

Butyrate-Producing Bacteria, Including Mucin Degraders, from the Swine Intestinal Tract

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To identify bacteria with potential for influencing gut health, 980 anaerobes were cultured from the swine intestinal tract and analyzed for butyrate production. Fifteen isolates in the order *Clostridiales* produced butyrate and had butyryl coenzyme A (CoA):acetate CoA transferase activity. Three of the isolates grew on mucin, suggesting an intimate association with host intestinal mucosa.

Butyrate and butyrate-producing microbes have been associated with gastrointestinal health in humans (1–4) and various animal species, including swine (5–7). Despite the likely importance of butyrate-producing microbes to the swine intestinal microbiome, the nature and characteristics of these bacteria remain largely unknown. Consequently, this investigation was aimed at isolating and identifying bacteria producing butyrate in the swine intestine. These are important steps toward our long-range goal of characterizing, monitoring, and manipulating butyrate-producing bacteria in the swine intestinal tract to benefit host animal health and agricultural productivity.

Under anaerobic culture conditions, 980 bacteria were isolated as single colonies from the feces, intestinal contents, and intestinal mucosal scrapings of three sows and their 21- to 108-day-old piglets (8). Various culture media were used to increase the likelihood of selecting diverse taxa (Table 1). These animals were also used in parallel studies (12). Also evaluated for butyrate production were seven mucin-degrading bacterial strains previously isolated from the swine intestinal compartments, including mucosal scrapings (11). The mucin degraders were obtained by serial passage in enrichment broth cultures containing swine gastric mucus and grew in medium containing mucus or mucins as sole carbon sources. After subculture purification by single colony transfers, the isolates were cultured in broth media to maximum growth (optical density at 620 nm [OD₆₂₀]), and culture supernatants were analyzed for butyrate by gas-liquid chromatography (13). Fifteen isolates that produced ≥5 mM butyrate were studied further (Table 1).

Nearly full-length 16S rRNA gene sequences were obtained after PCR amplification of genomic DNA preparations using the primers 8F (5'-AGAGTTTGGATCCTGGCTCAG-3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3'). Based on 16S rRNA gene sequence comparisons (Table 1; Fig. 1) and a >97% identity cutoff for species designation, eight isolates were strains of *Megasphaera elsdenii*, a commonly isolated swine intestinal anaerobe (14, 15). All *M. elsdenii* strains were isolated from the feces of piglets within 2 weeks after weaning. The other seven isolates were related to bacterial species in the genera *Eubacterium*, *Anaerostipes*, *Peptoniphilus*, *Roseburia*, and *Flavonifractor* (Table 1; Fig. 1). At least five isolates, 27-5-10, 68-3-10, 494a, 992a, and 499, appear to be new bacterial taxa (species or genera). Additional physiological, biochemical, and genetic characterizations, however, will be

required to confirm or establish the taxonomic identities of the isolates, other than *M. elsdenii* strains.

Butyrate-producing bacteria in the human intestine are predominantly members of clostridial clusters IV and XIVa (3, 16, 17). The last enzymatic step for butyrate formation in these anaerobes is commonly butyryl coenzyme A (CoA):acetate CoA transferase (But) (EC 2.8.3.8) rather than butyrate kinase (Buk) (EC 2.7.2.7) (3, 18). Cell-free lysates (prepared using a French press) of all 15 swine strains (Table 1) were negative in an assay for Buk activity (19) and lacked the Buk gene, as determined by PCR assay (18). In contrast, cell-free lysates of every strain were positive in an assay for But activity (20), with activities ranging from 0.07 to 7.77 μmol/min/mg protein (Table 1). Five strains (27-5-10, 494A, 499, 831b, and 992a) had But genes (Table 1), as determined in PCR assays using the previously described But primers (16) and the following amplification conditions: 5 min of denaturation at 95°C; 10 cycles of touchdown PCR of 95°C for 30 s, 53°C for 30 s (–0.4°C per cycle to 49°C), and 72°C for 30 s; 30 cycles of 95°C for 30 s, 49°C for 30 s, and 72°C for 30 s; and a final 10-min extension at 72°C. PCR amplicon sequences confirmed their identity as But genes, and the sequences were deposited in GenBank (accession numbers JX629256 to JX629270).

Notably, isolates 35-6-1 and 68-3-10 and the eight *M. elsdenii* strains did not have But genes detectable by degenerate PCR assay, although they all had But enzyme activity (Table 1). Previous studies using the same PCR primers for But genes (21, 22) would not have detected *M. elsdenii* or the two mucinolytic isolates. Our current findings combined with recent analyses of butyrate-producing bacteria from chicken ceca (23) suggest that But gene PCR assay with degenerate primers needs to be improved (broadened) to capture butyrate producer diversity in intestinal microbiomes of swine, chickens, and probably other animals. One approach could be arrays of PCRs using primer sets targeting specific But

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TABLE 1 Characteristics of butyrate-producing bacteria isolated from the swine intestine

Strain (origin) ^a	Isolation medium ^b	Butyrate production		Butyryl-CoA:acetate CoA transferase		Closest related species (16S rRNA % identity; accession no.) ^c
		Medium ^b	Concn (mM) ^c	Sp act ^d	PCR assay	
27-5-10 (P Ce M)	HM	PYM1	9.0 ± 0.2	1.98 ± 0.02	Pos	<i>Flavonifractor plautii</i> 265 ^T (95; Y18187)
35-6-1 (P Co M)	HM	PYGM1	6.8 ± 0.0	4.35 ± 0.10	Neg	<i>Peptoniphilus gorbachii</i> WAL 10418 ^T (97; DQ911241)
68-3-10 (P Co C)	HM	M2GSC	7.4 ± 0.0	0.07 ± 0.00	Neg	<i>Eubacterium nodatum</i> VPI D6 A-5 ^T (93; Z36274)
984a (P F)	DRFp	PYGM2	4.7 ± 0.5	1.07 ± 0.02	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
813a (P F)	DRFp	PYGM2	4.7 ± 0.6	1.30 ± 0.05	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
702 (P F)	DRFp	DRF7-4X	7.1 ± 0.2 ^f	2.50 ± 0.05	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
1161 (P F)	DRFla	DRFla	11.0 ± 4.5 ^f	4.79 ± 0.41	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
1123 (P F)	DRFla	DRFla	9.5 ± 0.5 ^f	1.92 ± 0.02	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
739a (P F)	DRFla	DRFla	8.7 ± 1.9 ^f	4.18 ± 0.02	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
655a (P F)	DRFla	DRFla	11.8 ± 3.7 ^f	1.93 ± 0.25	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
653a (P F)	DRFla	DRFla	13.6 ± 2.6 ^f	3.91 ± 0.09	Neg	<i>Megasphaera elsdenii</i> LC-1 ^T (99; NR_029207)
992a (P F)	DRF7	DRF7-4X	10.3 ± 0.3 ^f	4.32 ± 0.07	Pos	<i>Eubacterium hadrum</i> VPI 82-52 ^T (94; FR749934)
494a (S F)	DRF7	DRF7-2X	9.0 ± 0.3 ^f	2.42 ± 0.09	Pos	<i>Eubacterium hadrum</i> VPI 82-52 ^T (96; FR749934)
831b (P F)	DRF7	DRF7-2X	8.0 ± 0.1 ^f	7.77 ± 0.55	Pos	<i>Roseburia faecis</i> M72/1 ^T (97; AY305310)
499 (S F)	DRF7	DRF7-2X	2.7 ± 0.1 ^f	3.37 ± 0.04	Pos	<i>Roseburia hominis</i> A2-183 ^T (95; AJ270482)

^a P, postweaning pig, 3 to 15 weeks old; S, adult sow; Ce, cecum; Co, colon; F, feces; M, intestinal mucosa; C, intestinal compartment contents.

^b DRF, depleted rumen fluid medium (9). Media (1 liter) derived from DRF included DRFla (lactate and acetate, 2.5 g each), DRFp (Casamino Acids and Bacto tryptone, 2.5 g each), DRF7 (D-fructose, maltose, D-glucose, N-acetyl-D-glucosamine, D-ribose, cellobiose, L-arabinose, 0.25 g each). DRF7-2X and DRF7-4X contained, respectively, 2 and 4 times the concentrations of carbohydrates of DRF7. PYGM1 and PYGM2 have been described elsewhere (8). PYM1 is PYGM1 lacking glucose. M2GSC was modified from Miyazaki et al.'s formulation (10) to contain 300 ml depleted rumen fluid and 0.5 g L-cysteine/liter (29). HM medium has been described elsewhere (11).

^c Triplicate cultures were analyzed.

^d Expressed as μmoles/min/mg protein.

^e All are type strains of the species.

^f Strains also consuming acetate.

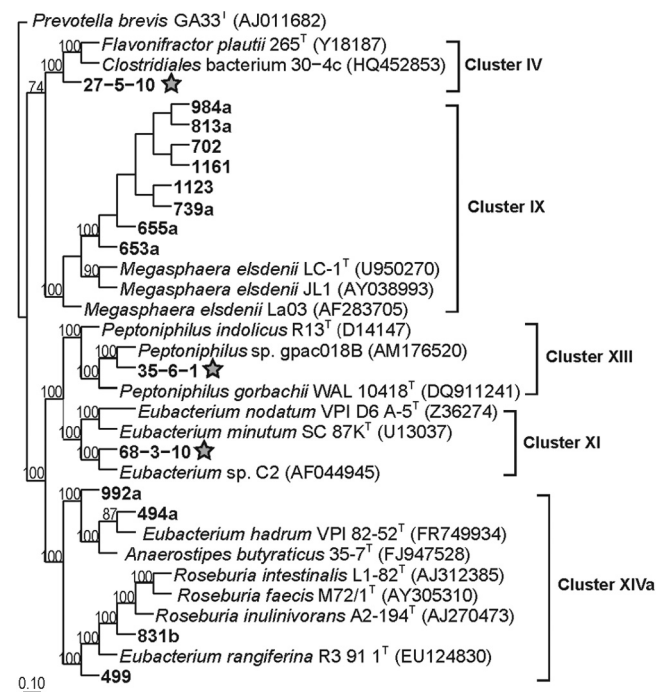


FIG 1 Maximum-likelihood phylogenetic tree of 16S rRNA gene sequences (1,231 bp) of swine butyrate producers (in bold), closely related cultured relatives, and select reference sequences, with superscript T denoting type strains. Clostridial clusters are labeled to the right of the tree. Stars indicates isolates that can degrade mucin. The tree is rooted with the *Prevotella brevis* GA33 16S rRNA sequence. Numbers by the nodes of the tree indicate the percentage bootstrap values of 1,000 resamplings and are noted only if the percentage was greater than 50%. The scale bar represents 10% sequence divergence.

genes. The availability of bacterial cultures, such as isolates 35-6-1 and 68-3-10 and *M. elsdenii* strains, for physiological and genomic analyses will facilitate this effort for swine.

All *M. elsdenii* strains were isolated from piglets shortly after weaning, a finding that is consistent with a previous report of early colonization of piglets by this anaerobe (15). *M. elsdenii* is a well-known lactate fermenter (to butyrate and propionate), present in ruminant and nonruminant mammalian digestive tracts (24, 25), including swine intestinal mucosal surfaces (26). *M. elsdenii* colonizes the human intestinal tract (27, 28), although it has not been identified as a numerically dominant butyrate producer in analyses of (adult) human feces (3). To our knowledge, colonization of infants by *M. elsdenii* remains to be studied.

A high proportion (three of seven) of the previously isolated mucin-degrading bacteria (11) were found to produce butyrate and to be phylogenetically diverse (Table 1; Fig. 1). Another mucinolytic bacterium, strain CL-84^T, was isolated from swine in the same study and has been designated a new bacterial species, *Cloacibacillus porcorum* CL-84^T in the phylum *Synergistetes* (11). *C. porcorum* CL-84^T produces butyrate when metabolizing serine. *C. porcorum* CL-84^T and mucin-degrading isolates 27-5-10 and 35-6-1 were isolated from mucosal surfaces of swine intestinal tissues. In recent *in vitro* simulations of the human intestinal mucosal environment, mucin agar became specifically colonized by butyrate-producing bacteria of *Clostridium* cluster XIVa (9). Mucinolytic bacterial species producing butyrate and inhabiting intestinal mucosal surfaces could easily be overlooked in studies of butyrate producers in fecal samples. Both their niche and their microhabitat predict that these bacteria interact intimately with host intestinal tissues, thereby influencing gut health.

Nucleotide sequence accession numbers. Newly determined

16S rRNA gene sequences have been deposited in GenBank under accession numbers [JX629256](#) to [JX629270](#).

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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