Microbial interaction between the succinate-utilizing bacterium *Phascolarctobacterium faecium* and the gut commensal *Bacteroides thetaiotaomicron*

Nao Ikeyama¹ | Takumi Murakami² | Atsushi Toyoda² | Hiroshi Mori² | Takao Iino¹ | Moriya Ohkuma¹ | Mitsuo Sakamoto¹,³

¹Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki, Japan
²Advanced Genomics Center, National Institute of Genetics, Mishima, Shizuoka, Japan
³PRIME, Japan Agency for Medical Research and Development (AMED), Tsukuba, Ibaraki, Japan

Correspondence
Mitsuo Sakamoto, Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan.
Email: sakamoto@riken.jp

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Abstract
A large variety of microbes are present in the human gut, some of which are considered to interact with each other. Most of these interactions involve bacterial metabolites. *Phascolarctobacterium faecium* hardly uses carbohydrates for growth and instead uses succinate as a substrate. This study investigated the growth behavior of the co-culture of the succinate-specific utilizer *P. faecium* and the succinogenic gut commensal *Bacteroides thetaiotaomicron*. Succinate production by *B. thetaiotaomicron* supported the growth of *P. faecium* and concomitant propionate production via the succinate pathway. The succinate produced was completely converted to propionate. This result was comparable with the monoculture of *P. faecium* in the medium supplemented with 1% (w/v) succinate. We analyzed the transcriptional response (RNA-Seq) between the mono- and co-culture of *P. faecium* and *B. thetaiotaomicron*. Comparison of the expression levels of genes of *P. faecium* between the mono- and co-cultured conditions highlighted that the genes putatively involved in the transportation of succinate were notably expressed under the co-cultured conditions. Differential expression analysis showed that the presence of *P. faecium* induced changes in the *B. thetaiotaomicron* transcriptional pattern, for example, expression changes in the genes for vitamin B₁₂ transporters and reduced expression of glutamate-dependent acid resistance system-related genes. Also, transcriptome analysis of *P. faecium* suggested that glutamate and succinate might be used as sources of succinyl-CoA, an intermediate in the succinate pathway. This study revealed some survival strategies of asaccharolytic bacteria, such as *Phascolarctobacterium* spp., in the human gut.

Keywords
*Bacteroides thetaiotaomicron*, *Phascolarctobacterium faecium*, RNA-Seq, succinate pathway, survival strategy
1 | INTRODUCTION

Microbe–microbe interactions in the human gut have been increasingly recognized and analyzed in multidisciplinary fields. However, key factors in the interactions remain incompletely understood. Bacterial metabolites that include short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate (succinate and lactate are considered SCFA precursors), are associated with human health and disease (Hosseini, Grootaert, Verstraete, & Van de Wiele, 2011; Koh, Vadder, Kovatcheva-Datchary, & Bäckhed, 2016). Succinate and lactate are utilized by certain groups of anaerobic bacteria (Louis & Flint, 2017).

The microbiota of the human gut consists of a variety of microorganisms. Many are unclassified or uncultured anaerobic bacteria. During our attempts to recover new microbes from human feces, we observed bacteria that barely use carbohydrates for growth and instead use succinate as a substrate.

Phascolarctobacterium faecium is an obligately anaerobic and Gram-negative bacterium that was first isolated from koala feces (Del Dot, Osawa, & Stackebrandt, 1993). Recently, it was reported that P. faecium abundantly colonizes the human gut (Wu et al., 2017). The functional role of P. faecium in the human gut is unknown. P. faecium utilizes succinate. It grows poorly on common blood agar, but adding succinate to the medium improves growth. In a previous study, we found that the P. faecium JCM 30894 genome lacked fumarate reductase, which is an enzyme that is necessary for the production of succinate (Ogata et al., 2019).

Among the human gut microbiota, the genus Bacteroides produces acetate and succinate as the main metabolites, so that a symbiotic relationship based on succinate is conceivable. Excess accumulation in the intestine of succinate induces diarrhea (Fernández-Veledo & Vendrell, 2019; Ferreyra et al., 2014), and the presence of succinate-utilizing bacteria may have beneficial effects on humans. Bacteroides thetaiotaomicron commonly inhabits the human gut and is capable of digesting polysaccharides (Flint, Bayer, Rincon, Lamed, & White, 2008; Porter, Luis, & Martens, 2018; Xu et al., 2003). B. thetaiotaomicron produces succinate as the main metabolite (Das, Ji, Kovatcheva-Datchary, Bäckhed, & Nielsen, 2018).

To determine microbial interactions in the human gut, we used P. faecium and B. thetaiotaomicron as the model organisms. The co-culture of P. faecium and B. thetaiotaomicron has not been previously studied. The trophic interaction between the mucus-degrading bacterium Akkermansia muciniphila and the butyrate-producing bacterium Anaerostipes caecae has been described (Chia et al., 2018). The authors demonstrated the use of metatranscriptomics (RNA-Seq) as an explorative approach to study the expression profiles of A. muciniphila in response to A. caecae. We also used metatranscriptomics to explore the interaction of succinate-producing and succinate-utilizing bacteria from the human gut.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and growth conditions

Phascolarctobacterium faecium JCM 30894 and B. thetaiotaomicron JCM 5827T were obtained from the Japan Collection of Microorganisms (JCM), RIKEN BioResource Research Center, Tsukuba, Japan. Normally, two strains were maintained on Egggerth Gagnon agar (Merck) supplemented with 5% (v/v) horse blood (EG; JCM Medium No. 14) for 2–4 days at 37°C in a gas atmosphere of H2, CO2, and N2 1:1:8 (v/v) ratio.

2.2 | Growth stimulation of P. faecium by B. thetaiotaomicron

Bacteroides thetaiotaomicron JCM 5827T was streaked on one-half of an EG plate using an inoculating loop. An inoculum of P. faecium JCM 30894 was similarly streaked on another half of the same EG plate. The monoculture of each strain was also performed as a control.

2.3 | Growth stimulation of P. faecium by the addition of succinate

Phascolarctobacterium faecium JCM 30894 from a 6-day plate culture were suspended in phosphate-buffered saline (PBS). A 1% (v/v) suspension (MacFarland standard 3 turbidity) was inoculated into Gifu Anaerobic Medium Broth (GAM Broth, Nissui Pharmaceutical Co., Tokyo, Japan) that was not supplemented or supplemented with 1% (w/v) succinate (adjusted to pH 7.0). The broth was cultured experiments that were performed in anaerobic serum bottles sealed with butyl-rubber stoppers at 37°C in an atmosphere of CO2 and N2 (1:9, v/v). Cultures were sampled at 0, 18, 20, 22, 24, 42, 44, 46, and 48 hr for analysis of metabolites and measurements of optical density at 660 nm (OD660). OD660 was measured using an Ultraspec 2100 pro spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). The pH of the medium was measured using a Twin pH compact pH meter (HORIBA, Kyoto, Japan).

2.4 | Co-culture

Co-culture experiments were performed in GAM broth using anaerobic serum bottles sealed with butyl-rubber stoppers at 37°C at the aforementioned culture conditions. B. thetaiotaomicron JCM 5827T cells from a 2-day plate culture were suspended in PBS. Suspensions (1% v/v; MacFarland standard 3) were added to GAM broth followed by 5 hr of incubation to allow accumulation of metabolites. A 1% (v/v) suspension (MacFarland standard 3) of P. faecium JCM 30894 was then added to the B. thetaiotaomicron cultures. Cultures were sampled at 0, 5, 23, 29, 47, and 53 hr for analysis of metabolites and measurements of OD660. For transcriptomic analysis, bacterial
pellets received after 2 days of incubation were suspended in TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) and stored at −20°C until used for RNA purification. A pure culture of each strain was also incubated for 2 days.

### 2.5 | Analysis of metabolites

One milliliter of bacterial culture was centrifuged, and the supernatant was used for high-performance liquid chromatography (HPLC) analysis. Metabolites were quantified using an HPLC system equipped with a model SPD-M20A diode array detector model (Shimadzu, Kyoto, Japan) and a Rezex ROA-Organic acid H⁺ (8%) column (Phenomenex, Torrance, CA, USA). The analytical conditions were as follows: eluent, 0.0025 N sulfuric acid; flow rate, 0.5 ml/min; detection, ultraviolet (UV) 210 nm; and column temperature 55°C. Succinate, propionate, and acetate were used as standards.

### 2.6 | RNA purification

Total RNA was isolated by using the TRIzol Max Bacterial RNA Isolation Kit (Life Technologies) and the RNaseasy Mini Kit (QIAGEN, Valencia, CA, USA) as described previously (Chia et al., 2018).

### 2.7 | RNA sequencing

RNA samples with RNA Integrity Number ≥6.4 were used for the preparation of sequencing libraries. The libraries were constructed using two methods. One was a combination of MICROBExpress Bacterial mRNA Enrichment Kit (Ex) (Thermo Fisher Scientific, Waltham, MA, USA) and TruSeq Stranded mRNA Library Prep (Illumina, San Diego, CA, USA). The other method used the NEBNext rRNA Depletion Kit (Bacteria) (Nx) (New England Biolabs, Inc., Ipswich, MA, USA) and TruSeq Stranded mRNA Library Prep, according to the manufacturer’s protocols. An Ex kit was used for the monoculture of each strain and co-culture of two strains. An Nx kit was used for the monoculture of *B. thetaiotaomicron* and co-culture. The final libraries were then sequenced on an Illumina HiSeq 2500 platform with 100 bp paired-end sequencing reads.

### 2.8 | Transcriptome analysis

Illumina adapter sequences and low-quality bases were trimmed from raw fastq reads with fastp v0.20 (Chen, Zhou, Chen, & Gu, 2018). Forward and reverse reads were independently quality filtered, and qualified reads were combined into one fastq file. Qualified reads were then mapped on the RefSeq genomes of *B. thetaiotaomicron* VPI 5482\(^T\) (JCM 5827\(^T\), GCF_000001105.1) and *P. faecium* JCM 30894 (GCF_003945365.1) using BWA-MEM v0.7.17 (Li & Durbin, 2009). The number of reads mapped within a protein-coding sequence (CDS, including those annotated as pseudogenes) was counted using htseq-count v0.11.2 (Anders, Pyl, & Huber, 2015) without MAPQ score filtering. *B. thetaiotaomicron* genes that were differentially expressed between the mono- and co-cultured conditions were assessed using DESeq2 v1.26.0 (Love, Huber, & Anders, 2014). Genes with total read counts <10 were eliminated before DESeq2 analysis. Because of the difficulty in preparing a sufficient number of RNA samples from monocultured *P. faecium* for DESeq2 analysis, we instead normalized the read counts of *P. faecium* genes to transcript per million (TPM) values (Wagner, Kin, & Lynch, 2012) and compared the expression level of each gene among samples. To estimate the function of genes, we referred to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology database of the two strains (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016) in addition to the RefSeq annotations. Fastq files obtained in this study have been deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers DRR228499–DRR228515.

### 3 | RESULTS

#### 3.1 | Effect of *B. thetaiotaomicron* on the growth of *P. faecium*

*Phascolarctobacterium faecium* JCM 30894 monoculture formed small pinpoint colonies on the EG medium (Figure 1). Upon co-culture with *B. thetaiotaomicron* JCM 5827\(^T\), *P. faecium* JCM 30894 grew well and formed slightly larger colonies compared with monoculture (especially around the colony border). There was no difference in the growth of *B. thetaiotaomicron* JCM 5827\(^T\) in mono- and co-culture conditions with *P. faecium* JCM 30894.

#### 3.2 | Effect of succinate on the growth of *P. faecium*

*Phascolarctobacterium faecium* JCM 30894 hardly grew in GAM broth with no evident turbidity. The addition of 1% (w/v) succinate significantly stimulated *P. faecium* growth (Figure 2a). This result agreed with previous observations (Ogata et al., 2019). *P. faecium* JCM 30894 began growing at approximately 22 hr. At 42 hr, the OD\(_{660}\) reached 0.200. The pH of the cultures without succinate was around 6.50–6.87, but that of cultures with succinate significantly increased to 7.37 after 42 hr with the growth of *P. faecium* JCM 30894 (p < 0.01, Figure 2b). Growth was stimulated further by the addition of succinate. After 120 hr, 5% (w/v) succinate solution (adjusted to pH 7.0) was added to *P. faecium* cultures to a final concentration of 1% (w/v). *P. faecium* JCM 30894 continued to grow (Figure 2c). At 42 hr (a total of 162 hr), the OD\(_{660}\) reached 0.352. In the presence of 1% (w/v) succinate, growth was observed with the consumption of succinate, and the production of propionate was detected (Figure 2d). Approximately 80 mM succinate was converted to 49 mM propionate.
3.3 | Co-culture of *P. faecium* and *B. thetaiotaomicron*

In a preliminary experiment, the growth of *B. thetaiotaomicron* JCM 5827<sup>T</sup> was investigated (Figure 3a). Based on these results, *P. faecium* JCM 30894 was inoculated into a 5-hour culture of *B. thetaiotaomicron* JCM 5827<sup>T</sup> (Figure 3c). *B. thetaiotaomicron* JCM 5827<sup>T</sup> grown as monoculture produced 41 mM succinate and 12.6 mM acetate, but no propionate was detected (Figure 3b). On the other hand, *P. faecium* and *B. thetaiotaomicron* co-culture produced propionate in addition to succinate and acetate (Figure 3c). In the co-culture, up to 15.0 mM succinate was detected after 23 hr of incubation. As the amount of propionate was increased, the amount of succinate decreased and was not detected after 47 hr. Alternatively, 18.2 mM propionate was detected. This result was comparable with the monoculture of *P. faecium* JCM 30894 in GAM broth supplemented with 1% (w/v) succinate (Figure 2d). No significant differences in growth were observed between the mono- and co-culture of *P. faecium* and *B. thetaiotaomicron* (Figure 3b,c). However, as in the succinate-amended culture described above (Figure 2b), there was a significant difference (*p* < 0.05) in the final pH of the monoculture (pH 4.9) and co-culture (pH 5.3; Figure 3d). At this point, it was unclear whether the growth of *P. faecium* JCM 30894 was promoted or not. To assess this, a portion of the co-culture was plated onto the EG medium. *P. faecium* JCM 30894 formed small colonies near the large colonies of *B. thetaiotaomicron* JCM 5827<sup>T</sup> (Figure 4). The colonies of *P. faecium* JCM 30894 were 1 to 2 mm in diameter and larger than those (0.1–0.2 mm) of the monoculture of this strain on the EG medium (Figure 1).

3.4 | Transcriptomes of co-culture of *P. faecium* and *B. thetaiotaomicron*

On average, 20 million reads were generated per sample, which is above the recommended sequence depth of 5-10 million reads for a single bacterial transcriptome (Haas, Chin, Nusbaum, Birren, & Livny, 2012). The Nx kit enabled the removal of more rRNA. The number of reads mapped to the CDSs was higher. In the co-culture sample, most of the reads were derived from *B. thetaiotaomicron*. An average of 9.78% (Ex kit) and 56.7% (Nx kit) of reads were mapped on the CDSs of
3.5 | Highly expressed genes of *P. faecium* co-cultured with *B. thetaiotaomicron*

The genes involved in the succinate pathway were expressed in mono- and co-culture. However, due to the insufficient number of reads, it was difficult to determine whether there were significant changes in the expression levels. In the co-culture, two genes encoding SLC13/DASS family transporters (PFJ30894_RS03075 and PFJ30894_RS04475) exhibited much larger TPM values compared to the monoculture. Furthermore, sodium/glutamate symporter (PFJ30894_RS00375), Glu/Leu/Phe/Val dehydrogenase (PFJ30894_RS04940), and a gene cluster consisting of PFJ30894_RS01150 (4Fe-4S dicluster domain-containing protein), PFJ30894_RS01155 (2-oxoacid:acceptor oxidoreductase subunit alpha), PFJ30894_RS01160 (2-oxoacid:ferredoxin oxidoreductase subunit beta), and PFJ30894_RS01165 (pyruvate/ketoisovalerate oxidoreductase gamma subunit) were highly expressed in the co-culture (Table A2). Moreover, genes encoding chaperones and stress response factors also exhibited larger TPM values in the co-culture than in the monoculture (Table 1).

3.6 | Differential expression between *B. thetaiotaomicron* in monoculture and co-culture with *P. faecium*

The genome of *B. thetaiotaomicron* possesses 4794 CDSs, of which 4786 (99.8%) were expressed in at least one sample. We used an
adjusted p-value <0.05 as the cutoff for significantly regulated genes. Genes with the same direction of variation between the Ex and Nx kits were defined as “consistent change” and those with a different direction of variation as “inconsistent change.” A total of 1495 genes were consistent change, of which 538 were log₂ fold change (FC) ≥1 in both kits. In the co-culture with P. faecium, a gene cluster consisting of BT_RS13000 (glutamate decarboxylase), BT_RS13005 (glutaminase A), BT_RS13010 (two-pore domain potassium channel family protein), and BT_RS13015 (glutamate:GABA antiporter gadC) was downregulated in B. thetaiotaomicron. In contrast, a gene cluster for an ABC-binding cassette (ABC) transporter consisting of BT_RS02750 (HlyD family efflux transporter periplasmic adaptor subunit), BT_RS02755 (ATP-binding cassette domain-containing protein), BT_RS02760 (ABC transporter permease), and BT_RS02765 (ABC transporter permease) was upregulated in the co-culture (Table A3). Another gene set for an ABC transporter (BT_RS10625, BT_RS10630, and BT_RS10635) and neighboring BT_RS10605 (TonB-dependent receptor plug domain-containing protein) were also upregulated in the co-culture. Degnan, Barry, Mok, Taga, and Goodman (2014) identified that these genes encode BtuCDF (an ABC transporter) and BtuB (an outer membrane transporter, designated as btuB3), transporters for vitamin B₁₂ and its analogous corrinoids. Two other gene loci that encode BtuB (btuB1 and btuB2) in the B. thetaiotaomicron genome and another gene set for BtuCDF were consecutively coded with btuB2. ButB1 (BT_RS07540) was also upregulated in the co-culture, although the adjusted p-value in the Ex kit was slightly larger than 0.05. In contrast, btuB2 (BT_RS09905) was downregulated in the co-culture (Table 2). Besides, a cluster of genes encoding subunits of ATP synthase was upregulated in the monoculture (Table 3).
4 | DISCUSSION

In this study, we investigated the growth behavior of the co-cultured *P. faecium* and *B. thetaiotaomicron*. The pH values for succinate and propionate are 4.16 and 4.87, respectively. The pH of the culture supplemented with succinate increased from 6.6 to 7.4 after 42 hr. As inferred from the above-mentioned pH values for each organic acid, this is consistent with a decrease in succinate and increase in propionate. The pH of the co-culture also increased slightly from 5.0 to 5.3 after 29 hr (Figure 3d). The collective results support the view that the pH change is associated with the conversion of succinate to propionate.

The genus *Phascolarctobacterium* contains three known species (including *P. faecium*). *Phascolarctobacterium succinatutens* was isolated from human feces (Watanabe, Nagai, & Morotomi, 2012). *Phascolarctobacterium wakonense* was isolated from common marmoset feces (Shigeno, Kitahara, Shime, & Benno, 2019). These two species, as well as *P. faecium*, grew well in medium supplemented with succinate. This finding may be one of the characteristics of this genus. In the human gut, *Phascolarctobacterium* spp. convert succinate to propionate, which is a health-promoting microbial metabolite (Hosseini et al., 2011).

The three different biochemical pathways for propionate production include the succinate, acrylate, and propanediol pathways (Reichardt et al., 2014; Vidra & Németh, 2018). *Bacteroides* spp. possess the succinate pathway (Macy, Ljungdahl, & Gottschalk, 1978; Reichardt et al., 2014). This pathway is also present in *Phascolarctobacterium* spp. (Ogata et al., 2019; Reichardt et al., 2014). However, due to the lack of fumarate reductase, it is presumed that *P. faecium* JCM 30894 is unable to produce succinate, a key metabolite of the succinate pathway (Ogata et al., 2019). Therefore, co-existence with succinate-producing bacteria, such as *Bacteroides*, is essential for *Phascolarctobacterium* spp. to inhabit the human gut and produce propionate. Although *Bacteroides* spp. can convert succinate to propionate by the succinate pathway, it has been reported that succinate accumulates in cultures of *Bacteroides fragilis* under growth conditions where phosphoenolpyruvate carboxylase is repressed at high CO2 partial pressures and high dilution rates (Caspari & Macy, 1983). Furthermore, one of the conversion reactions from succinate to propionate involves methylmalonyl-CoA mutase, which requires vitamin B12 (Louis & Flint, 2017). Succinate accumulates in B12-depleted cultures of *Prevotella ruminicola* (Strobol, 1992). The genomes of *Bacteroides* spp. and *Phascolarctobacterium* spp. have been assessed for the presence of biosynthesis pathways for eight B vitamins: biotin, cobalamin (vitamin B12), folate, niacin, pantothenate, pyridoxine, riboflavin, and thiamin (Magnúsdóttir, Ravcheev, de Crécy-Lagarde, & Thiele, 2015; Ogata et al., 2019). *B. thetataomicron* VPI 5482 (JCM 5827) lacked the upstream genes required for vitamin B12 biosynthesis. On the other hand, *Phascolarctobacterium* spp. (JCM 30894 and *P. succinatutens* YIT 12067) were predicted to possess a complete vitamin B12 biosynthesis pathway. Therefore, when grown as

![Image](B. thetataomicron JCM 5827.png)

**FIGURE 4** Colonies of *Phascolarctobacterium faecium* and *Bacteroides thetaiotaomicron* in co-culture

| Locus tag   | Strand | RefSeq annotation          | KEGG Orthology | MoEx TMP | CoEx TPM | CoNx TPM |
|-------------|--------|-----------------------------|----------------|----------|----------|----------|
| PFJ30894_RS00455 | −      | Bacteriocin family protein | −              | 4076.86  | 9225.09  | 13,777.01|
| PFJ30894_RS00630 | +      | Universal stress protein   | −              | 1066.11  | 3010.79  | 4517.97  |
| PFJ30894_RS05605 | +      | Heat-inducible transcription repressor HrcA | K03705 | 3021.14  | 8547.96  | 13,627.11|
| PFJ30894_RS06510 | +      | Nucleotide exchange factor GrpE | K03687 | 4062.34  | 8751.30  | 4761.36  |
| PFJ30894_RS06515 | +      | Molecular chaperone DnaK   | K04043 | 923.46   | 2476.38  | 5102.12  |
| PFJ30894_RS06520 | +      | Molecular chaperone DnaJ   | K03686 | 324.39   | 1099.11  | 2476.24  |
| PFJ30894_RS03240 | −      | Universal stress protein   | −              | 418.42   | 53,544.17| 80,951.83|
| PFJ30894_RS07940 | +      | Recombinase RecA           | K03553 | 815.27   | 6812.12  | 10,200.12|
| PFJ30894_RS07945 | +      | Hypothetical protein       | K03565 | 305.71   | 4065.23  | 6452.08  |
| PFJ30894_RS11160 | −      | Chaperonin GroEL           | K04077 | 914.48   | 4093.71  | 5986.45  |
| PFJ30894_RS11165 | −      | Co-chaperone GroES         | K04078 | 1842.30  | 4516.56  | 8424.61  |

Note: Bold type indicates TPM values ≥2 in the co-culture (CoEx, CoNx) as compared with the monoculture (MoEx).
TABLE 2 Differential expression of genes for transporters of vitamin B\textsubscript{12} in B. thetaiotaomicron.

| Locus tag | Strand | RefSeq annotation | KEGG Orthology | Ex log\textsubscript{2} FC | Nx log\textsubscript{2} FC | Ex adjP | Nx adjP |
|-----------|--------|-------------------|----------------|------------------|-----------------|--------|--------|
| BT_RS07540 | +      | TonB-dependent receptor | -              | 0.53             | 0.7             | 6.49E-02 | 6.14E-09 |
| BT_RS09890 | -      | ABC transporter ATP-binding protein | K02013         | 1.22             | -0.43           | 6.88E-02 | 1.61E-01 |
| BT_RS09895 | -      | Iron ABC transporter permease | K02015         | 0.4              | -1.4            | 3.35E-01 | 5.82E-24 |
| BT_RS09900 | -      | ABC transporter substrate-binding protein | K02016         | -0.03            | -0.64           | 9.20E-01 | 9.07E-05 |
| BT_RS09905 | -      | TonB-dependent receptor plug domain-containing protein | K02014         | -0.81            | -1.36           | 3.16E-08 | 4.00E-38 |
| BT_RS10605 | -      | TonB-dependent receptor plug domain-containing protein | K02014         | 0.68             | 0.79            | 2.32E-03 | 2.50E-12 |
| BT_RS10625 | +      | ABC transporter substrate-binding protein | K02016         | 1.01             | 0.69            | 1.60E-02 | 7.91E-05 |
| BT_RS10630 | +      | Iron ABC transporter permease | K02015         | 1.48             | 0.63            | 6.09E-03 | 5.90E-04 |
| BT_RS10635 | +      | ABC transporter ATP-binding protein | K02013         | 1.95             | 1.58            | 2.24E-04 | 3.10E-14 |

Note: Negative values indicate upregulation in the monoculture, and positive values indicate upregulation in the co-culture.

TABLE 3 Differential expression of genes encoding subunits of ATP synthase in B. thetaiotaomicron

| Locus tag | Strand | RefSeq annotation | KEGG Orthology | Ex log\textsubscript{2} FC | Nx log\textsubscript{2} FC | Ex adjP | Nx adjP |
|-----------|--------|-------------------|----------------|------------------|-----------------|--------|--------|
| BT_RS03560 | +      | FOF1 ATP synthase subunit beta | K02112         | -1.77            | -0.97           | 3.21E-135 | 1.26E-22 |
| BT_RS03565 | +      | ATP synthase F1 subunit epsilon | K02114         | -1.62            | 0.15            | 1.23E-53 | 6.43E-01 |
| BT_RS03570 | +      | Hypothetical protein | -              | -0.87            | -1.32           | 1.14E-04 | 5.89E-16 |
| BT_RS03575 | +      | FOF1 ATP synthase subunit A | K02108         | -0.86            | -0.54           | 8.84E-16 | 3.40E-06 |
| BT_RS03580 | +      | ATP synthase F0 subunit C | K02110         | -0.52            | -0.10           | 2.00E-02 | 3.14E-01 |
| BT_RS03585 | +      | FOF1 ATP synthase subunit B | K02109         | -0.82            | -0.45           | 2.63E-11 | 1.29E-02 |
| BT_RS03590 | +      | FOF1 ATP synthase subunit delta | K02113         | -0.46            | -0.44           | 1.52E-03 | 1.00E-02 |
| BT_RS03595 | +      | FOF1 ATP synthase subunit alpha | K02111         | -0.51            | -0.14           | 9.44E-07 | 2.97E-01 |
| BT_RS03600 | +      | FOF1 ATP synthase subunit gamma | K02115         | -0.77            | -0.50           | 4.52E-12 | 1.70E-04 |

Note: Negative values indicate upregulation in the monoculture, and the positive value indicates upregulation in the co-culture.

In a monoculture, B. thetaiotaomicron JCM 5827\textsuperscript{T} probably accumulated succinate, but not propionate. In contrast, in the co-culture of two species, P. faecium JCM 30894 can convert succinate to propionate, as this strain harbors the vitamin B\textsubscript{12} biosynthesis pathway. Although B. thetaiotaomicron does not have complete gene sets for vitamin B\textsubscript{12} biosynthesis, it instead harbors three genes (btuB1–3) encoding transporters for vitamin B\textsubscript{12} and its analogous corrinoids. These genes are essential for the colonization of B. thetaiotaomicron in the intestine of germ-free mice (Degnan et al., 2014). Interestingly, in the co-culture, btuB1 and btuB3 were upregulated, but btuB2 was downregulated. Degnan et al. (2014) reported that these three transporters exhibited different preferences among several corrinoid species. These results suggest that B. thetaiotaomicron changes the expression patterns of these transporter genes in response to the corrinoids provided by P. faecium. Previous experiments observed in the presence of vitamin B\textsubscript{12} suggested that propionate production was associated with the conservation of biologically useful energy (Strobel, 1992). P. faecium may exist in an energy-limited environment, and maximizing energy conservation during the production of propionate may be one strategy this bacterium uses to survive in the human gut. The conversion of the energy of the decarboxylation reaction into sodium ion (Na\textsuperscript{+}) gradients by methylmalonyl-CoA decarboxylase is the biological use of decarboxylation energy (Dimroth, 1987; Hilpert & Dimroth, 1982). The central energy conservation step in Propionigenium modestum (Schink & Pfennig, 1982) is the conversion of the energy of methylmalonyl-CoA decarboxylation into a Na\textsuperscript{+} gradient, which in turn drives ATP synthesis via Na\textsuperscript{+}-activated ATPase (Dimroth, 1997; Dimroth & Schink, 1998; Hilpert, Schink, & Dimroth, 1984).
In this study, although the difference in the expression of the genes involved in the succinate pathway of *P. faecium* was not clear, high expressions of two genes encoding SLC13/DASS family transporter were observed in the co-culture. One of the genes (PFJ30894_RS03075) is encoded consecutively with the gene cluster of the succinate pathway. The SLC13 transporter is part of the divergent anion;Na⁺ symporter (DASS) family (Mulligan, Fitzgerald, Wang, & Mindell, 2014). VcINDY, an SLC13 homologue from *Vibrio cholerae*, couples a Na⁺ gradient to the transport of succinate, a C₄-dicarboxylate (Mulligan et al., 2014). Therefore, it seems that *P. faecium* upregulated the SLC13 transporter to transport succinate produced by *B. thetaiotaomicron* into the cell.

It has been suggested that lower pH achieved in the co-culture of *B. thetaiotaomicron* and *Bifidobacterium adolescentis* could potentially slow the growth and metabolism of *B. thetaiotaomicron* (Das et al., 2018). Furthermore, it has been reported that *B. thetaiotaomicron* DSM 2079T (=JCM 5827T) showed a growth rate at pH 5.5 of approximately 40% of the growth at pH 6.7 (Duncan, Louis, Thomson, & Flint, 2009). As mentioned above, the pH of the co-culture of *P. faecium* and *B. thetaiotaomicron* decreased from 7.0 to 5.0, but then increased to 5.3. Increased pH in co-culture would improve the growth of *B. thetaiotaomicron*. Consequently, the co-existence of these two species seems to be beneficial for each species. In the presence of *P. faecium*, *B. thetaiotaomicron* downregulated glutamate-dependent acid resistance system-related genes involved in glutaminase A and glutamate decarboxylase activity and the antiporter GadC. Strategies adopted to face acid encounters include amino acid-dependent systems (Lu et al., 2013; Pennacchietti, D’Alonzo, Freddi, Occhialini, & De Biase, 2018). In particular, the glutamate-dependent acid resistance system is extremely powerful. *B. thetaiotaomicron* possesses this system (Pennacchietti et al., 2018). The difference in pH values between the monoculture (pH 4.9) and co-culture (pH 5.3) resulted in the downregulation of the glutamate-dependent acid resistance system-related genes in the co-culture. On the other hand, co-cultured *P. faecium* highly expressed genes for chaperones and stress response factors. *P. faecium* did not grow in the monoculture but could grow in the co-culture. Active transcription of chaperonene genes could be associated with acid stress caused by succinate and acetate produced in the co-culture. The molecular mechanisms adopted by Gram-positive and Gram-negative bacteria for coping with acid stress have been reviewed (Lund, Tramonti, & De Biase, 2014). ATPase (ATP synthase) is involved in acid resistance for *Escherichia coli* (Sun, Fukamachi, Saito, & Kobayashi.
In this study, B. thetaiotaomicron upregulated ATP synthase genes in the monoculture. This result is consistent with that of the aforementioned glutamate-dependent acid resistance system, which is one of the mechanisms of protection against acidic stress.

B. thetaiotaomicron may release compounds including glutamate and ammonium into the medium in response to acidic stress. Therefore, it is conceivable that P. faecium also transports glutamate into the cell using the Na⁺ gradient formed during succinate metabolism. The transported glutamate is converted to 2-oxoglutarate by glutamate dehydrogenase, and 2-oxoglutarate is converted to succinyl-CoA by 2-oxoglutarate oxidoreductase. As mentioned above, high expression of these enzyme genes was observed in the co-culture with B. thetaiotaomicron, so that glutamate and succinate might be used as sources of succinyl-CoA, an intermediate in the succinate pathway.

In the presence of P. faecium, B. thetaiotaomicron upregulated a gene set for an ABC transporter, putatively acting as an efflux pump. It has been reported that B. fragilis may induce efflux pump gene expression when encountered with secreted antibiotics or other potentially toxic components by competing with surrounding organisms (Ghotaslou, Yekani, & Memar, 2018). Thus, B. thetaiotaomicron may have expressed a defense mechanism in the presence of P. faecium.

Co-culture experiments of P. succinatutens and the xylan-utilizing and succinate-producing bacterium Paraprevotella xylaniphila have also been reported (Watanabe et al., 2012). In the co-culture, the numbers of P. succinatutens cells increase and succinate is converted to propionate. These findings may indicate one of the survival strategies of asaccharolytic Phascolarctobacterium spp. in the human gut. This idea is supported by the greater abundance of Phascolarctobacterium along with the increased abundance of Bacteroides in rats fed a high-fat diet (Lecomte et al., 2015). The abundance of B. fragilis and Bacteroides vulgatus was positively correlated with both changes in body weight and fat mass. A previous study demonstrated that P. faecium colonizes the human gut in early life and develops to a high level in healthy adults, followed by a decrease in elderly individuals (Wu et al., 2017). The authors described that elderly individuals and those <1 year of age consumed relatively less fat and had a relatively low body weight. As inferred from rat experiments described above, this may result in a decrease in Bacteroides and the decrease in the available succinate for P. faecium.

In conclusion, we reveal some survival strategies of asaccharolytic bacteria, such as Phascolarctobacterium spp., in the human gut. The encounter between P. faecium and B. thetaiotaomicron in the human gut may result in a beneficial conversion of succinate to propionate. An overview of the microbial interaction between the succinate-utilizing bacterium P. faecium and the gut commensal B. thetaiotaomicron is shown in Figure 5.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Nao Ikeyama: Formal analysis (lead); Investigation (lead); Writing-original draft (lead); Writing-review & editing (lead). Takumi Murakami: Formal analysis (equal); Writing-original draft (equal). Atsushi Toyoda: Funding acquisition (equal); Investigation (equal); Writing-original draft (equal). Hiroshi Mori: Formal analysis (equal); Funding acquisition (equal); Writing-original draft (equal). Takao Iino: Formal analysis (equal); Writing-review & editing (equal). Moriya Ohkuma: Funding acquisition (equal); Writing-review & editing (equal). Mitsuo Sakamoto: Conceptualization (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Project administration (lead); Resources (lead); Supervision (lead); Validation (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead).

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

The datasets used and analyzed during the current study are included in this published article.

ORCID

Takumi Murakami https://orcid.org/0000-0002-4738-0464
Hiroshi Mori https://orcid.org/0000-0003-0806-7704
Mitsuo Sakamoto https://orcid.org/0000-0002-3622-4642

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APPENDIX

**TABLE A1** The number of reads mapped on the protein-coding sequences (CDSs)

| Sample       | Total read count | B. thetaiotaomicron (4794 CDSs) | Read-mapped CDS count | P. faecium (2277 CDSs) | Read-mapped CDS count |
|--------------|------------------|----------------------------------|-----------------------|------------------------|-----------------------|
|              |                  | CDS-mapped read count            |                       |                        |                       |
| Bt_A_Ex      | 17,156,707       | 655,289 (3.82)                   | 4113 (85.79)          |                        |                       |
| Bt_B_Ex      | 18,043,654       | 805,156 (4.46)                   | 4238 (88.40)          |                        |                       |
| Bt_C_Ex      | 17,306,996       | 762,024 (4.40)                   | 4206 (87.73)          |                        |                       |
| Bt_D_Ex      | 20,798,562       | 1,055,905 (5.08)                 | 4350 (90.74)          |                        |                       |
| Pf_Ex        | 16,883,728       | -                                | 1,027,297 (6.08)      | 2277 (100)             |                       |
| Co_A_Ex      | 17,945,740       | 1,587,043 (8.84)                 | 4769 (99.48)          | 6377 (0.04)            | 574 (25.21)           |
| Co_B_Ex      | 16,255,269       | 1,705,601 (10.49)                | 4759 (99.27)          | 7205 (0.04)            | 637 (27.98)           |
| Co_C_Ex      | 18,441,787       | 1,731,455 (9.39)                 | 4785 (99.81)          | 6836 (0.04)            | 628 (27.58)           |
| Co_D_Ex      | 17,332,280       | 1,803,122 (10.40)                | 4777 (99.65)          | 7031 (0.04)            | 695 (30.52)           |
| Bt_A_Nx      | 24,059,327       | 11,871,401 (49.34)               | 4746 (99.00)          |                        |                       |
| Bt_B_Nx      | 23,968,675       | 11,706,125 (48.84)               | 4747 (99.02)          |                        |                       |
| Bt_C_Nx      | 23,567,147       | 12,057,528 (51.16)               | 4749 (99.06)          |                        |                       |
| Bt_D_Nx      | 23,261,196       | 10,812,167 (46.48)               | 4726 (98.58)          |                        |                       |
| Co_A_Nx      | 23,624,293       | 15,116,851 (63.99)               | 4758 (99.25)          | 85,823 (0.36)          | 1361 (59.77)          |
| Co_B_Nx      | 23,804,062       | 12,425,946 (52.20)               | 4747 (99.02)          | 66,204 (0.28)          | 1353 (59.42)          |
| Co_C_Nx      | 23,020,409       | 13,891,365 (60.34)               | 4755 (99.19)          | 77,322 (0.34)          | 1363 (59.86)          |
| Co_D_Nx      | 22,196,464       | 11,162,589 (50.29)               | 4740 (98.87)          | 58,955 (0.27)          | 1298 (57.00)          |

Note: Percentage of mapped reads and CDSs are indicated in parentheses.

Bt: B. thetaiotaomicron, Pf: P. faecium, Co: Co-culture, Ex: MICROBExpress Bacterial mRNA Enrichment Kit, Nx: NEBNext rRNA Depletion Kit.
**TABLE A2** Transcript per million (TPM) value of the genes of *Phascolarctobacterium faecium*.

| Locus tag            | Strand | RefSeq annotation                           | KEGG Orthology | MoEx TPM (average) | CoEx TPM (average) | CoNx TPM (average) |
|----------------------|--------|---------------------------------------------|----------------|--------------------|--------------------|--------------------|
| **Genes involved in the succinate pathway** |        |                                             |                |                    |                    |                    |
| PFJ30894_RS01590     | +      | Cobalamin B_{12}-binding domain-containing protein | K01849         | 295.72             | 0                  | 0                  |
| PFJ30894_RS01595     | +      | Methylmalonyl-CoA carboxyltransferase        | K01604         | 338.18             | 0                  | 0                  |
| PFJ30894_RS03730     | +      | Succinate CoA transferase                    | K18118         | 333.54             | 60.91              | 32.15              |
| PFJ30894_RS03735     | +      | Methylmalonyl-CoA mutase                     | K01848         | 224.08             | 0                  | 31.48              |
| PFJ30894_RS04255     | +      | Methylmalonyl-CoA mutase                     | K01847         | 207.62             | 0                  | 0                  |
| PFJ30894_RS04260     | +      | Methylmalonyl-CoA mutase                     | K01847         | 234.07             | 0                  | 0                  |
| PFJ30894_RS04410     | +      | Acetyl-CoA hydrolase/transferase family protein | K18118         | 1104.25            | 525.48             | 989.68             |
| PFJ30894_RS04415     | +      | Methylmalonyl-CoA mutase family protein      | K01848         | 1474.25            | 762.62             | 774.18             |
| PFJ30894_RS04420     | +      | Cobalamin B_{12}-binding domain-containing protein | K01849         | 765.35             | 169.18             | 671.45             |
| PFJ30894_RS04430     | +      | Methylmalonyl-CoA epimerase                  | K05606         | 534.06             | 209.5              | 448.4              |
| PFJ30894_RS04435     | +      | Methylmalonyl-CoA carboxyltransferase        | K01604         | 356.74             | 0                  | 91.02              |
| PFJ30894_RS04440     | +      | OadG family protein                          | K23352         | 228.87             | 67.9               | 115.87             |
| PFJ30894_RS04445     | +      | Biotin/lipoyl-binding protein                | K23351         | 551.21             | 121.18             | 128.31             |
| PFJ30894_RS04450     | +      | Sodium ion-translocating decarboxylase subunit beta | K20509         | 277.59             | 279.98             | 242.45             |
| PFJ30894_RS04455     | +      | Methylmalonyl-CoA carboxyltransferase        | K01604         | 311.37             | 14.59              | 82.38              |
| PFJ30894_RS04460     | +      | Sodium pump decarboxylase                    | K23352         | 138.97             | 0                  | 0                  |
| PFJ30894_RS04465     | +      | Biotin/lipoyl-binding protein                | K23351         | 141.67             | 0                  | 0                  |
| PFJ30894_RS04470     | +      | Sodium ion-translocating decarboxylase subunit beta | K20509         | 157.25             | 0                  | 15.65              |
| **Genes for SLC13 family transporters** |        |                                             |                |                    |                    |                    |
| PFJ30894_RS03075     | +      | Citrate transporter                          | K14445         | 253.98             | 2764.97            | 7569.79            |
| PFJ30894_RS04475     | +      | SLC13/DASS family transporter                | K14445         | 1135.71            | 996.63             | 3053.47            |
| **Genes involved in the glutamate metabolism** |        |                                             |                |                    |                    |                    |
| PFJ30894_RS00375     | −      | Sodium/glutamate symporter                   | K03312         | 439.74             | 955.46             | 2475.13            |
| PFJ30894_RS01150     | +      | 4Fe-4S dcluster domain-containing protein    | K00176         | 359.17             | 357.32             | 501.28             |
| PFJ30894_RS01155     | +      | 2-oxoacid:acceptor oxidoreductase subunit alpha | K00174         | 335.97             | 426.04             | 689.65             |
| PFJ30894_RS01160     | +      | 2-oxoacid:ferredoxin oxidoreductase subunit beta | K00175         | 279.4              | 305.7              | 651.11             |
| PFJ30894_RS01165     | +      | Pyruvate/ketosiovalerate oxidoreductase gamma subunit | K00177         | 276.8              | 634.28             | 915.82             |
| PFJ30894_RS04940     | +      | Glu/Leu/Phe/Val dehydrogenase                | K00260         | 2622.01            | 6570.4             | 13,455.59          |

*Note: Bold type indicates TPM values ≥2 in the co-culture (CoEx, CoNx) as compared with the monoculture (MoEx).*
| Locus tag | Strand | RefSeq annotation | KEGG Orthology | Ex log2FC | Nx log2FC | Ex adjP | Nx adjP |
|-----------|--------|------------------|----------------|-----------|-----------|---------|---------|
| Genes for the glutamine/glutamate-dependent acid resistance system | | | | | | | |
| BT_RS13000 | + | Glutamate decarboxylase | K01580 | -1.2 | -1.15 | 7.04E-50 | 1.28E-12 |
| BT_RS13005 | + | Glutaminase A | K01425 | -1.99 | -1.95 | 1.04E-120 | 2.22E-28 |
| BT_RS13010 | - | Two-pore domain potassium channel family protein | - | -1.72 | -1.32 | 2.42E-18 | 3.05E-29 |
| BT_RS13015 | + | Amino acid permease | K20265 | -0.92 | -1.32 | 7.01E-06 | 1.13E-18 |
| Genes for a putative efflux ABC transporter | | | | | | | |
| BT_RS02750 | + | HlyD family efflux transporter periplasmic adaptor subunit | K01993 | 0.3 | 3.25 | 1.98E-01 | 7.87E-113 |
| BT_RS02755 | + | ATP-binding cassette domain-containing protein | K01990 | 2.5 | 5.23 | 1.13E-29 | 0 |
| BT_RS02760 | + | ABC transporter permease | K01992 | 6.68 | 6.66 | 2.97E-112 | 0 |
| BT_RS02765 | + | ABC transporter permease | K01992 | 3.21 | 4.14 | 5.49E-178 | 0 |

Note: Negative values indicate upregulation in the monoculture, and positive values indicate upregulation in the co-culture.