Characterization of the phosphotransacetylase-acetate kinase pathway for ATP production in Porphyromonas gingivalis

Yasuo Yoshida, Mitsunari Sato, Takamasa Nonaka, Yoshiaki Hasegawa and Yuichiro Kezuka

*Department of Microbiology, School of Dentistry, Aichi Gakuin University, Nagoya, Japan; †Division of Structural Biology, Department of Pharmaceutical Sciences, School of Pharmacy, Iwate Medical University, Yahaba, Japan

ABSTRACT
Acetyl phosphate (AcP) is generally produced from acetyl coenzyme A by phosphotransacetylase (Pta), and subsequent reaction with ADP, catalyzed by acetate kinase (Ack), produces ATP. The mechanism of ATP production in Porphyromonas gingivalis is poorly understood. The aim of this study was to explore the molecular basis of the Pta-Ack pathway in this microorganism. Pta and Ack from P. gingivalis ATCC 33277 were enzymatically and structurally characterized. Structural and mutational analyses suggest that Pta is a dimer with two substrate-binding sites in each subunit. Ack is also dimeric, with a catalytic cleft in each subunit, and structural analysis indicates a dramatic domain motion that opens and closes the cleft during catalysis. ATP formation by Ack proceeds via a sequential mechanism. Reverse transcription-PCR analysis demonstrated that the pta (PGN_1179) and ack (PGN_1178) genes, tandemly located in the genome, are cotranscribed as an operon. Inactivation of pta or ack in P. gingivalis by homologous recombination was successful only when the inactivated gene was expressed in *trans*. Therefore, both pta and ack genes are essential for this microorganism. Insights into the Pta-Ack pathway reported herein would be helpful to understand the energy acquisition in *P. gingivalis*.

Porphyromonas gingivalis, a black-pigmented Gram-negative rod-type bacterium, is a keystone pathogen linked to the onset and progression of periodontitis [1,2]. Although periodontitis is localized to tissues surrounding teeth, it is associated with serious systemic conditions such as cardiovascular disease [3,4], diabetes [5], and rheumatoid arthritis [6].

The main routes for ATP and energy production in *P. gingivalis* remain to be fully elucidated. *P. gingivalis* is unable to ferment carbohydrates such as glucose, although small quantities of glucose monomers can be utilized for polyomer biosynthesis [7]. Therefore, *P. gingivalis* relies on catabolism of amino acids to generate metabolic energy [8,9]. In many bacteria, the enzymes phosphotransacetylase (Pta, EC 2.3.1.8) and acetate kinase (Ack, EC 2.7.2.1) form a key pathway for production of ATP from excess acetyl coenzyme A (acetyl-CoA) [10]. Pta produces acetyl phosphate (AcP) from acetyl-CoA, and AcP is subsequently converted to acetate by Ack, with the concomitant production of ATP through substrate-level phosphorylation.

AcP functions as a signaling molecule that is capable of serving as a phosphate donor for two-component signal transduction systems, thereby connecting central metabolism with environment sensing and signal transduction [11,12]. Interestingly, inactivation of the Pta-Ack pathway in *Escherichia coli* results in biofilm formation that is quantitatively and architecturally distinct from that in parental strains [12–14]. However, disruption of the Pta-Ack pathway does not affect cell growth in many bacteria, including *E. coli* [15], Streptococcus mutans [16], Streptococcus pneumoniae [13], Clostridium acetobutylicum [17], Clostridium tyrobutyricum [18], and Bacillus subtilis [19]. Indeed, the presence of alternate pathways has been demonstrated for several bacterial, including *B. subtilis* [20], and a search of all available Ack sequences showed that more than 300 species possess multiple Ack enzymes [21]. By contrast, a recent study revealed the indispensable role of the Pta-Ack pathway in *Staphylococcus aureus* for maintaining energy and metabolic homeostasis during overflow metabolism [22].

Since *P. gingivalis* produces a large amount of short chain fatty acids, including acetate, propionate, and butyrate [23], it is postulated that the Pta-Ack pathway, which is associated with acetate production, is conserved in *P. gingivalis* [8,9] (Figure 1). Despite the importance of Pta and Ack in carbon cycling and energy metabolism in *P. gingivalis*, these enzymes have not been characterized in this microorganism, even though the coding genes have been assigned based on sequence homology [24,25]. In the current
study, recombinant Pta (PgPta) and Ack (PgAck) from *P. gingivalis* ATCC 33277, encoded by pta (PGN_1179) and ack (PGN_1178), respectively, were prepared and enzymatically characterized. In addition, crystal structures were determined by X-ray crystallography. Based on the structures, several putative functional residues were identified and their contribution to catalytic activity was evaluated by site-directed mutagenesis. Furthermore, we examined the essentiality of pta and ack in *P. gingivalis*.

### Materials and methods

#### Bacterial strains and growth conditions

All bacterial strains used in this study are listed in Table 1. *P. gingivalis* ATCC 33277 and its derivatives were grown in modified GAM broth (Nissui, Tokyo, Japan), or on Brucella HK agar (Kyokuto Pharmaceutical Industrial, Tokyo, Japan) supplemented with 5% rabbit blood at 37°C in an anaerobic atmosphere (80% N₂, 10% H₂, 10% CO₂). Ampicillin or tetracycline was added to a final concentration of 10 µg/mL and 1 µg/mL, respectively, when needed. *E. coli* DH5α (Invitrogen, Carlsbad, CA) and BL21 (DE3) (Novagen, Madison, WI) strains were grown aerobically at 37°C in 2 × YT or LB medium (Thermo Fisher JP, Tokyo, Japan) supplemented with 100 µg/mL ampicillin, as required.

#### Expression of recombinant PgPta and PgAck

Recombinant PgPta and PgAck were overproduced using *E. coli* after cloning each coding region between appropriate restriction sites in pGEX-6P-1 (GE Healthcare Japan, Hino, Japan) and pET28a (Merck, Darmstadt, Germany), respectively. Primers used for cloning are listed in the Supplemental Table. PgPta has a glutathione S-transferase (GST)-tag cleavable by PreScission protease, whereas PgAck possesses an N-terminal (His)₆-tag followed by a thrombin cleavage site. After verification by DNA sequencing, the resulting pPR1179-Gex and pET1178-Pet constructs (Table 1) were used to transform competent *E. coli* BL21 (DE3) cells. Transformants were cultivated at 37°C to an optical density of ~0.3, and isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 0.3 mM to induce expression of recombinant proteins. Culturing was continued at

![Figure 1. Proposed metabolic pathways for use of glutamate and aspartate in *P. gingivalis* based on previously described pathways [8,9,67,68]. Broken lines indicate expected pathways that were not supported by the experimental evidence.](image-url)
Table 1. Bacterial strains and plasmids used.

| Strains and plasmids | Relevant characteristics | References |
|----------------------|--------------------------|------------|
| **Strains**          |                          |            |
| E. coli DS5a         | F’ end a hsdR17 (F’ mcrA mcrB1418 supE44 thi-1 recA1 gyrA96 d800a2ZM1S) | Invitrogen |
| BL21 (DE3) P. gingivalis | F’ ompT hsdSB (rB- mB- uB+) gal dcm metE131 | Novagen |
| ATCC 33277 P. gingivalis | Parent strain | ATCC |
| PGAGU1179P P. gingivalis | ATCC33277 harboring the Pgptt gene in pComp1179 | This study |
| PGAGU1178P P. gingivalis | ATCC33277 harboring the Pgap gene in pComp1178 | This study |
| PGAGU124 P. gingivalis | ATCC33277 with the cepA cassette replacing PGN_1180 | This study |
| PGAGU125 P. gingivalis | ATCC33277 with the cepA cassette replacing pta | This study |
| PGAGU126 P. gingivalis | ATCC33277 with the cepA cassette replacing ack | This study |
| PGAGU127 P. gingivalis | ATCC33277 with the cepA cassette replacing PGN_1177 | This study |
| **Plasmids**         |                          |            |
| pMCL200             | Cm<sup>+</sup>, cloning vector | [33] |
| pCEPA               | Ap<sup>+</sup> in E. coli and P. gingivalis; pCR-Blunt II TOPO (Thermo Fisher) containing cepA with its promoter and terminator | [32] |
| pKO1180-cepA        | Cm<sup>+</sup> and Ap<sup>+</sup>, pMCL200 derivative containing cepA flanked by the upstream and downstream regions of PGN_1180 | This study |
| pKO1179-cepA        | Cm<sup>+</sup> and Ap<sup>+</sup>, pMCL200 derivative containing cepA flanked by the upstream and downstream regions of pta | This study |
| pKO1177-cepA        | Cm<sup>+</sup> and Ap<sup>+</sup>, pMCL200 derivative containing cepA flanked by the upstream and downstream regions of ack | This study |
| pTrAG402            | Ap<sup>+</sup> and Tc<sup>+</sup> in E. coli, Tc<sup>+</sup> in P. gingivalis; shuttle vector | [27] |
| pComp1179           | Ap<sup>+</sup> in E. coli, Tc<sup>+</sup> in P. gingivalis; pTRA402 derivative containing pta | This study |
| pComp1178           | Ap<sup>+</sup> in E. coli, Tc<sup>+</sup> in P. gingivalis; pTRA402 derivative containing ack | This study |
| pGEX-6P-1 Healthcare | Ap<sup>+</sup>; GST fusion expression vector | GE |
| pET28a              | Km<sup>+</sup>, (His)<sup>+</sup>, fusion expression vector | Merck |
| pPR1179-Gex         | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying pta | This study |
| pPR1179-Gex         | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the R89A-Pgpta mutant | This study |
| pPR1179-R135A-Gex   | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the R135A-Pgpta mutant | This study |
| pPR1179-D309A-Gex   | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the D309A-Pgpta mutant | This study |
| pPR1179-S311A-Gex   | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the S311A-Pgpta mutant | This study |
| pPR1179-R312A-Gex   | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the R3212A-Pgpta mutant | This study |
| pPR1179-D318A-Gex   | Ap<sup>+</sup>; pGEX-6P-1 derivative carrying the D318A-Pgpta mutant | This study |
| pET1178-Pet         | Ap<sup>+</sup>; pET28a derivative carrying Pgap | This study |
| pET1178              | Ap<sup>+</sup>; pET28a derivative carrying the R91A-Pgpta mutant | This study |
| pET1178-R91A-Pet     | Ap<sup>+</sup>; pET28a derivative carrying the R91A-Pgpta mutant | This study |
| pET1178-R241A-Pet    | Ap<sup>+</sup>; pET28a derivative carrying the R241A-Pgpta mutant | This study |
| pET1178-E385A-Pet    | Ap<sup>+</sup>; pET28a derivative carrying the E385A-Pgpta mutant | This study |

37°C for ~3 h, and cells were harvested by centrifugation at 4°C.

Site-directed mutagenesis of _pta_ and _ack_ genes in _P. gingivalis_ was performed using overlap extension PCR [26] or a KOD-Plus-Mutagenesis Kit (Toyobo, Osaka, Japan) with primers listed in the Supplemental Table, as previously described [27]. Overproduction of mutant enzymes was performed using the same procedure for wild-type (WT) enzyme described above.

### Purification of recombinant PgPta and PgAck

To purify recombinant _PgPta_, harvested cells were resuspended in phosphate-buffered saline (PBS) and lysed by ultrasonication on ice. The lysate was centrifuged at 30,000 × _g_ for 1 h, and the GST fusion protein in the supernatant was absorbed onto an affinity matrix glutathione-Sepharose 4B column and cleaved with PreScission protease (GE Healthcare Japan) according to the manufacturer’s protocol. The tag-digested protein solution was loaded onto a MonoQ 10/100 GL column (GE Healthcare Japan) and eluted with a six column volume linear gradient of NaCl from 100 to 500 mM. Fractions containing recombinant _PgPta_ were pooled, concentrated, and loaded onto a HiLoad 16/60 Superdex 200 pg column (GE Healthcare Japan), pre-equilibrated with 10 mM Tris/HCl pH 7.6 containing 10 mM NaCl.

For purification of recombinant _PgAck_, harvested cells were resuspended in 50 mM potassium phosphate (pH 8.0) containing 250 mM NaCl, and lysed by ultrasonication on ice. The lysate was centrifuged at 30,000 × _g_ for 1 h, and recombinant _PgAck_ was purified using TALON Superflow resin (GE Healthcare Japan) according to the manufacturer’s protocol. The (His)<sub>6</sub>-tag was cleaved by thrombin (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instructions, and the tag-digested protein was further purified using MonoQ 10/100 GL and HiLoad 16/60 Superdex 200 pg columns as described above for _PgPta_.

Purified enzymes were stored at ~20°C after adding an equal volume of 80% glycerol. Protein concentrations were determined as described previously [28] and protein purity was assessed by SDS-PAGE and gel filtration chromatography using a Superdex 200 HR 10/30 column (GE Healthcare) at a flow rate of 0.3 mL/min in PBS. Molecular mass estimation of purified enzymes (240 μg/200 μL) was also performed by gel filtration chromatography using a calibrated column and protein standards (molecular weight range of 12,000–200,000) purchased from Sigma-Aldrich.
Colorimetric enzyme assays

i) *PgPta* activity. The ability of *PgPta* to produce AcP from acetyl-CoA was investigated by spectrophotometrically quantifying the consumption of acetyl-CoA during the reaction as previously described [29] with several minor modifications. Briefly, each reaction (100 µL) consisted of 100 mM Tris/phosphate (pH 9.0), 20 mM KCl, varying concentrations of acetyl-CoA (50–200 µM), and 1.8 ng/mL recombinant *PgPta*. After reactions were initiated by addition of enzyme and precisely incubated for 3 min at 37°C, samples were spectrophotometrically examined by measuring the absorbance at 233 nm (A233). The concentration of acetyl-CoA was calculated using a standard curve, and kinetic parameters of *PgPta* were calculated by fitting directly to initial rate vs. substrate concentration (V vs. S) curves based on the Michaelis-Menten equation. To analyze the optimal pH, reaction mixtures consisting of 50 mM Tris/phosphate buffer were adjusted to pH 6.0, 7.0, 8.0, 9.0, or 10.0. Data were obtained from three independent experiments.

ii) *PgAck* activity. The activity of *PgAck* was determined by monitoring NADPH production as previously described [30] with several modifications. Reactions (150 µL) consisted of 50 mM Tris/HCl (pH 8.0), 2 mM dithiothreitol, 3.2 mM glucose, 1.3 mM NADP, 3.7 U/mL hexokinase (Sigma-Aldrich), 0.93 U/mL glucose-6-phosphate dehydrogenase (Sigma-Aldrich), 1.3 mM MgCl2, 67 ng/mL recombinant *PgAck*, varying concentrations of lithium potassium AcP (0.0667, 0.133, 0.267, and 0.667 mM), and ADP (0.267, 0.667, 1.33, and 2.67 mM). Reactions were initiated by the addition of substrate and incubation for 5 min at 37°C, and the amount of ATP in the reaction mixture was determined by spectrophotometrically quantifying NADPH at A340 using standard curves. Initial velocities of *PgAck* were determined at four fixed ADP concentrations at different AcP concentrations, and double reciprocal (Lineweaver-Burk) plots were obtained. Secondary plots of the y intercept (velocity reciprocal) vs. the inverse concentration of ADP were used to obtain Vmax value and Km value for ADP. Secondary plots of reciprocal slopes from Lineweaver-Burk plots vs. the inverse concentration of ADP provided Km/Vmax values, which were used to calculate Km values for AcP. Assay mixtures used for divalent metal ion screening contained 1.3 mM MgCl2, 1.3 mM ZnCl2, 1.3 mM CaCl2, or 1.3 mM EDTA instead of 1.3 mM MnCl2. To analyze the optimal pH, reaction mixtures consisted of 50 mM Tris/HCl buffer with the pH adjusted to 6.0, 7.0, 8.0, 9.0, or 10.0. Data were obtained from three independent experiments.

Reverse transcription (RT)-PCR

RT-PCR analysis was carried out as previously described [31]. Briefly, total RNA was isolated from *P. gingivalis* using ISOGEN-LS reagent (Nippon Gene, Tokyo, Japan) in accordance with the manufacturer’s instructions. Total RNA samples were treated with RNase-free DNase (TaKaRa Bio, Kusatsu, Japan) to remove traces of chromosomal DNA. Total RNA was reverse-transcribed with random hexadeoxoryribonucleotide primers (TaKaRa Bio) using PrimeScript Reverse Transcriptase (TaKaRa Bio) according to the manufacturer’s instructions. Gene-specific primers used for RT-PCR are listed in the Supplemental Table. Reaction mixtures without reverse transcriptase served as negative controls to evaluate the presence of contaminating genomic DNA in samples.

Construction of gene-deficient mutants by allelic replacement

Mutant strains of *P. gingivalis* lacking *PGN_1180*, *pta*, or *PGN_1177* genes were constructed by replacing each gene with a 2,123 bp cassette containing the ampicillin resistance gene, *cepA*, flanked by upstream and downstream DNA sequences targeting the *P. gingivalis* gene of interest. Each targeting sequence was separately amplified using *P. gingivalis* ATCC 33277 genomic DNA as a template, and *cepA* was amplified from pCEPA [32]. Primers used for PCR are listed in the Supplemental Table. Three amplicons were linked using an In-fusion HD Cloning kit (TaKaRa Bio) and subcloned into pMCL200 [33]. The construct was sequenced to rule out unintended base changes. Each linearized plasmid was electroporated into *P. gingivalis* ATCC 33277 and its derivatives as previously described [34]. Briefly, an overnight culture in modified GAM broth was diluted 1:5 in 5 ml of fresh modified GAM broth and incubated for 8 h at 37°C to obtain competent cells in log phase. The cells were collected, washed three times with 0.1 mM HEPES, washed once and resuspended with 10% glycerol, and then stored at ~80°C until use. The linearized plasmids (approximately 5 µg) were added to the competent cells and the mixtures were pulsed with a Gene Pulser apparatus (Bio-Rad Laboratories, Hercules, CA) at 2.5 kV with a time constant of 5 ms. The cell suspensions were immediately added to 10 ml of modified GAM broth prewarmed anaerobically, and then incubated anaerobically at 37°C for 24 h without antibiotics. Aliquots of the cell suspension were plated on Brucella HK agar with 5% rabbit blood containing appropriate antibiotics and incubated anaerobically for 10–15 days at 37°C. PCR analysis of genomic DNA from mutant strains was performed to confirm appropriate gene insertion in ampicillin-resistant mutant colonies.
Conditional mutant strains were also constructed from *P. gingivalis* ATCC 33277 using pComp1179 or pComp1178 (Table 1). Plasmids contained pta or ack inserted between BamHI and SalI sites in pTRAG402 [27] under the control of the strong ragA promoter [35].

**Structure determination of recombinant PgPta and PgAck**

To perform crystallization experiments, each purified protein was concentrated to 10 mg/mL. Crystallization experiments were performed at 20ºC using the hanging-drop vapor diffusion method. Purified protein solution (0.8 µL) was mixed with reservoir solution (0.8 µL) and the resulting droplet was equilibrated against 200 µL reservoir solution in a well. Crystals of PgPta were obtained using reservoir solution containing 20% (w/v) polyethylene glycol (PEG) 3350 and 0.2 M ammonium tartrate dibasic. Crystals of PgPta in complex with acetyl-CoA were prepared by cocrystallization using droplets containing 5 mM acetyl-CoA equilibrated against the same reservoir. Before crystallization of PgAck, magnesium chloride was added to the protein solution at a final concentration of 10 mM. PgAck was crystallized using reservoir solution containing 0.2 M lithium sulfate monohydrate, 0.1 M Tris/HCl (pH 8.5), and 24% (w/v) PEG 3350.

X-ray diffraction data were collected using beamline NE3A at the Photon Factory Advanced Ring (Ibaraki, Japan) at 95 K. Crystals of PgPta and PgAck were cryoprotected by soaking briefly in mother liquor containing 10% (v/v) 2-methyl-2,4-pentanediol and 30% (w/v) PEG 3350, respectively. Diffraction data were indexed, integrated, and scaled using DIALS [36] and SCALA [37] as implemented in XIA2 [38]. The structures of PgPta and PgAck were solved by molecular replacement using MOLREP [39]. Search models were generated using the homology modeling server SWISS-MODEL [40] based on the structure of Pta from *Methanosarcina thermophila* (MtPta) [Protein Data Bank (PDB) ID: 2AF4] [41] and Ack from *Thermotoga maritima* (PDB ID: 2IIR) for PgPta and PgAck, respectively. The resultant structures were progressively refined by fitting to the electron density maps using COOT [42]. Manual adjustment of structures was interspersed with refinement cycles using REFMAC5 [43]. Final statistics for X-ray diffraction data and crystallographic refinement are summarized in Table 2.

**Statistical analyses**

Differences between groups were assessed by one-way analysis of variance and Student-Newman-Keuls multiple comparison post-tests or Student’s t-tests.

### Table 2. Statistics for X-ray diffraction data and crystallographic refinement of PgPta and PgAck.

| Enzyme | PgPta (PGN_1179) | PgAck (PGN_1178) |
|--------|-----------------|-----------------|
| Form   | Acetyl-CoA-bound | Substrate-free   |
| Intensity statistics | | | |
| Beamline | NE3A | NE3A |
| Wavelength (Å) | 1.0000 | 1.0000 |
| Space group | P2₁2₁2₁ | P2₁ |
| Cell dimensions | 95.69/130.02/a, b, c | 82.77/98.47/a, b, c |
| Rmerge (%) | 56.20 | 102.77/91.64 |
| Completeness (%) | 53.4–1.94/53.4–1.94 | 93. (24.0)/93. (24.0) |
| I/σ | 16.9 (5.5)/16.9 (5.5) | 11.9 (5.8)/11.9 (5.8) |
| Multiplicity | 12.6 (11.4)/12.6 (11.4) | 6.5 (6.5)/6.5 (6.5) |

* Values in parentheses are statistics for the highest resolution shell.

### Accession numbers

Atomic coordinates and structure factors for PgPta in complex with acetyl-CoA, and PgAck, have been deposited at the PDB under accession codes 6IOX and 6IOY, respectively.

### Results

**Purification and enzymatic characterization of recombinant PgPta and PgAck proteins**

The final step in the production of ATP from acetyl-CoA in *P. gingivalis* is believed to be carried out by the enzymes associated with the Pta-Ack pathway (Figure 1). A homology search revealed that the amino acid sequences of PgPta (PGN_1179) and PgAck (PGN_1178) from *P. gingivalis* share 45% and 47% identity, respectively, with MtPta and MtAck from *M. thermophila* (Supplemental Figures S1 and S2), which are well-characterized [44–46]. To enzymatically characterize PgPta and PgAck, recombinant proteins were expressed in *E. coli* and purified, and homogeneity was confirmed by SDS-PAGE (Figure 2(a)). The molecular masses of denatured PgPta and PgAck were in agreement with the predicted values (35.8 kDa and 43.2 kDa, respectively).
In gel filtration chromatography experiments, PgPta and PgAck eluted as single peaks at retention volumes corresponding to molecular masses of 80.5 and 108 kDa, respectively (Figures 2(b) and 2(c)), suggesting that PgPta is a homodimer in solution, and PgAck is a homodimer or homotrimer.

Next, the enzymatic activity of the recombinant proteins was characterized. The phosphotransacetylase activity of recombinant PgPta was evaluated by spectrophotometrically quantifying acetyl-CoA consumed during the reaction. The maximum activity of recombinant PgPta was observed at pH 9.0 (Figure 3(a)). Kinetic parameters of PgPta were determined using the Michaelis-Menten equation (Tables 3 and 3(b)), yielding $k_{\text{cat}}$ and $K_m$ values of $1,487 \pm 63$ s$^{-1}$ and $63.8 \pm 7.5$ µM, respectively. Meanwhile, the acetate kinase activity of PgAck was determined by quantifying ATP produced from ADP and AcP. The optimal pH of PgAck was pH 8.0 (Figure 4(a)). Among the divalent metal ions tested (Mn$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, and Ca$^{2+}$), Mn$^{2+}$ and Mg$^{2+}$ supported the high PgAck activity (Figure 4(b)). The activity of PgAck in the presence of Mg$^{2+}$ was ~80% of that in the presence of Mn$^{2+}$. No detectable activity was observed in the presence of EDTA. Initial reaction velocities of PgAck were determined at four fixed ADP concentrations at different AcP concentrations, and Lineweaver-Burk (double reciprocal) plots revealed a clear intersecting line pattern (Figure 4(c)), indicating that PgAck acts via a sequential mechanism [45]. The $V_{\text{max}}$ (145 ± 1 µmol/min/mg) and $K_m$ values for ADP (188 ± 22 µM) and AcP (22.2 ± 0.2 µM) were estimated from secondary plots (Figures 4(d) and 4(e)). The kinetic parameters are summarized in Table 3.
Structure of PgPta and enzymatic activity of its mutants

The structure of PgPta in complex with acetyl-CoA was determined at 2.0 Å resolution (Figure 5(a) and Table 2). The asymmetric unit of the P2_12_12 cell contains one dimer. The subunit is an α/β protein composed of two α/β domains (Figure 5(b)); N-terminal domain I (residues 1–145 and 302–336) and C-terminal domain II (residues 146–301), based on the previous definition [44]. Domain II is responsible for dimerization (Figure 5(a)). The N- and C-terminal domains are positioned side by side, and the domain arrangement results in the formation of a cleft with a putative active site at the interface.

Three distinct conformations of the subunit structure have been reported for MtPta [44]; a change in the position of domain I relative to domain II results in distinct open, closed, and partially closed conformations. Structural comparison of the subunits of PgPta in complex with acetyl-CoA with those of MtPta showed that both subunits of PgPta are in the open conformation, with a root mean square deviation (rmsd) of 0.89 Å (314 Ca atoms) and 0.90 Å (318 Ca atoms) for subunits A and B, respectively. However, the extent of the conformational change observed in PgPta is much less than that in PgAck (described below).
Structural analysis revealed an acetyl-CoA molecule bound to each subunit (Figure 5(a)), with seven direct hydrogen bonds or electrostatic interactions between the bound acetyl-CoA and subunit B (Figure 5(b)). The adenine base is recognized by two hydrogen bonds with the main-chain amide and carbonyl groups of Val150. The 3’-phosphate interacts with the side chains of Arg89 and Lys92 in subunit B. Arg135 forms an electrostatic interaction with the β-phosphate. The second amide nitrogen is hydrogen-bonded to the carbonyl group of Ala175. The interactions provided by Lys92 and Ala175 are missing in subunit A due to differences in χ angles of side chains and/or the bound acetyl-CoA. This results in higher B-factor values for acetyl-CoA in subunit A (74 Å²) than subunit B (52 Å²).

Six amino acid residues (Arg89, Arg135, Asp309, Ser311, Arg312, and Asp318) positioned at the cleft and the acetyl-CoA binding site were mutated and recombinant proteins were prepared (Supplemental Figure S3) to investigate their contributions to catalytic activity. All the prepared mutants except D318A gave a single peak in the gel filtration (Supplemental Figure S3), indicating the mutations did not affect the dimerization. The D318A mutation displayed a broad biphase pattern. However, it seems that more than half of the D318A mutant is still dimer. The crystal structure clearly shows that Arg89 and Arg135 interact with the 3’-phosphate and β-phosphate of acetyl-CoA, respectively (Figure 5(b)). Replacement of Arg89 and Arg135 resulted in a 58% and 21% decrease in specific activity, respectively (Figure 5(c)). The other four mutated residues are located at the cleft, not in contact with Arg89 and Arg135. The polypeptide region in which Asp309, Ser311, and Arg312 are located forms part of the side of the cleft on the domain I side, with Ser311 and Arg312 positioned at the edge of the cleft, and Asp309 located near the bottom of the cleft. Mutation of these residues resulted in the complete loss of enzyme activity. Finally, Asp318 is located at the edge of the cleft on the opposite side from the observed acetyl-CoA binding site. The D318A mutant displayed only 11% of the specific activity of the WT enzyme.

**Structure of PgAck and enzymatic activity of its mutants**

The structure of PgAck was refined at 1.9 Å resolution (Table 2). Four subunits (A, B, C, and D) are present in the asymmetric unit of the P2₁ cell. Structural analysis by the PISA server [47] was performed to identify potential subunit interfaces and evaluate their significance for multimer formation. Interfaces between subunits A and B, and subunits C and D (interface area of ~3000 Å²) received significantly higher scores than others (data not shown), suggesting that PgAck forms a homodimer (Figure 6(a)), with two homodimers present in the asymmetric unit. This implies a dimeric state in solution, consistent with the ambiguous dimer or trimer predicted by gel filtration chromatography (Figure 2(b)). The structure of the PgAck subunit is composed of two α/β domains; the N-terminal domain (residues 1–149 and 384–398) and the C-terminal domain (residues 150–383, Figure 6(b)). The secondary structural elements of PgAck are well conserved with those of MtAck [48]. The C-terminal domain of each subunit is responsible for dimerization, and the dimer is related by the 2-fold symmetry axis (Figure 6(a)). A putative catalytic cleft is formed between the domains.

Comparison of the four subunits in the asymmetric unit revealed distinct conformations, with rmsd values ranging from 0.81 to 5.19 Å using all 398 Ca atoms (Figure 6(c)). The structure of the C-terminal domain is almost invariant among the subunits, and the positions of the N-terminal domain relative to the C-terminal domain are different, leading to opening and closing of the cleft (Figure 6(c)). The cleft in subunit D is the most closed, with two sulfate ions bound, presumably derived from lithium sulfate in the mother liquor. Compared with subunit D, the cleft in the other subunits is more open to varying extents and contains no bound ligands. Pro37 at the edge of the cleft is shifted 17.6, 14.0, and 11.7 Å in subunits A, B, and C, respectively, from the corresponding position in subunit D. The degree of opening is ordered subunits A, B, C, and D (highest to lowest). Enzyme activity assays revealed that Mg²⁺ or Mn²⁺ is required for catalytic activity of PgAck (Figure 4(b)). Interestingly, although we crystallized PgAck in the presence of Mg²⁺ (and Mn²⁺, data not shown), electron density corresponding to a divalent metal cation was not observed.

We performed a mutational analysis of three residues positioned at the cleft; based on the structure of PgAck and previous mutational studies on MtAck [46,49–51], and we selected Arg91, Arg241, and Glu385 for mutation. Arg91 and Arg241 interact with SO₄²⁻ 401 in the crystal structure (Figure 6(b)), while Glu385 is positioned ~7 Å away from both sulfate ions. Following preparation of the mutant proteins (Supplemental Figure S3), enzymatic activity was compared (Figure 6(d)). All three mutations dramatically decreased the specific activity; R91A and R241A exhibited only 0.6% and 3.9% of the activity of the WT enzyme, and E385A displayed no detectable activity.

Gel filtration analysis of the mutants showed that each mutant protein mostly forms homodimer in common with the WT enzyme (Supplemental Figure S3).
However, the peaks of E385A and R91A had obvious front shoulders with molecular mass of 217 kDa, which corresponds to almost the double of the estimated mass of the dimer (108 kDa). This result indicated that approximately 20% and 40% of the E385A and R91A mutants, respectively, appeared to be tetramer.

Transcriptional analysis of Pta and Ack genes in P. gingivalis

Transcriptional regulation of the pta and ack regions in P. gingivalis was characterized by RT-PCR (Figure 7). DNA fragments with expected length were effectively amplified using the primers (Supplemental Table) and the genomic DNA from P. gingivalis (Supplemental Figure S4). Transcripts corresponding to the regions spanning the borders of pta and ack were detectable, demonstrating that these genes are cotranscribed in this microorganism. PCR fragments corresponding to the regions spanning the border of PGN_1180 and pta were not amplified. The function of the PGN_1180 protein is unknown. By contrast, the gene (PGN_1177) immediately downstream from ack was oppositely transcribed. Thus, only pta and ack were transcribed together as an operon. No significant products were amplified using cDNA prepared without reverse transcriptase, indicating that the RT-PCR products were not derived from contaminating chromosomal DNA (Figure 7).

Both pta and ack genes are essential in P. gingivalis

To examine the physiological roles of pta and ack genes in P. gingivalis ATCC 33277, we attempted to construct chromosomal knockout mutants of each gene using a standard allelic replacement procedure that involves the insertion of an ampicillin resistance gene into the target gene (Supplemental Figure S5). However, attempts to obtain mutant P. gingivalis strains in which pta or ack was inactivated were unsuccessful (Table 4). Furthermore, when an erythromycin resistance cassette was used instead of an ampicillin resistance gene, no null mutants were obtainable (data not shown). By contrast, using the same procedure, it was possible to obtain null mutants of both PGN_1180 and the PGN_1177 genes, which are located upstream from pta and downstream from ack, respectively (Table 4).
Knowledge of cell physiology and the conversion of nutrients to ATP, the universal molecular currency of energy transfer, is indispensable for understanding how bacteria survive, grow, reproduce, and express virulence factors. However, ATP production in P. gingivalis remains poorly understood. Indeed, the present study represents the first characterization of the Pta-Ack pathway associated with ATP production in P. gingivalis.

The $k_{cat}$ value of PgPta (1,487 ± 63 s$^{-1}$) is comparable to that of MtPta (1,500 ± 30 s$^{-1}$) [52] and is more than 10-fold higher than those of other Pta orthologues [53,54]. By contrast, the $K_m$ of PgPta (63.8 ± 7.5 μM) for acetyl-CoA is within the range reported for other Pta orthologues (8.6 to 600 μM) [53–56]. The pH optimum of PgPta was 9.0, which is slightly higher than the pH 8.0 reported previously for other Pta orthologues [29,54].

Structural analysis of PgPta revealed two acetyl-CoA molecules bound to the dimer (Figure 5(a)), and the acetyl-CoA binding site in each subunit is unique and identical. In MtPta, there are two CoA binding sites (1 and 2) in each subunit with different affinities [41]. Replacement of Ser309 and Arg310 located at binding site 1 in MtPta greatly affected the $k_{cat}$ value [52], whereas mutation of Arg87 and Arg133 positioned at site 2 mainly impacted the $K_m$ value [57]. Given the number of interactions between the bound CoA and each site, CoA at site 1 is thought to be more tightly bound to MtPta than CoA at site 2. Therefore, in MtPta, site 1 (the high-affinity site) is suggested to be the active site [41]. Site-directed mutagenesis of Arg89 and Arg135 in site 2 of PgPta (Arg87 and Arg133 in MtPta) had moderate effects on specific activity (Figure 5(c)), indicating that they are also not essential for catalysis. By contrast, mutation of residues at site 1 (D309A, S311A, R312A, and D318A) completely or dramatically decreased the catalytic activity. These findings suggest that site 1 was also the probable active site in PgPta. Contrary to predictions, acetyl-CoA molecules were bound only to site 2 in both subunits of PgPta (Figures 5(a) and 5(b)), even though crystal packing of PgPta does not appear to interfere with binding of acetyl-CoA to site 1. Since interference of the binding of acetyl-CoA to site 1 of PgPta has not been observed in solution, such contradictory findings might be due to crystallization artifacts. Dynamic aspects, such as domain motion, can affect substrate binding and are difficult to estimate from the static picture revealed by a crystal structure. Further analyses to investigate the binding of acetyl-CoA to PgPta are therefore required.

The pH optimum for PgAck (pH 8.0) is consistent with that of most other Ack enzymes reported to date.

**Discussion**

### Figure 7. Genetic organization and transcriptional analysis of the pta-ack region of P. gingivalis ATCC 33277. (a) Gene arrangement within the pta-ack region of P. gingivalis ATCC 33277 chromosomal DNA. Large and small arrows indicate open reading frames and RT-PCR primers, respectively. (b) RT-PCR analysis of pta and ack gene expression. PCR amplicons were separated by 0.8% agarose gel electrophoresis. The predicted size (in bp) of each PCR amplicon is shown in parentheses. Lanes marked (+) or (–) indicate standard RT-PCR reactions or negative controls without cDNA, respectively. Lanes marked (+) or (–) indicate standard RT-PCR reactions or negative controls without cDNA, respectively. The positions of DNA size standards (in kb) are indicated on the left.

### Table 4. P. gingivalis transformation efficiency.

| Recipient strain | Gene targeted for inactivation | Transformation efficiency (no. of transformants/μg DNA) |
|------------------|-------------------------------|---------------------------------------------------|
| ATCC 33277       | PGN_1180                      | 19.3 ± 5.18                                       |
| ATCC 33277       | pta                           | n.d. *                                           |
| ATCC 33277       | ack                           | n.d. *                                           |
| ATCC 33277       | PGN_1177                      | 33.2 ± 11.8                                      |
| ATCC 33277 harboring pta in the plasmid |                      | 10.9 ± 3.13                                      |
| ATCC 33277 harboring ack in the plasmid |                      | 15.7 ± 2.46                                      |

* Not detected.

Next, we attempted to construct chromosomal knockout mutants of pta and ack genes in the P. gingivalis ATCC 33277 background using pComp1179 and pComp1178, respectively, (Table 1) for expression of the respective genes in P. gingivalis under the control of the ragA promoter [27]. The introduction of pta and ack genes in trans led to disruption of the corresponding genes in the chromosomal DNA of P. gingivalis, and the transformation efficiencies for pta- and ack-deficient mutants were comparable with those for PGN_1180- and PGN_1177-deficient mutants (Table 4). The growth of all the mutant strains was similar to that of the parent strain (Supplemental Figure S6).
[21,53]. Under the conditions tested, \( P_g \) Ack displayed highest activity in the presence of \( \text{Mn}^{2+} \), and was less efficient with \( \text{Mg}^{2+} \). Similarly, other Ack enzymes also function optimally with \( \text{Mn}^{2+} \) or \( \text{Mg}^{2+} \) [58]. The reaction velocities of \( P_g \) Ack exhibited conventional Michaelis-Menten kinetics, consistent with most other Ack enzymes [53]. Double reciprocal plots (Figure 4(c)) indicate that ATP formation by \( P_g \) Ack proceeds via a sequential mechanism.

There are four crystallographically independent \( P_g \) Ack subunits in the asymmetric unit with distinct conformations, corresponding to the degree of opening and closing of the catalytic cleft (Figure 6(c)). Such conformational changes are a common feature of the acetate and sugar kinase/Hsc70/actin (ASKHA) superfamily [59]. Structural analysis of \( M_t \) Ack with transition state analogs ADP, AlF\(_3\) (a mimic of the meta-phosphate transition state), and acetate strongly suggest that catalysis proceeds via a direct in-line phosphoryl transfer mechanism [51]. ADP, AlF\(_3\), and acetate are aligned in a linear array in the cleft in \( M_t \) Ack, and the catalytic cleft is wide open to accommodate all three ligands. By contrast, the cleft of subunit D is almost closed in the \( P_g \) Ack structure determined without any ligands such as ADP, AlF\(_3\), and acetate. This structure likely represents complete cleft closure because there is a direct contact between residues Glu32 and Arg285 positioned on opposite sides of the interface of the two domains (Figure 6(b)). Comparison of the \( M_t \) Ack subunit with bound transition state analogs and subunit D of \( P_g \) Ack reveals a shift in the N-terminal domain of 22 Å (Ca-Ca distance between residue 37 of the two enzymes) upon cleft closure. This estimated value is larger than expected (\( \leq 15 \) Å) for \( M_t \) Ack and propionate kinase, other members of the ASKHA superfamily [60]. Therefore, these results strongly indicate a dramatic domain motion in \( P_g \) Ack during catalysis via a direct in-line phosphoryl transfer mechanism.

There are several conserved residues at the active site of Ack enzymes [30], and their roles and contributions to enzymatic activity and/or substrate binding have been extensively examined for \( M_t \) Ack [46,49,50,60,61]. Arg91 and Arg241 in \( M_t \) Ack interact with the carboxyl group of acetate and are catalytically essential; R91A and R241A mutants of \( M_t \) Ack displayed \( k_{\text{cat}} \) values only 0.3 and 0.4% that of the WT enzyme, respectively [51]. The specific activities of \( P_g \) Ack mutants R91A and R214A showed a similar trend to the corresponding \( M_t \) Ack mutants, indicating their importance in catalysis. The E385A mutant of \( P_g \) Ack lost all enzyme activity (Figure 6(d)). The equivalent Glu384 in \( M_t \) Ack is believed to be responsible for divalent metal cation binding [46,60]. As shown in Figure 4(b), \( P_g \) Ack favors \( \text{Mn}^{2+} \) or \( \text{Mg}^{2+} \), either of which is indispensable for catalysis. Therefore, Glu385 in \( P_g \) Ack is a candidate metal-binding residue. As the result of gel filtration, the E385A and R91A mutants are like to be partly tetramer. Although it is unclear if the possible oligomeric transition affects the catalytic activity, a large proportion (60–80%) of the R91A and E385A mutants are present as dimer like the WT enzyme. Therefore, the no or little activity of the mutants would be mostly due to the single amino acid mutation.

Bacterial genes are often organized in operons with regulatory units that control genes/proteins with associated functions. The RT-PCR analysis demonstrated that \( p_t \) and \( ack \) genes constitute an operon in \( P. \) gingivalis (Figure 7), as has also been shown for homologous genes in several bacteria including \( E. \) coli [15] and \( Corynebacterium \) glutamicum [62]. Transcription of the PGN_1180 gene is independent from \( p_t \) and \( ack \). The PGN_1180 protein, the function of which has not been assigned, is therefore presumably not associated with ATP production.

Our experiments using conditional mutant strains clearly revealed that both \( p_t \) and \( ack \) are essential genes in \( P. \) gingivalis, at least under the growth conditions tested (Table 4). A previous experiment using a transposon insertion mutant library demonstrated that 463 genes, including \( p_t \) and \( ack \), are essential for growth of \( P. \) gingivalis ATCC 33277 in vitro [63], whereas another independent experiment using the same bacterial strain and a similar method did not identify these genes as essential [64]. Of the 459 genes found to be essential by Klein et al. [63], only 281 genes (61%) were predicted to be essential by Hutcherson et al. [64]. It should be noted that the essentiality of genes can change depending on the growth conditions.

All living cells rapidly turn over ATP to satisfy their energy demands, and consequently ATP must be produced from ADP in a process requiring energy. In general, ATP is biosynthesized via substrate-level phosphorylation by enzymes in the cytoplasm, or through electron transfer by ATPases located on mitochondrial (eukaryotes) or cytoplasmic (prokaryotes) membranes. Since ATP production and substrate-level phosphorylation processes are directly coupled, the reactions necessarily liberate the amount of energy required to phosphorylate ADP [65]. Coupling to the phosphorylation of ADP is limited to a small number of molecules for which it is thermodynamically feasible: 1,3-bisphosphoglycerate, succinyl-CoA, phosphoenolpyruvate, arginine phosphate, carbamoyl phosphate, creatine phosphate, butyryl phosphate, and AcP, catalyzed by phosphoglycerate kinase, succinyl-CoA synthetase, pyruvate kinase, arginine kinase, carbamate kinase, creatine kinase, butyrate kinase, and Ack, respectively [65]. Homology searches using complete genome
sequences revealed that, apart from phosphoglycerate kinase and PgAck, homologs of these enzymes are not present in *P. gingivalis* ATCC 33277 or W83 [24,25]. A homolog of phosphoglycerate kinase, one of the major enzymes in glycolysis, is present in the genome of *P. gingivalis* ATCC 33277 (PGN_0433). Previous experiments using radio-labeled glucose revealed only 5% uptake of glucose from the test medium by *P. gingivalis*, most of which contributes to cell carbohydrates and their derivatives, suggesting that glucose utilization by this organism is very poor, and carbohydrates do not appear to readily support growth [7]. Therefore, the PGN_0433 protein might not necessarily function as a phosphoglycerate kinase. In addition, apart from PgAck, no other analogs were identified in the genome of *P. gingivalis* ATCC 33277 or W83. Further studies are therefore needed to clarify the exact mechanism of ATP production in *P. gingivalis*, but the essentiality of both PgPta and PgAck implies that the Pta-Ack pathway plays an important role in ATP production in this microorganism.

**Acknowledgments**

We are grateful to Ms. Satoko Kumagai for assistance in sample preparation and protein crystallization. Synchrotron radiation experiments were performed at beamline NE3A in the Photon Factory Advanced Ring (Tsukuba, Japan) under proposal number 2017G675.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This study was supported in part by Grants-in-Aid for Scientific Research to Y.Y. (grant number JP17K11634) and to Y.K. (grant number JP16K11485) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; KAKENHI [JP16K11485]; KAKENHI [JP17K11634].

**References**

[1] Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018;16(12):745–759.

[2] Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10(10):717–725.

[3] Scannapieco FA. Position paper of The American academy of periodontology; periodontal disease as a potential risk factor for systemic diseases. J Periodontol. 1998;69(7):841–850.

[4] Dietrich T, Jimenez M, Krall Kaye EA, et al. Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. Circulation. 2008;117(13):1668–1674.

[5] Campus G, Salem A, Uzzau S, et al. Diabetes and periodontal disease: a case-control study. J Periodontol. 2005;76(3):418–425.

[6] de Pablo P, Chapple IL, Buckley CD, et al. Periodontitis in systemic rheumatic diseases. Nat Rev Rheumatol. 2009;5(4):218–224.

[7] Shah HN, Williams RAD. Utilization of glucose and amino acids by *Bacteroides intermedius* and *Bacteroides ginvialis*. Curr Microbiol. 1987;15(5):241–246.

[8] Takahashi N, Sato T, Yamada T. Metabolic pathways for cytotoxic end product formation from glutamate- and aspartate-containing peptides by *Porphyromonas gingivalis*. J Bacteriol. 2000;182(17):4704–4710.

[9] Takahashi N, Sato T. Preferential utilization of dipeptides by *Porphyromonas gingivalis*. J Dent Res. 2001;80(5):1425–1429.

[10] Wolfe AJ. The acetate switch. Microbiol Mol Biol Rev. 2005;69(1):12–50.

[11] Klein AH, Shulla A, Reimann SA, et al. The intracellular concentration of acetyl phosphate in *Escherichia coli* is sufficient for direct phosphorylation of two-component response regulators. J Bacteriol. 2007;189(15):5574–5581.

[12] Wolfe AJ, Chang DE, Walker JD, et al. Evidence that acetyl phosphate functions as a global signal during biofilm development. Mol Microbiol. 2003;48(4):977–988.

[13] Ramos-Montañez S, Kazmierczak KM, Hentchel KL, et al. Instability of ack (acetate kinase) mutations and their effects on acetyl phosphate and ATP amounts in *Streptococcus pneumoniae* D39. J Bacteriol. 2010;192(24):6390–6400.

[14] Pruss BM, Verma K, Samanta P, et al. Environmental and genetic factors that contribute to *Escherichia coli* K-12 biofilm formation. Arch Microbiol. 2010;192(9):715–728.

[15] Kakuda H, Shiroishi K, Hosono K, et al. Construction of Pta-Ack pathway deletion mutants of *Escherichia coli* and characteristic growth profiles of the mutants in a rich medium. Biosci Biotechnol Biochem. 1994;58(12):2232–2235.

[16] Kim JN, Ahn SJ, Burne RA. Genetics and physiology of acetate metabolism by the Pta-Ack pathway of *Streptococcus mutans*. Appl Environ Microbiol. 2015;81(15):5015–5025.

[17] Kuit W, Minton NP, López-Contreras AM, et al. Disruption of the acetate kinase (ack) gene of *Clostridium acetobutylicum* results in delayed acetate production. Appl Microbiol Biotechnol. 2012;94(3):729–741.

[18] Zhu Y, Liu X, Yang ST. Construction and characterization of *pta* gene-deleted mutant of *Clostridium tyrobutyricum* for enhanced butyric acid fermentation. Biotechnol Bioeng. 2005;90(2):154–166.

[19] Grundy FJ, Waters DA, Allen SH, et al. Regulation of the *Bacillus subtilis* acetate kinase gene by CcpA. J Bacteriol. 1993;175(22):7348–7355.

[20] Grundy FJ, Waters DA, Takova TY, et al. Identification of genes involved in utilization of acetate and acetoin in *Bacillus subtilis*. Mol Microbiol. 1993;10(2):259–271.

[21] Chan SH, Norregaard I, Solem C, et al. Acetate kinase isozymes confer robustness in acetate metabolism. PloS One. 2014;9(3):e92256.
[55] Pelroy RA, Whiteley HR. Kinetic properties of phosphotransacetylase from *Veillonella alcalescens*. J Bacteriol. 1972;111(1):47–55.

[56] Campos-Bermudez VA, Bologna FP, Andreo CS, et al. Functional dissection of *Escherichia coli* phosphotransacetylase structural domains and analysis of key compounds involved in activity regulation. FEBS J. 2010;277(8):1957–1966.

[57] Rasche ME, Smith KS, Ferry JG. Identification of cysteine and arginine residues essential for the phosphotransacetylase from *Methanosarcina thermophila*. J Bacteriol. 1997;179(24):7712–7717.

[58] Winzer K, Lorenz K, Durre P. Acetate kinase from *Clostridium acetobutylicum*: a highly specific enzyme that is actively transcribed during acidogenesis and solventogenesis. Microbiology. 1997;143(Pt 10):3279–3286.

[59] Hurley JH. The sugar kinase/heat shock protein 70/actin superfamily: implications of conserved structure for mechanism. Annu Rev Biophys Biomol Struct. 1996;25:137–162.

[60] Gorrell A, Ferry JG. Investigation of the *Methanosarcina thermophila* acetate kinase mechanism by fluorescence quenching. Biochemistry (Mosc). 2007;46(49):14170–14176.

[61] Ingram-Smith C, Barber RD, Ferry JG. The role of histidines in the acetate kinase from *Methanosarcina thermophila*. J Biol Chem. 2000;275(43):33765–33770.

[62] Reinscheid DJ, Schnicke S, Rittmann D, et al. Cloning, sequence analysis, expression and inactivation of the *Corynebacterium glutamicum pta-ack* operon encoding phosphotransacetylase and acetate kinase. Microbiology. 1999;145(Pt 2):503–513.

[63] Klein BA, Tenorio EL, Lazinski DW, et al. Identification of essential genes of the periodontal pathogen *Porphyromonas gingivalis*. BMC Genomics. 2012;13:578.

[64] Hutcherson JA, Gogeneni H, Yoder-Himes D, et al. Comparison of inherently essential genes of *Porphyromonas gingivalis* identified in two transposon-sequencing libraries. Mol OralMicrobiol. 2016;31(4):354–364.

[65] Müller V, Hess V. The minimum biological energy quantum. Front Microbiol. 2017;8:2019.

[66] Lovell SC, Davis IW, Arendall WB 3rd, et al. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins. 2003;50(3):437–450.

[67] Hendrickson EL, Xia Q, Wang T, et al. Pathway analysis for intracellular *Porphyromonas gingivalis* using a strain ATCC 33277 specific database. BMC Microbiol. 2009;9:185.

[68] Nelson KE, Zinder SH, Hance I, et al. Phylogenetic analysis of the microbial populations in the wild herbivore gastrointestinal tract: insights into an unexplored niche. Environ Microbiol. 2003;5(11):1212–1220.