Taxonomic analysis of two dominant OTUs

The most conspicuous difference in the gut microbiota induced by ACA was a site-specific effect in two populations of bacteria both classified as members of family *Muribaculaceae* (See main-text Fig. 3). OTUs were classified based on an approximately 240 bp fragment of the 16S rRNA gene in the V4 hypervariable region. Using this fragment, we applied several lines of evidence to confirm that OTU-1 and OTU-4 are both members of the *Muribaculaceae* and that they are genetically distinct from cultured relatives. Classification of sequences using the method of Wang et al. [1] and the SILVA non-redundant database as a reference [2], identified both OTU-1 and OTU-4 as members of the family with 100% bootstrap support. While use of the RDP training set [3] Version 14 instead assigned these sequences to the family Porphyromonadaceae this is presumably because the *Muribaculaceae* are not recognized as a taxon in the RDP (previously reported by [4]), nor are alternative names for the clade ("S24-7" or "*Homeothermaceae*").

A follow-up phylogenetic analysis of representative amplicon sequences from two dominant OTUs was carried out using approximate maximum likelihood estimation implemented in the FastTree software (version 2.1.8, [5]) using the generalized time reversible model with twenty discrete rate categories (`-gtr -gamma` options). Approximate maximum likelihood phylogenetic estimation, using a selection of type strains in the order Bacteroidales, places OTU-1 and OTU-4 in a clade with representatives of the *Muribaculaceae* with >95% support for the topology of that node (see Fig. 1). While such a short sequence fragment is unlikely to perfectly recapitulate phylogeny—indeed, tree topology was generally weakly supported and was sensitive to both the choice of reference sequences and the evolutionary model used—we are nonetheless satisfied with the evidence for assignment of both OTU sequences to this clade; besides exceptions in the Porphyromonadaceae, Marinilabiliaceae, and *Bacteroides*, our phylogenetic reconstruction largely matches a recently proposed taxonomy of the Bacteroidales [6].

OTU-1 and OTU-4 represent uncultured genera. Over the analyzed sequence they have 89% and 92% identity, respectively, to *Muribaculum intestinalis* strain YL27, the first cultured representative of the *Muribaculaceae* [8]. A BLAST search against the NCBI non-redundant nucleotide collection did not find higher sequence similarity to any other cultured bacteria. Representative sequences for OTU-1 and OTU-4 share nucleotides at only 22 out of 244 positions (91%).

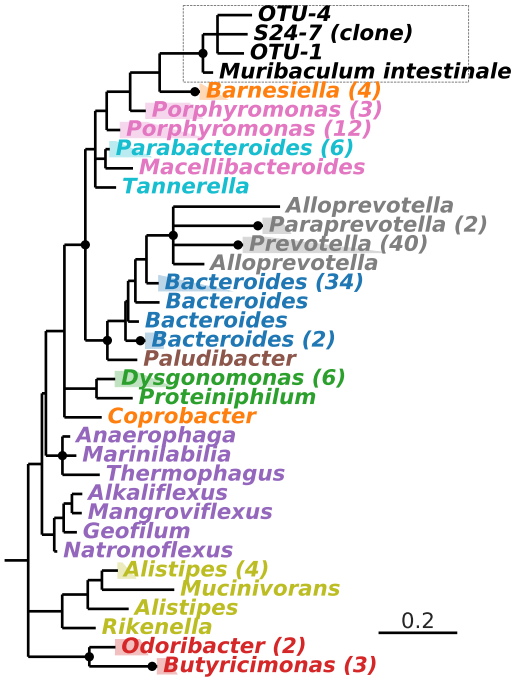

Figure 1: Phylogenetic characterization of OTU-1 and OTU-4 based on approximately 240 bp of the 16S rRNA gene V4 hypervariable region and more than 130 type strain reference sequences spanning the diversity of order Bacteroidales. Branch lengths are in units of expected substitutions per site. The tree is rooted by a Flavobacteriales out-group (not shown). Reference taxa are labeled with genus designations according to the SILVA database. When multiple representatives from the same genus have been folded together, the number of sequences is reported in parentheses. Nodes with Shimodaira-Hasegawa local support over 95% are indicated with black circles and nodes with support less than 70% have been collapsed to polytomies. The dashed box encloses taxa inferred to be within the *Muribaculaceae*. The taxon labeled 'S24-7 (clone)' (GenBank: AJ400263.1) is the environmental sequence by which the clade was originally identified, and by which it was historically named [7], while *Muribaculum intestinalis* (GenBank: KR364784.1, DSM-28989) is the first cultured representative [8]. Label colors indicate a recently proposed family membership of each reference: Prevotellaceae (gray), Barnesiellaceae (orange), Porphyromonadaceae (pink), Dysgonomonadaceae (green), Bacteroidaceae (blue), Tannerellaceae (light blue), Marnilabilaceae (purple), Marinifilaceae (red), Paludibacteraceae (brown), and Rikenellaceae (yellow) [6].

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