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Butyryl-CoA:acetate CoA-transferase gene associated with the genus *Roseburia* is decreased in the gut microbiota of Japanese patients with ulcerative colitis

Ryohei SHINOHARA[1a], Kengo SASAKI[2*a], Jun INOUE[3], Namiko HOSHI[3], Itsuko FUKUDA[4, 5], Daisuke SASAKI[2], Akihiko KONDO[2, 6] and Ro OSAWA[4, 5]

[1]*Division Food and Drug Evaluation Science, Department of Social/Community Medicine and Health Science, Graduate School of Medicine, Kobe University, 7-5-2 Kusunoki-cho Chuo-ku, Kobe, Hyogo 650-0017, Japan*

[2]*Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan*

[3]*Division of Gastroenterology, Department of Internal Medicine, Graduate School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan*

[4]*Department of Bioresource Science, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan*

[5]*Research Center for Food Safety and Security, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan*

[6]*RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan*

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Microbial production of butyrate is impaired in patients with ulcerative colitis (UC); however, this inhibition is not well understood in Japanese UC patients. Therefore, we quantitatively analyzed genes encoding butyryl-CoA:acetate CoA-transferase (*but*) and butyrate kinase (*buk*) in the gut microbiota of Japanese patients with UC and healthy volunteers (HVs). *But* showed higher levels than *buk*. Moreover, patients with UC showed significantly decreased levels of *but* associated with *Roseburia* sp./*Eubacterium rectale* compared with HVs. *But*, which is associated with *Faecalibacterium* sp., was maintained in patients with UC, with an unchanged relative abundance of *Faecalibacterium* sp. microorganisms in patients with UC compared with HVs.

Key words: microbiota, ulcerative colitis, butyrate, butyryl-CoA:acetate CoA-transferase, *Roseburia* sp., *Faecalibacterium* sp.

Ulcerative colitis (UC) is a chronic, relapsing, immune-mediated disease [1]. Patients with UC exhibit mucosal inflammation that extends from the rectum to the proximal segments of the colon [2]. The human gastrointestinal tract is reported to harbor 3.8×10^{13} bacteria in a 70-kg reference man [3]; these bacteria interact with each other and their host, significantly influencing human health and physiology. Several alterations (dysbiosis) have been reported in the gut microbial profile of patients with UC [4]. A previous study revealed a significant reduction in the numbers of two of the most important groups, *Roseburia hominis* (belonging to clostridial cluster XIVa) and *Faecalibacterium prausnitzii* (belonging to clostridial cluster IV), in the intestinal flora of patients with UC compared with healthy individuals [5]. After the relapse of UC, the population of fecal *F. prausnitzii* recovers in patients who achieve remission [6]. Moreover, butyrate-producing commensals contribute to mitigating intestinal diseases [7].

Bacteria produce short-chain fatty acids such as acetate, propionate, and butyrate which regulate adaptive immune responses [8]. We previously found that butyrate production is reduced in human microbiota models (Kobe University Human Intestinal Microbiota Model [KUHIMM]) of patients with UC compared with healthy individuals [9]. Butyrate contributes to the differentiation of naïve T cells into FoxP3+ regulatory T-cells, which serve as anti-inflammatory effectors [10]. It also inhibits the differentiation of naïve T cells into interferon-γ-producing cells [11]. Thus, butyrate mediates gut homeostasis and epithelium integrity.

The microbiota of healthy people mainly synthesizes butyrate via acetyl-coenzyme A (CoA) to form acetoacetyl-CoA, which is reduced in a stepwise manner to butyryl-CoA [12]. Two pathways execute the final step of butyrate formation from butyryl-CoA via butyryl-CoA:acetate CoA-transferase (encoded by *but*) or butyrate kinase (encoded by *buk*) [13]. These genes serve as biomarkers for identifying butyrate-producing communities [14]. In a healthy human colon, the *but* pathway predominates [15]. In the USA,

[a]Ryohei Shinohara and Kengo Sasaki contributed equally to this work.

*Corresponding author. Kengo Sasaki (E-mail: sikengo@people.kobe-u.ac.jp)

Table 1. Primers used in this study are illustrated

Primer Name (Forward)	Base sequence	Primer Name (Reverse)	Base sequence	Genomic DNA s for standard curves[a]	Reference
G_buk_F	tgctgtWgttggWagaggYgga	G_buk_R	gcaacIgcYttttgatttaatgcatgg	*Clostridium perfringens* JCM 1290^T	[16]
G_Fprsn_F	gacaagggccgtcaggtcta	G_Fprsn_R	ggacaggcagatRaagctcttgc	*Faecalibacterium prausnitzii* JCM31915	
G_RosEub_F	tcaaatcMggIgactgggtWga	G_Ros_R G_Eub_R	tcgataccggacatatgccaKgag tcataaccgcccatatgccatgag	*Roseburia intestinalis* JCM17583^T	
1132F	atggYtgtcgtcagctcgtg	1108R	Gggttgcgctcgttgc	*Faecalibacterium prausnitzii* JCM31915	

G_buk_F/R – *buk* genes of *Clostridium acetobutylicum*, *C. butyricum*, and *C. perfringens*; G_Fprsn – *but* gene of *Faecalibacterium prausnitzii*; G_RosEub, G_Ros_R, G_Eub_R – *but* genes of *Eubacterium rectale* and *Roseburia* sp.; 1132F, 1108R – universal primers for 16S.
[a] 16S rRNA gene copy numbers: 10 for *Clostridium perfringens* [29], 9 for *Faecalibacterium prausnitzii* [30], and 1 for *Roseburia intestinalis* (GenBank: FP929049.1).

patients with UC who underwent a colectomy followed by ileal pouch anal anastomosis harbor abnormal butyrate-producing communities predominated by *buk* [16]. Few detectable levels of *but* are similar to reference *but* genes of *F. prausnitzii* and *Roseburia* sp. in the intestinal microbiome of US patients with UC [16]. Additionally, differences in diet influence the composition of butyrate-producing bacteria [14, 17]. Thus, the results for US patients may not directly apply to Japanese patients with UC because of dietary differences.

Here, we analyzed the butyrate synthesis pathways that function in fecal microbial communities of Japanese patients with UC and compared the results with those of healthy individuals. We further analyzed butyrate synthesis using the KUHIMM, as this model reproducibly maintains the microbiota composition [18], reflecting the metabolic activity of butyrate production in the human colon [9, 18]. The results contribute to the characterization of Japanese patients with UC from the perspective of gene dynamics.

We studied 12 Japanese patients with a history of UC and 12 healthy volunteers (HVs) as previously described [9]. Written informed consent was obtained from all participants. The study was performed in accordance with the principles of the Declaration of Helsinki and guidelines of our institution and was approved by the Institutional Ethics Review Board of Kobe University (research code, 1902; approved May 10, 2016). The study was performed in accordance with the guidelines approved by the Medical Ethics Committee of Kobe University. The KUHIMM was initiated by inoculation of each fecal sample into a medium-containing vessel, as previously described [18]. Fecal samples were cultured for 30 hr. Microbial genomic DNA was extracted from the fecal samples and fermentation cultures as previously described [19]. Purified DNA was eluted into TE buffer (10 mM Tris-HCl, 1.0 mM EDTA) and stored at −20°C.

The levels of *but* and *buk* were determined by quantitative PCR using our primer sets previously designed by Vital *et al.* [16] (Table 1). Amplification was performed using TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara Bio, Inc., Kusatsu, Japan) with 2 µL template DNA per reaction (total volume 20 µL). Annealing temperatures and final primer concentrations were used according to Vital *et al.* [16] (primers described in Table 1) as follows: G_buk (64°C, 0.83 µM), G_Fprsn (70°C, 0.83 µM), G_Ros/Eub (62°C, 0.83 µM), G_Ros_R and G_Eub_R (60°C, 0.42 µM each), and total 16S (60°C, 0.67 µM). Thermocycling was performed as follows: 2 min at 50°C, 10 min at 95°C, 45 sec at 95°C; 45 sec at appropriate annealing temperatures, and 45 sec at 72°C. Elongation at 72°C was omitted from the reactions when using the 16S rRNA gene (×40) as a template. Samples were analyzed in duplicate. Genomic DNAs of *Clostridium perfringens* JCM 1290^T, *F. prausnitzii* JCM 31915, and *Roseburia intestinalis* JCM 17583^T were used to generate standard curves to determine target concentrations. We used an MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA) as previously described to determine the sequences of the 16S rRNA genes [9]. The Mann-Whitney *U* test was used for statistical analysis. P values <0.05 were considered statistically significant.

The butyrate-producing bacterial community is associated with functional resistance in patients with UC [12, 16]. Therefore, we performed quantitative PCR analysis to determine the levels of *but* and *buk* using fecal samples and KUHIMMs from Japanese HVs and patients with UC. The ratios of the *but* and *buk* levels to that of the 16S rRNA gene are shown in Fig. 1. Interestingly, the ratio of *but*, which is associated with *Roseburia* sp./*E. rectale* in patients with UC, was significantly decreased in the fecal community (p=0.0376, Mann-Whitney *U* test) and in the KUHIMMs (p=0.0373, Mann-Whitney *U* test) compared with in the HVs. However, the ratios of the *but* levels in the fecal community associated with *F. prausnitzii* did not significantly differ in patients with UC and HVs (p=0.524, Mann-Whitney *U* test) and in the KUHIMM (p=0.258, Mann-Whitney *U* test). In contrast, in the fecal community, the ratios of *buk* levels were significantly lower compared to those of *but* in HVs and patients with UC. These findings were confirmed using the KUHIMM. The ratio of *buk* did not significantly differ between HVs and patients with UC, in the fecal community and KUHIMM.

We next analyzed the bacterial 16S rRNA gene sequences of fecal communities and those of the KUHIMMs of HVs and patients with UC. The relative abundance of members of the

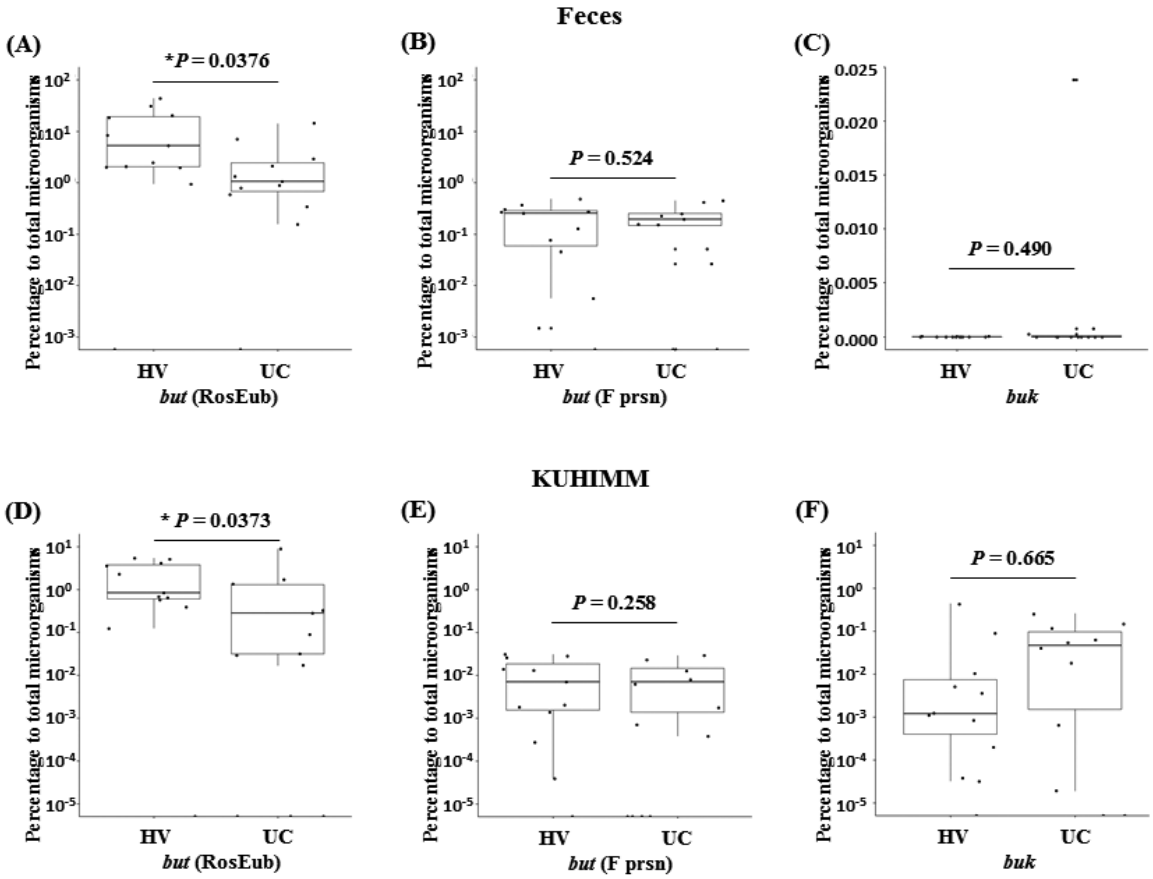

Fig. 1. Quantitative PCR analysis of butyryl-CoA:acetate CoA-transferase (*but*) and butyrate kinase (*buk*) genes in feces and in the KUHIMM. (A, D) *but* in *Roseburia* sp./*E. rectale*, *but* (RosEub) (B, E) *but* in *F. prausnitzii*, *but* (F prsn), and (C,F) *buk* in *C. butyricum*, *C. acetobutylicum*, and *C. perfringens*. The percentages were calculated by quantitative PCR analyses (= 100 × [*but* or *buk* copy numbers]/[16S rRNA gene copy numbers]). Experiments were performed using DNA samples from (A–C) fecal samples and (D–F) KUHIMM fermentation cultures. * indicates significant difference, *p<0.05.

Lachnospiraceae, which includes *Roseburia*, was decreased in patients with UC compared with HVs in fecal communities and KUHIMMs, as described previously [9]. These results corresponded to the present results acquired by quantitative PCR for *but* associated with *Roseburia* sp. The relative abundance of *Faecalibacterium* in the fecal communities and KUHIMMs did not significantly differ between those of HVs and patients with UC. These results were consistent with the quantitative PCR results for *but* in *Faecalibacterium* sp. (Fig. 2).

This is the first study to analyze butyrate-producing bacteria in Japanese patients with UC (Fig. 3). *But* predominated in Japanese patients with UC and HVs. However, *buk* predominates in US patients with UC [16], and *but* generally predominates in healthy individuals [17], as described in a study of people residing in the United Kingdom [15]. The levels of these genes associated with serious adverse consequences for US patients with UC were compared with those in their Japanese counterparts because the *but* pathway yields more butyrate compared with the *buk* pathway [15]. Moreover, *but*, which is associated with *Roseburia* sp./*E. rectale* (clostridial cluster XIVa), was decreased, while *but*, which is associated with *Faecalibacterium* sp. (clostridial cluster IV), was maintained in Japanese patients with UC; however, *but* levels associated with *Roseburia* sp./*E. rectale* and *Faecalibacterium* sp. are decreased in US patients with UC [16].

It is important to consider the factors that differentially affect the populations of butyrate-producing bacteria harbored by Japanese and US patients with UC. For example, Van den Abbeelle *et al.* [20] found that *Roseburia* sp. is the predominant producer of butyrate in the mucin layer. This result is consistent with findings showing that the colonic mucus thickness was increased in mice fed dietary fiber [21]; additionally, *Roseburia* sp. is sensitive to the composition of dietary fiber, which is altered after intake of a low-fiber diet by humans [17]. These findings indicate that the mucus layer is degraded in Japanese and US patients with UC, leading to alterations in the levels of *but* associated with *Roseburia* sp.

In contrast, the gut microbiome of Japanese people is comprised of a larger population of *Bifidobacterium*

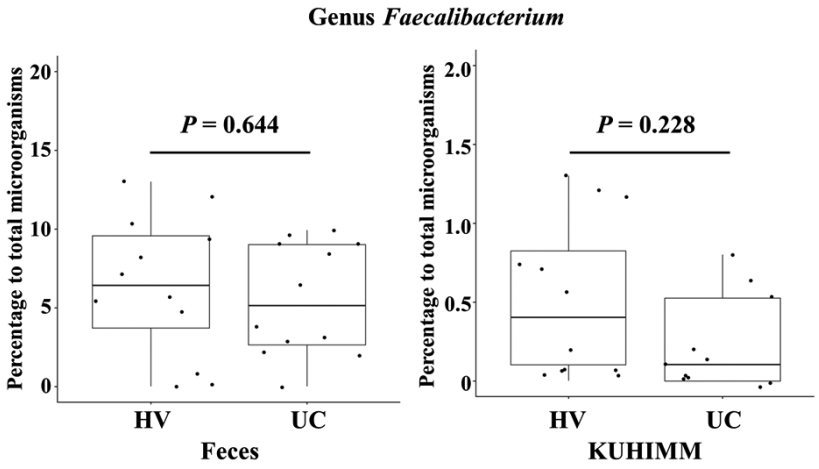

Fig. 2. Relative abundances of members of *Faecalibacterium*. Relative abundances of members of *Faecalibacterium* in (A) fecal samples and (B) corresponding fermentation cultures of healthy volunteers (HVs) and patients with UC.

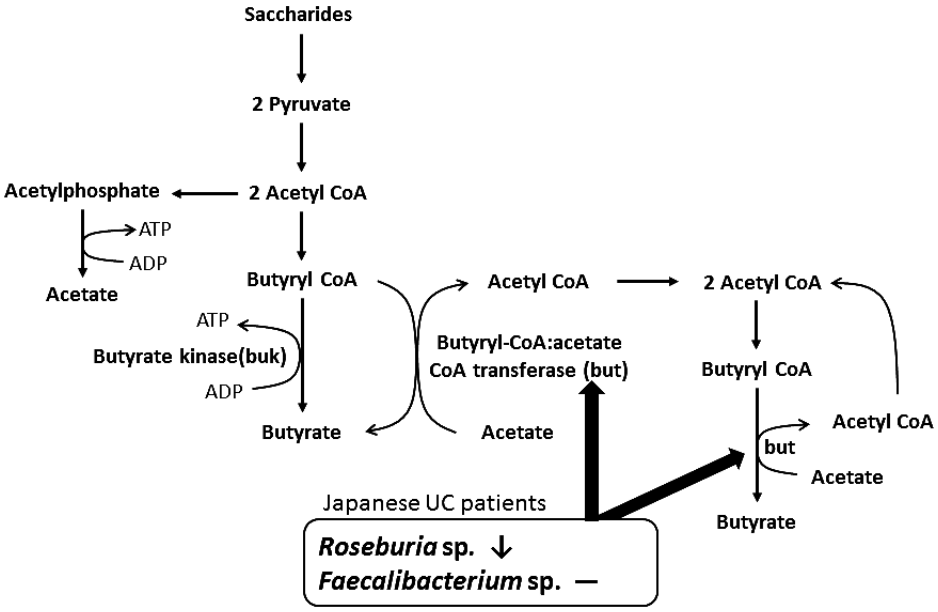

Fig. 3. Relationship between butyrate-producing bacteria and pathways for acetate and butyrate formation in Japanese patients with UC. *But* associated with *Roseburia* sp. was decreased, and that associated with *Faecalibacterium* sp. was maintained. Pathways for acetate and butyrate formation are from Khan e*t al.* [31] and Duncan *et al.* [23], respectively.

compared with that in people residing in other countries [22]. Further, *Roseburia* sp. and *F. prausnitzii* use acetate as a substrate for butyrate production when they are cultured in media containing glucose as the sole carbon source [23]. Moreover, acetate-producing *Bifidobacterium longum* and acetate-converting, butyrate-producing bacteria cross-feed when cultured in media containing oligofructose [24]. These findings indicate that cross-feeding occurs between species of *Bifidobacterium* and butyrate-producing bacteria grown on a prebiotic substrate in the human colon. We previously showed that the number of *Bifidobacterium* was not decreased in Japanese patients with UC (relative abundance: 12.0%) compared with that in healthy individuals (relative abundance: 4.3%), in studies of the human microbiota using 16S rRNA gene amplicons [9]. *Bifidobacterium* species contribute to the reproduction of *Faecalibacterium* species, which proliferate in the lumen [25]. In contrast, cross-feeding between species of *Bifidobacterium* species and *Roseburia* species, which

are reported to colonize the mucus rather than a luminal environment [25] as described above, may be weak in the lesioned mucus layer of patients with UC [26]. This occurs in Japanese patients with UC and thus does not influence the ratio of *but* associated with *Faecalibacterium* sp.

In conclusion, the present study revealed a decrease in *but* associated with *Roseburia* species rather than with *Faecalibacterium* species, demonstrating the importance of restoring *Roseburia* species in Japanese patients with UC. This may be accomplished by dietary consumption of probiotics and prebiotics, which stimulate *Bifidobacterium* species and butyrate-producing colon bacteria [27, 28].

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