Supplemental file 1.

The detailed method of T-RFLP

The PCR primer set used for terminal-restriction fragment length polymorphism (T-RFLP) was Thio820F and 1387R (32). Thio820F was labeled with phosphoramidite fluorochrome 5-carboxyfluorescein at the 5’ end (5’ 6-FAM) (TAKARA BIO, Ohtsu, Japan). After PCR amplification, fluorescently labeled PCR products (300 μl) were purified by using WIZARD PCR Preps DNA purification system (Promega) and were eluted in a final volume of 50 μl. Each digest contained 10 μl of a purified PCR product, 20U of restriction enzymes (*Hha* and *EcoR* I: NIPPON GENE, Toyama, Japan.), and 1.5 μl of the restriction buffer (10 × H Buffer; NIPPON GENE). Incubation was done at 37°C for 6 h. After incubation, 12 μl of the *Hidi*-formamide and ROX size standards (Applied Biosystems) were added as the template to 1 μl of the restriction digest. All reaction mixtures were incubated at 95°C for 5 min, and then cooled on ice for 10 min. The size of terminal 16S rRNA gene fragments present in the restriction digest was determined with an automatic-sequencer (Prism 310 genetic analyzer; Applied Biosystems) using 1 μl of the template. Data were analyzed using the GeneScan Analysis program V.3.1.2 (Applied Biosystems). Peak height was also measured for semi-quantitative comparison.