria</i>
AF371674	664	<i>Clostridium populeti</i>	1,172/1,242 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371478	664	<i>Lactobacillus gasserii</i>	1,493/1,502 (99.4%)	<i>Lactobacillales</i>
AF371570	664	<i>Ruminococcus lactaris</i>	1,128/1,223 (92.2%)	<i>Clostridium</i> subcluster XIVa
AF371672	664	<i>Clostridium populeti</i>	1,156/1,222 (94.6%)	<i>Clostridium</i> subcluster XIVa
AF371490	665	<i>Pediococcus parvulus</i>	1,406/1,435 (98.0%)	<i>Lactobacillales</i>
AF371589	715	<i>Ruminococcus gnavus</i>	866/919 (94.2%)	<i>Clostridium</i> subcluster XIVa
AF371782	734	<i>Papillibacter cinnamivorans</i>	1,135/1,224 (92.7%)	<i>Clostridium</i> cluster IV
AF371783	734	<i>Papillibacter cinnamivorans</i>	1,137/1,224 (92.9%)	<i>Clostridium</i> cluster IV
AF371784	734	<i>Papillibacter cinnamivorans</i>	1,138/1,224 (93.0%)	<i>Clostridium</i> cluster IV
AF371789	734	<i>Papillibacter cinnamivorans</i>	1,131/1,213 (93.2%)	<i>Clostridium</i> cluster IV
AF371790	734	<i>Bacteroides capillosus</i>	1,148/1,239 (92.7%)	<i>Clostridium</i> cluster IV
AF371724	748	<i>Faecalibacterium prausnitzii</i>	1,376/1,414 (97.3%)	<i>Clostridium</i> cluster IV
AF371726	748	<i>Faecalibacterium prausnitzii</i>	1,416/1,445 (98.0%)	<i>Clostridium</i> cluster IV
AF371727	748	<i>Faecalibacterium prausnitzii</i>	1,405/1,434 (98.0%)	<i>Clostridium</i> cluster IV
AF371728	748	<i>Faecalibacterium prausnitzii</i>	1,399/1,428 (98.0%)	<i>Clostridium</i> cluster IV
AF371729	748	<i>Faecalibacterium prausnitzii</i>	1,415/1,445 (97.9%)	<i>Clostridium</i> cluster IV
AF371730	748	<i>Faecalibacterium prausnitzii</i>	1,385/1,427 (97.1%)	<i>Clostridium</i> cluster IV
AF371731	748	<i>Faecalibacterium prausnitzii</i>	1,386/1,427 (97.1%)	<i>Clostridium</i> cluster IV
AF371732	748	<i>Faecalibacterium prausnitzii</i>	1,405/1,421 (98.9%)	<i>Clostridium</i> cluster IV
AF371733	748	<i>Faecalibacterium prausnitzii</i>	1,427/1,442 (99.0%)	<i>Clostridium</i> cluster IV
AF371760	748	<i>Acetanaerobacterium elongatum</i>	1,091/1,189 (91.8%)	<i>Clostridium</i> cluster IV
AF371721	749	<i>Subdoligranulum variabile</i>	1,316/1,366 (96.3%)	<i>Clostridium</i> cluster IV
AF371722	749	<i>Subdoligranulum variabile</i>	1,166/1,221 (95.5%)	<i>Clostridium</i> cluster IV
AF371690	749	<i>Clostridium glycolicum</i>	1,389/1,420 (97.8%)	<i>Clostridium</i> cluster XI
AF371716	749	<i>Subdoligranulum variabile</i>	1,319/1,347 (97.9%)	<i>Clostridium</i> cluster IV
AF371717	749	<i>Subdoligranulum variabile</i>	1,336/1,356 (98.5%)	<i>Clostridium</i> cluster IV
AF371718	749	<i>Subdoligranulum variabile</i>	1,297/1,319 (98.3%)	<i>Clostridium</i> cluster IV
AF371719	749	<i>Subdoligranulum variabile</i>	1,332/1,359 (98.0%)	<i>Clostridium</i> cluster IV
AF371943	749	<i>Subdoligranulum variabile</i>	1,149/1,229 (93.5%)	<i>Clostridium</i> cluster IV
AF371715	750	<i>Subdoligranulum variabile</i>	1,313/1,356 (96.8%)	<i>Clostridium</i> cluster IV
AF371685	750	<i>Eubacterium brachy</i>	1,123/1,212 (92.7%)	<i>Clostridium</i> subcluster XI
AF371585	751	<i>Pseudobutyriivibrio ruminis</i>	1,221/1,313 (93.0%)	<i>Clostridium</i> subcluster XIVa
AF371586	752	<i>Clostridium scindens</i>	1,140/1,240 (91.9%)	<i>Clostridium</i> subcluster XIVa
AF371654	752	<i>Ruminococcus gnavus</i>	1,259/1,346 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371835	752	<i>Clostridium disporicum</i>	1,367/1,432 (95.5%)	<i>Clostridium</i> cluster I
AF371768	752	<i>Clostridium methylpentosum</i>	1,124/1,249 (90.0%)	<i>Clostridium</i> cluster IV
AF371655	753	<i>Ruminococcus gnavus</i>	1,265/1,347 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371762	753	<i>Clostridium methylpentosum</i>	1,110/1,239 (89.6%)	<i>Clostridium</i> cluster IV
AF371765	753	<i>Clostridium methylpentosum</i>	1,102/1,223 (90.1%)	<i>Clostridium</i> cluster IV
AF371786	753	<i>Bacteroides capillosus</i>	1,142/1,222 (93.5%)	<i>Clostridium</i> cluster IV
AF371787	753	<i>Bacteroides capillosus</i>	1,144/1,222 (93.6%)	<i>Clostridium</i> cluster IV
AF371767	753	<i>Clostridium methylpentosum</i>	821/915 (89.7%)	<i>Clostridium</i> cluster IV
AF371764	753	<i>Clostridium leptum</i>	1,072/1,184 (90.5%)	<i>Clostridium</i> cluster IV
AF371775	753	<i>Clostridium leptum</i>	1,177/1,253 (93.9%)	<i>Clostridium</i> cluster IV
AF371845	753	<i>Clostridium perfringens</i>	1,421/1,425 (99.7%)	<i>Clostridium</i> cluster I
AF371616	754	<i>Roseburia intestinalis</i>	1,429/1,462 (97.7%)	<i>Clostridium</i> subcluster XIVa
AF371614	754	<i>Roseburia intestinalis</i>	1,350/1,427 (94.6%)	<i>Clostridium</i> subcluster XIVa
AF371561	754	<i>Clostridium amygdalinum</i>	1,278/1,355 (94.3%)	<i>Clostridium</i> subcluster XIVa
AF371590	754	<i>Clostridium amygdalinum</i>	1,164/1,221 (95.3%)	<i>Clostridium</i> subcluster XIVa

Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371653	754	<i>Ruminococcus gnavus</i>	1,263/1,348 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371650	754	<i>Ruminococcus gnavus</i>	1,262/1,354 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371651	754	<i>Ruminococcus gnavus</i>	1,263/1,354 (93.3%)	<i>Clostridium</i> subcluster XIVa
AF371659	754	<i>Eubacterium hallii</i>	1,384/1,460 (94.8%)	<i>Clostridium</i> subcluster XIVa
AF371770	754	<i>Bacteroides capillosus</i>	1,098/1,224 (89.7%)	<i>Clostridium</i> cluster IV
AF371629	754	<i>Roseburia intestinalis</i>	1,282/1,358 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371838	754	<i>Clostridium disporicum</i>	1,406/1,433 (98.1%)	<i>Clostridium</i> cluster I
AF371839	754	<i>Clostridium disporicum</i>	1,405/1,434 (98.0%)	<i>Clostridium</i> cluster I
AF371664	755	<i>Ruminococcus gnavus</i>	1,231/1,343 (91.7%)	<i>Clostridium</i> subcluster XIVa
AF371667	755	<i>Ruminococcus gnavus</i>	1,283/1,407 (91.2%)	<i>Clostridium</i> subcluster XIVa
AF371615	755	<i>Roseburia intestinalis</i>	1,366/1,398 (97.7%)	<i>Clostridium</i> subcluster XIVa
AF371549	755	<i>Ruminococcus productus</i>	1,280/1,360 (94.1%)	<i>Clostridium</i> subcluster XIVa
AF371625	755	<i>Lachnobacterium bovis</i>	1,228/1,335 (92.0%)	<i>Clostridium</i> subcluster XIVa
AF371563	755	<i>Clostridium indolis</i>	1,354/1,448 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371842	756	<i>Clostridium chartatabidum</i>	1,415/1,454 (97.3%)	<i>Clostridium</i> cluster I
AF371559	756	<i>Ruminococcus torques</i>	1,249/1,342 (93.1%)	<i>Clostridium</i> subcluster XIVa
AF371562	757	<i>Clostridium celerecrescens</i>	1,367/1,456 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371761	769	<i>Clostridium methylpentosum</i>	1,116/1,226 (91.0%)	<i>Clostridium</i> cluster IV
AF371766	772	<i>Clostridium methylpentosum</i>	878/969 (90.6%)	<i>Clostridium</i> cluster IV
AF371723	780	<i>Faecalibacterium prausnitzii</i>	1,310/1,353 (96.8%)	<i>Clostridium</i> cluster IV
AF371737	913	<i>Eubacterium siraeum</i>	1,163/1,229 (94.6%)	<i>Clostridium</i> cluster IV
AF371743	913	<i>Faecalibacterium prausnitzii</i>	1,084/1,212 (89.4%)	<i>Clostridium</i> cluster IV
AF371741	913	<i>Faecalibacterium prausnitzii</i>	1,172/1,323 (88.6%)	<i>Clostridium</i> cluster IV
AF371744	913	<i>Eubacterium siraeum</i>	1,083/1,207 (89.7%)	<i>Clostridium</i> cluster IV
AF371747	916	<i>Ruminococcus albus</i>	1,119/1,184 (94.5%)	<i>Clostridium</i> cluster IV
AF371844	918	<i>Clostridium paraputrificum</i>	1,398/1,455 (96.1%)	<i>Clostridium</i> cluster I
AF371687	919	<i>Mogibacterium neglectum</i>	1,322/1,403 (94.2%)	<i>Clostridium</i> subcluster XI
AF371688	919	<i>Eubacterium brachy</i>	1,095/1,202 (91.1%)	<i>Clostridium</i> subcluster XI
AF371644	920	<i>Eubacterium eligens</i>	1,274/1,323 (96.3%)	<i>Clostridium</i> subcluster XIVa
AF371631	920	<i>Roseburia intestinalis</i>	1,283/1,350 (95.0%)	<i>Clostridium</i> subcluster XIVa
AF371567	921	<i>Clostridium saccharolyticum</i>	1,303/1,390 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371573	922	<i>Butyrivibrio crossotus</i>	1,166/1,241 (94.0%)	<i>Clostridium</i> subcluster XIVa
AF371560	922	<i>Clostridium celerecrescens</i>	1,288/1,427 (90.3%)	<i>Clostridium</i> subcluster XIVa
AF371537	925	<i>Gemella haemolysans</i>	1,377/1,450 (95.0%)	<i>Firmicutes</i>
AF371748	930	<i>Eubacterium siraeum</i>	874/966 (90.5%)	<i>Clostridium</i> cluster IV
AF371759	931	<i>Acetanaerobacterium elongatum</i>	1,243/1,351 (92.0%)	<i>Clostridium</i> cluster IV
AF371752	931	<i>Clostridium methylpentosum</i>	818/879 (93.1%)	<i>Clostridium</i> cluster IV
AF371751	933	<i>Anaerofilum agile</i>	1,086/1,219 (89.1%)	<i>Clostridium</i> cluster IV
AF371753	933	<i>Clostridium methylpentosum</i>	817/878 (93.1%)	<i>Clostridium</i> cluster IV
AF371720	933	<i>Subdoligranulum variabile</i>	1,281/1,356 (94.5%)	<i>Clostridium</i> cluster IV
AF371736	934	<i>Eubacterium siraeum</i>	1,273/1,358 (93.7%)	<i>Clostridium</i> cluster IV
AF371755	934	<i>Ruminococcus albus</i>	1,278/1,325 (96.5%)	<i>Clostridium</i> cluster IV
AF371756	934	<i>Ruminococcus albus</i>	1,125/1,188 (94.7%)	<i>Clostridium</i> cluster IV
AF371750	935	<i>Ruminococcus albus</i>	814/880 (92.5%)	<i>Clostridium</i> cluster IV
AF371769	936	<i>Clostridium leptum</i>	1,171/1,303 (89.9%)	<i>Clostridium</i> cluster IV
AF371781	936	<i>Papillibacter cinnamivorans</i>	1,147/1,224 (93.7%)	<i>Clostridium</i> cluster IV
AF371788	936	<i>Papillibacter cinnamivorans</i>	1,137/1,213 (93.7%)	<i>Clostridium</i> cluster IV
AF371934	936	<i>Sporobacter termitidis</i>	1,241/1,337 (92.8%)	<i>Clostridium</i> cluster IV
AF371942	936	<i>Clostridium orbiscindens</i>	1,251/1,353 (92.5%)	<i>Clostridium</i> cluster IV
AF371595	937	<i>Hespellia stercorisuis</i>	1,356/1,444 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371534	937	<i>Marinibacillus marinus</i>	889/965 (92.1%)	<i>Firmicutes</i>
AF371535	937	<i>Marinibacillus marinus</i>	889/965 (92.1%)	<i>Firmicutes</i>
AF371536	937	<i>Marinibacillus marinus</i>	889/965 (92.1%)	<i>Firmicutes</i>
AF371605	938	<i>Ruminococcus gnavus</i>	1,327/1,433 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371630	938	<i>Roseburia intestinalis</i>	1,279/1,342 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371555	938	<i>Roseburia intestinalis</i>	1,331/1,438 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371749	938	<i>Ruminococcus albus</i>	811/880 (92.2%)	<i>Clostridium</i> cluster IV



Table 1 continued

Accession no.	Size of T-RFs (nucleotides)	Species that showed the most high-score in BLAST homology search	Homology (%)	Phylogenetic groups
AF371848	939	<i>Shigella flexneri</i>	1,457/1,461 (99.7%)	<i>Enterobacteriaceae</i>
AF371606	939	<i>Roseburia intestinalis</i>	1,391/1,450 (95.9%)	<i>Clostridium</i> subcluster XIVa
AF371607	939	<i>Roseburia intestinalis</i>	1,392/1,450 (96.0%)	<i>Clostridium</i> subcluster XIVa
AF371627	939	<i>Lachnobacterium bovis</i>	1,356/1,451 (93.5%)	<i>Clostridium</i> subcluster XIVa
AF371554	939	<i>Clostridium celerecrescens</i>	1,333/1,437 (92.8%)	<i>Clostridium</i> subcluster XIVa
AF371596	939	<i>Ruminococcus torques</i>	1,361/1,418 (96.0%)	<i>Clostridium</i> subcluster XIVa
AF371597	939	<i>Ruminococcus torques</i>	1,357/1,420 (95.6%)	<i>Clostridium</i> subcluster XIVa
AF371591	939	<i>Clostridium nexile</i>	1,329/1,398 (95.1%)	<i>Clostridium</i> subcluster XIVa
AF371592	939	<i>Ruminococcus torques</i>	1,341/1,420 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371851	939	<i>Enterobacter ludwigii</i>	1,446/1,453 (99.5%)	<i>Enterobacteriaceae</i>
AF371852	939	<i>Enterobacter ludwigii</i>	1,450/1,451 (99.9%)	<i>Enterobacteriaceae</i>
AF371593	939	<i>Ruminococcus lactaris</i>	1,261/1,335 (94.5%)	<i>Clostridium</i> subcluster XIVa
AF371849	940	<i>Shigella flexneri</i>	1,452/1,457 (99.7%)	<i>Enterobacteriaceae</i>
AF371628	940	<i>Roseburia intestinalis</i>	1,375/1,467 (93.7%)	<i>Clostridium</i> subcluster XIVa
AF371640	940	<i>Ruminococcus gnavus</i>	1,350/1,437 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371637	940	<i>Clostridium populeti</i>	1,141/1,222 (93.4%)	<i>Clostridium</i> subcluster XIVa
AF371608	941	<i>Roseburia intestinalis</i>	1,388/1,456 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371582	941	<i>Ruminococcus lactaris</i>	1,234/1,329 (92.9%)	<i>Clostridium</i> subcluster XIVa
AF371621	955	<i>Pseudobutyriivibrio ruminis</i>	1,319/1,429 (92.3%)	<i>Clostridium</i> subcluster XIVa
AF371622	955	<i>Pseudobutyriivibrio ruminis</i>	1,324/1,430 (92.6%)	<i>Clostridium</i> subcluster XIVa
AF371642	956	<i>Eubacterium eligens</i>	1,380/1,459 (94.6%)	<i>Clostridium</i> subcluster XIVa
AF371543	956	<i>Ruminococcus schinkii</i>	1,297/1,359 (95.4%)	<i>Clostridium</i> subcluster XIVa
AF371641	957	<i>Eubacterium eligens</i>	1,377/1,460 (94.3%)	<i>Clostridium</i> subcluster XIVa
AF371643	958	<i>Eubacterium eligens</i>	1,376/1,457 (94.4%)	<i>Clostridium</i> subcluster XIVa
AF371850	990	<i>Shigella flexneri</i>	1,104/1,145 (96.4%)	<i>Enterobacteriaceae</i>
AF371931	990	<i>Shigella flexneri</i>	1,429/1,437 (99.4%)	<i>Enterobacteriaceae</i>
AF371936	990	<i>Escherichia coli</i>	1,445/1,450 (99.7%)	<i>Enterobacteriaceae</i>
AF371853	990	<i>Actinobacillus minor</i>	1,421/1,455 (97.7%)	<i>Gammaproteobacteria</i>
AF371854	990	<i>Actinobacillus minor</i>	1,427/1,453 (98.2%)	<i>Gammaproteobacteria</i>
AF371600	990	<i>Hespellia stercorisuis</i>	1,308/1,428 (91.6%)	<i>Clostridium</i> subcluster XIVa
AF371601	990	<i>Clostridium amygdalinum</i>	1,262/1,356 (93.1%)	<i>Clostridium</i> subcluster XIVa
AF371623	990	<i>Roseburia intestinalis</i>	1,328/1,442 (92.1%)	<i>Clostridium</i> subcluster XIVa
AF371580	990	<i>Clostridium saccharolyticum</i>	1,255/1,347 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371581	990	<i>Clostridium saccharolyticum</i>	1,256/1,347 (93.2%)	<i>Clostridium</i> subcluster XIVa
AF371544	990	<i>Ruminococcus schinkii</i>	1,288/1,353 (95.2%)	<i>Clostridium</i> subcluster XIVa
AF371542	990	<i>Ruminococcus schinkii</i>	1,288/1,351 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371725	990	<i>Faecalibacterium prausnitzii</i>	1,118/1,144 (97.7%)	<i>Clostridium</i> cluster IV
AF371745	990	<i>Acetanaerobacterium elongatum</i>	1,165/1,306 (89.2%)	<i>Clostridium</i> cluster IV
AF371746	990	<i>Acetanaerobacterium elongatum</i>	1,069/1,183 (90.4%)	<i>Clostridium</i> cluster IV
AF371758	990	<i>Acetanaerobacterium elongatum</i>	1,130/1,229 (91.9%)	<i>Clostridium</i> cluster IV
AF371773	990	<i>Clostridium leptum</i>	703/750 (93.7%)	<i>Clostridium</i> cluster IV
AF371785	990	<i>Sporobacter termitidis</i>	1,138/1,237 (92.0%)	<i>Clostridium</i> cluster IV
AF371886	990	<i>Prevotella buccalis</i>	1,139/1,243 (91.6%)	<i>Prevotella</i>
AF371656	990	<i>Clostridium celerecrescens</i>	1,203/1,318 (91.3%)	<i>Clostridium</i> subcluster XIVa
AF371551	990	<i>Eubacterium cellulosolvens</i>	1,366/1,454 (93.9%)	<i>Clostridium</i> subcluster XIVa
AF371603	990	<i>Lachnobacterium bovis</i>	1,346/1,447 (93.0%)	<i>Clostridium</i> subcluster XIVa
AF371757	990	<i>Acetanaerobacterium elongatum</i>	1,075/1,171 (91.8%)	<i>Clostridium</i> cluster IV
AF371546	990	<i>Roseburia intestinalis</i>	1,226/1,329 (92.2%)	<i>Clostridium</i> subcluster XIVa
AF371617	990	<i>Roseburia intestinalis</i>	1,346/1,413 (95.3%)	<i>Clostridium</i> subcluster XIVa
AF371855	990	<i>Actinobacillus minor</i>	1,430/1,452 (98.5%)	<i>Gammaproteobacteria</i>

**Table 2 Correspondence between T-RFs that are produced by *Bs*/I-digestion of 16S rDNA sequences from human feces or pig gastrointestinal tract and taxon**

Human <sup>a</sup>		Pig	
T-RFs (nucleotides)	Taxon	T-RFs (nucleotides)	Taxon
109	<i>Clostridium</i> subcluster XIVa	83	<i>Clostridium</i> subcluster XIVa
113, 114, 115	<i>Clostridium</i> cluster IX, <i>Megamonas</i>	114, 115	<i>Clostridium</i> cluster XI, <i>Clostridium</i> subcluster XIVa
125, 126, 127, 128	<i>Bifidobacterium</i>	126, 127	<i>Bifidobacterium</i> , <i>Actinobacteria</i>
141	<i>Prevotella</i>	149	<i>Deltaproteobacteria</i>
172	<i>Clostridium</i> cluster IV	172	<i>Clostridium</i> cluster IV, <i>Prevotella</i>
317	<i>Prevotella</i>	317	<i>Prevotell</i>
328	<i>Lactobacillales</i>	328, 330	<i>Lactobacillales</i>
335	<i>Clostridium</i> cluster XI	335	<i>Clostridium</i> cluster XI
361		361	<i>Deferribacteres</i>
365, 366	<i>Bacteroides</i>	364, 365, 366, 367	<i>Prevotella</i> , <i>Bacteroides</i>
369, 370	<i>Clostridium</i> cluster IV	368, 369, 370	<i>Clostridium</i> cluster IV, <i>Clostridium</i> cluster III, <i>Clostridium</i> cluster IX, <i>Clostridium</i> cluster I
418	<i>Clostridium</i> cluster XVIII		
466, 467, 468	<i>Bacteroides</i>	467, 468, 470, 471	<i>Clostridium</i> cluster IV, <i>Clostridium</i> subcluster XI, <i>Bacteroides</i> , <i>Epsilonproteobacteria</i>
488, 489, 490, 491	<i>Clostridium</i> subcluster XIVa	486, 489, 490, 491, 492	<i>Clostridium</i> subcluster XIVa
501, 502	<i>Clostridium</i> subcluster XIVa	499, 500, 501, 502, 504	<i>Clostridium</i> subcluster XIVa, <i>Clostridium</i> cluster I, <i>Clostridium</i> cluster XI
514	<i>Clostridium</i> subcluster XIVa	513, 514, 515	<i>Clostridium</i> subcluster XIVa
517	<i>Lactobacillales</i>	517, 518, 519	<i>Clostridium</i> cluster IX, <i>Lactobacillales</i> , <i>Clostridium</i> subcluster XIVa
		529, 530	<i>Clostridium</i> subcluster XIVa
		591	<i>Clostridium</i> cluster IV
643	<i>Clostridium</i> cluster XVII	624	<i>Clostridiales</i>
654	<i>Clostridium</i> cluster XVIII	644	<i>Clostridium</i> cluster XVII
659, 661, 662, 663	<i>Lactobacillales</i>	656, 657, 658	<i>Betaproteobacteria</i>
		661, 662, 663, 664, 665	<i>Lactobacillales</i> , <i>Clostridium</i> subcluster XIVa, <i>Gammaproteobacteria</i>
		715	<i>Clostridium</i> subcluster XIVa
		734	<i>Clostridium</i> cluster IV
747, 748, 749, 750	<i>Clostridium</i> cluster IV	748, 749, 750, 751, 752, 753	<i>Clostridium</i> cluster IV, <i>Clostridium</i> subcluster XIVa, <i>Clostridium</i> cluster XI, <i>Clostridium</i> cluster I
753, 754, 755, 756, 757	<i>Clostridium</i> subcluster XIVa	754, 755, 756, 757	<i>Clostridium</i> subcluster XIVa, <i>Clostridium</i> cluster I
		769, 772	<i>Clostridium</i> cluster IV
		780	<i>Clostridium</i> cluster IV
847	<i>Bacteroides</i>		
919, 920, 923	<i>Clostridium</i> cluster XI, subcluster XIVa	913	<i>Clostridium</i> cluster IV
		916, 918, 919, 920, 921, 922	<i>Clostridium</i> subcluster XIVa, <i>Clostridium</i> cluster XI
		925, 930, 931, 933, 934, 935, 936	<i>Clostridium</i> cluster IV
935, 937, 938, 939, 940	<i>Clostridium</i> subcluster XIVa	937, 938, 939, 940, 941	<i>Clostridium</i> subcluster XIVa, <i>Enterobacteriaceae</i>
	<i>Enterobacteriales</i>		
954, 955, 956, 957, 959	<i>Clostridium</i> subcluster XIVa	955, 956, 957, 958,	<i>Clostridium</i> subcluster XIVa
995	<i>Clostridium</i> subcluster XIVa	990	<i>Clostridium</i> subcluster XIVa, <i>Enterobacteriaceae</i> , <i>Gammaproteobacteria</i>

<sup>a</sup> Data with respect to human have been previously published<sup>3)</sup>