Identification and characterization of a haem biosynthesis locus in Veillonella

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Haemin/haem is one of the essential nutrients required by periodontopathogens such as Porphyromonas gingivalis to grow in vitro. In the oral cavity, this nutrient is believed to be provided by the crevicular fluid, a serum-like exudate produced during gum inflammation. However, P. gingivalis is also present in the healthy dental biofilm where inflammation is absent. This study was designed to answer the question: what organism(s) in the healthy dental biofilm provides haemin/haem to those periodontal pathogens? We report here that veillonellae, a group of bridging species in dental biofilm development, harbour a complete gene cluster for haem biosynthesis. Haemin production was detected from cell lysate, suggesting that the haem biosynthesis pathway is functional in veillonellae. Using the only transformable strain Veillonella atypica OK5, we inactivated specific key genes in the haem biosynthesis pathway. Inactivation of hemE, encoding the enzyme uroporphyrinogen decarboxylase, not only abolished haemin production but also significantly decreased OK5-supported growth of P. gingivalis. A luciferase gene reporter to the hemEHG operon demonstrated up-regulation of operon expression by P. gingivalis. Analysis of all sequenced genomes of oral bacteria in the HOMD database identified three genera (Veillonella, Propionibacterium and Aggregatibacter) that have a complete haem biosynthesis gene cluster, suggesting that they all could be potential haemin/haem providers in the dental biofilm.

INTRODUCTION

The dental biofilm is a multispecies community consisting of more than 700 microbial species, and each human mouth may harbour as many as 120 species (Hajishengallis & Lamont, 2012; Huang et al., 2011). Dental biofilm develops in a sequential process with initial colonizers attaching to the tooth surface, followed by early, middle and late colonizers joining via cell–cell co-aggregation (Kolenbrander et al., 2006, 2010; Zhou et al., 2015b). In health, the dental biofilm protects the human host from pathogen infections; however, under certain environmental conditions, ecological dysbiosis occurs triggering chronic inflammatory disease such as periodontitis (Hajishengallis & Lamont, 2012). The middle/late colonizer Porphyromonas gingivalis is considered a keystone periodontopathogen due to its role in skewing human immune response and biofilm ecology (Lamont & Yilmaz, 2012; Zhou et al., 2015c). In vitro, this species requires haemin or haem for growth, and this nutrient is also crucial for other later colonizers, such as Tannerella forsythia, and is believed to be provided by the crevicular fluid during gum inflammation (Hajishengallis, 2011; Lamont & Jenkinson, 1998; Sharma, 2010). Interestingly, P. gingivalis is also present in the early dental biofilm, where inflammation is absent (Periasamy & Kolenbrander, 2009). Thus, an intriguing question arises: where does haemin/haem come from in the early biofilm where saliva is the major nutrient source?

We hypothesized that, in the early biofilm, some early colonizing bacteria may have the capacity to synthesize haemin/haem, which would then be utilized by the later colonizers. One such early colonizing bacterium is the Veillonella species, which utilize lactic acid, produced by initial colonizers (such as streptococci) as major carbon and energy source. Veillonella species are amongst the most prevalent and numerically dominant species in both supragingival (above the gum line) and subgingival (below the gum line) dental biofilms (Aas et al., 2005; Becker et al., 2002; Zhou et al., 2015c). In vitro, they were found to support the growth of many middle/late colonizers including P. gingivalis when 25% saliva was used as the sole nutrient source (Periasamy & Kolenbrander, 2009, 2010). Although these studies provided strong evidence for micro-organisms such as Veillonella as bridging species to support the colonization and growth of later colonizers, determining the mechanism of this function has become possible only recently by the development of the only genetic transformation system in the Veillonella genus (Liu et al., 2012; Zhou et al., 2015a, c).
In this study, we used the transformable strain *Veillonella atypica* OK5 to probe the mechanism that enabled *Veillonella* species to support growth of *P. gingivalis*. We identified a complete gene cluster for haem biosynthesis in every species/strain of the *Veillonella* genus, and we demonstrated that, by genetic mutagenesis, the haem biosynthesis pathway is not only functional in *Veillonella* but also required for supporting growth of *P. gingivalis*. We further demonstrate regulation of the *hem* biosynthesis operon by *P. gingivalis*.

**METHODS**

**Bacterial strains and growth conditions.** The bacterial strains and plasmids used in this study are listed in Table 1. *V. atypica* OK5 and its derivatives were grown in BHI broth (Difco) with 0.6 % sodium lactate (BHL) or on BHL agar plates. For transformation, cells were grown in Todd–Hewitt broth (Difco) with 0.6 % sodium lactate (THL), and transformants were selected on BHL plates supplemented with tetracycline (Sigma) at 2.5 µg ml⁻¹. For testing the effect of *hem* mutation on cell growth, we used a chemically defined medium (He et al., 2008) supplemented with 0.1 % vegetable proteose peptone (Fluka) and 0.6 % lactate (semi-CDM). *P. gingivalis* ATCC 32327 was grown in TSB (trypticase soy broth) supplemented with yeast extract (1 mg ml⁻¹) or on Brucella blood agar plates with haemin and vitamin K (Hardy Diagnostics). All bacterial strains were grown anaerobically (85 % N₂, 10 % CO₂ and 5 % H₂) at 37 °C. *Escherichia coli* cells were grown in Luria–Bertani (Difco) broth with aeration at 37 °C. *E. coli* strains carrying plasmids were grown in Luria–Bertani containing 10 µg ml⁻¹ tetracycline.

**Constructions of insertional mutagenesis mutants.** PCR primers used in this study are listed in Table 2. To construct insertional inactivation plasmids for the *hemG* and *hemE* genes, we amplified the internal 800 bp and 600 bp regions of target genes with PCR using primer pairs *hemG*-F/*hemG*-R and *hemE*-F/*hemE*-R, respectively. The PCR product was double digested with Sfll and PstI and then ligated with plasmid pBST (Zhou et al., 2015c), which was digested with the same enzymes. The recombinant plasmids pBST-*hemG* and pBST-*hemE* were confirmed by PCR and sequencing. The confirmed plasmids were then transformed into *V. atypica* OK5 using the established protocol (Zhou et al., 2015a). Resulting transformants were selected on tetracycline plates and confirmed by PCR.

**Determination of haemin production in Veillonella.** Overnight cultures of OK5 wild-type, *hemG* and *hemE* were diluted 1:100 into fresh BHL or semi-CDM. When grown to an OD₆₀₀~0.6, all cultures were harvested by centrifuging at 12 000 g for 10 min at 4 °C. Bacterial pellets were weighed then re-suspended using iCed Hemin Assay Buffer (Hemin Assay Kit, Sigma–Aldrich). Cells were lysed using FastPrep-24 (MP Biomedicals) at 4 °C and lysates were centrifuged at 12 000 g for 10 min at 4 °C. Supernatants were utilized to measure haemin concentration according to the manufacturer’s protocol (Hemin Assay Kit, Sigma–Aldrich).

**Co-culture assay.** Overnight cultures of OK5 wild-type, *hemG*, *hemE* and *P. gingivalis* ATCC 32327 were centrifuged to remove the supernatants, and the cell pellets were washed with BHL or semi-CDM twice then re-suspended in fresh BHL or semi-CDML supplemented with 1.2 µM vitamin K (BHLK or semi-CDMLK) to an OD₆₀₀ of 1.0. All cultures were then diluted 1:50 into 2 ml fresh BHLK or semi-CDMLK. For the mixed culture, diluted cultures of *Veillonella* strains and *P. gingivalis* were mixed in a 1:1 ratio, and the mixed culture was incubated in an anaerobic chamber at 37 °C for 48 h. The *P. gingivalis* single culture was supplemented with haemin (5 µg ml⁻¹) and used as control. For c.f.u. ml⁻¹ quantification, samples were taken at 0 h and 48 h, serially diluted, sonicated to break cell aggregates and plated on Brucella blood agar plates supplemented with haemin and vitamin K. The plates were incubated in the anaerobic chamber at 37 °C for 4 days. *P. gingivalis* colonies were distinguished from *Veillonella* by colony morphology and the production of black pigment.

**Construction of hemG reporter strain.** The *hemG*-lac reporter was constructed as follows. An 878 bp fragment of the *hemG* and luciferase genes was PCR amplified using OK5 chromosomal DNA and plasmid pFW5-luc as templates and primer pairs *hemG*-luc-up-F/*hemG*-luc-up-R and *hemG*-luc-down-F/*hemG*-luc-down-R, respectively (Table 2). The two amplicons were then ligated by overlapping PCR to create *hemG*-lac integrated cassette using primer pair *hemG*-luc-up-F/*hemG*-luc-down-R. The PCR product was double digested with EcoRI and *Bam*HI and then ligated with plasmid pBST, which was digested with the same enzymes. The recombinant plasmid pBST-*hemG*-lac was confirmed by sequencing and then transformed into OK5 (Liu et al., 2012; Zhou et al., 2015a). The transformants were selected on BHL plates supplemented with tetracycline (2.5 µg ml⁻¹). Transformants were further confirmed by PCR and sequencing.

**Table 1.** Bacterial strains and plasmids used in this study

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<tr>
<th>Strains and plasmids</th>
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<td><strong>Strains</strong></td>
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<td><em>E. coli</em> DH5a</td>
<td>Cloning strain</td>
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<td><em>V. atypica</em> OK5</td>
<td>Wild-type</td>
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<td><em>hemG</em></td>
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<td><em>hemE</em></td>
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<td>OK5-<em>hemG</em>-luc</td>
<td><em>hemEHG</em> operon luciferase reporter</td>
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<td><em>P. gingivalis</em> ATCC 32327</td>
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<td><strong>Plasmids</strong></td>
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<td>pBST</td>
<td>Suicide vector for <em>V. atypica</em>, Tc⁴</td>
<td>Zhou et al. (2015c)</td>
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<td>pBST-<em>hemG</em></td>
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<td>pBST-<em>hemG</em>-luc</td>
<td>Construction of <em>hemG</em> luciferase reporter</td>
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Luciferase assays. An overnight culture of OK5-hemG-luc reporter strain was centrifuged to remove supernatant and re-suspended with fresh BHIL media to an OD$_{600}$ ~1.0. Suspended culture then was 1:50 diluted into fresh BHIL broth supplemented with vitamin K (1.2 µM) (BHILK). For co-culture test, 2 days culture of _P. gingivalis_ ATCC 33277 was centrifuged and re-suspended with fresh BHIL to an OD$_{600}$ ~1.0 and then diluted 1:50 into the fresh OK5-hemG-luc culture. All cultures were grown in an anaerobic chamber for 48 h. Luciferase assays were performed by adding 25 µl of 1 mM d-luciferin (Sigma) solution (suspended in 0.1 M citrate buffer, pH 6.0) into 100 µl samples, and luciferase activities were measured using a TD 20/20 luminometer (Turner Biosystems). Colony-forming units (c.f.u. ml$^{-1}$) were obtained by plate counting. Luciferase activity was expressed as relative light units/ c.f.u. x 10$^6$ (for mixed species culture).

Statistical analysis. The Student’s test was used for statistical analyses and significance was designated as P $<$ 0.05.

RESULTS

Identification of a haem biosynthesis pathway in _Veillonella_

By searching the KEGG metabolic pathway for porphyrin and chlorophyll metabolism against the only available genome sequence of _Veillonella parvula_ DSM2008 in the KEGG database, we identified all genes involved in the haem biosynthesis pathway in the DSM2008 genome (Fig. 1). The same search against the genome of _P. gingivalis_ did not identify a complete gene set, although homologues of some genes in the pathway are present (Fig. 1a). To see if the same gene cluster is also present in other species/stains in the _Veillonella_ genus, we used the DNA and protein sequences of the key gene _hemE_ as query to search for homologues in the draft sequences of 11 _Veillonella_ strains including 3 strains of _V. atypica_, 2 strains of _Veillonella dispar_, 4 strains of _V. parvula_ and 2 strains of unassigned species in the HOMD database. As haem and vitamin B12 biosynthesis pathways share the same intermediate uroporphyrinogen III, _HemE_ is the first enzyme in the haem-specific pathway catalysing the conversion of uroporphyrinogen III into coproporphyrinogen III (Fig. 1a). Our search revealed that _hemE_ is present in all strains. Next, we aligned the DNA sequence of _V. atypica_ Col7a with the draft sequence of _V. atypica_ OK5 (P. Zhou and F. Qi, unpublished data), and we identified the same gene in the OK5 genome. Thus, the _hemE_ gene is present in all 12 (including _V. atypica_ OK5) draft genome sequences of _Veillonella_.

Further genomic analysis of the _V. parvula_ DSM2008 genome revealed that the nine genes of the haem biosynthesis pathway are localized on three operons: _hemA-C-D-B-L_, _hemE-H-G_ and _hemF_ (Fig. 1b). In addition, a putative transcription regulator is located as the last gene in the _hemA_ operon, which also includes _cysG_, encoding the first enzyme that utilizes the same substrate (uroporphyrinogen III) to synthesize precursoin 2, the first intermediate in the branching pathway leading to B12 biosynthesis (Fig. 1a). The _hemF_ operon includes also two other genes not involved in the haem biosynthesis pathway. Interestingly, the same operon organization is also found in all 12 _Veillonella_ strains regardless of what species they belong to (data not shown). This result indicates that the haem biosynthesis pathway is conserved in the genus _Veillonella_.

Inactivation of the haem biosynthesis pathway

As the haem biosynthesis pathway is highly conserved in the genus _Veillonella_, we used the transformable strain _V. atypica_ OK5 as a model to study its function in the biology and ecology of _Veillonella_. We chose _hemE_, encoding haemin synthetase, as our target because it is the last gene in the _hemEHG_ operon; therefore insertional inactivation of this gene would not exert a polar effect on other genes of this operon. The resulting mutant strain _hemG_ grew slower in BHIL medium compared with the wild-type, but the same cell mass was reached after 24 h (Fig. 2a). As expected, the mutant strain did not produce haemin (Fig. 2b). However, this mutant could still grow in semi-CDM containing 0.1 % vegetable-derived peptone (veggie peptone), which was assayed by haemin detection kit not to contain haemin (data not shown). This result indicated that haemin is probably not an essential but an enhancing nutrient for _Veillonella_ growth under laboratory conditions. Whether the haem biosynthesis pathway is essential in the oral biofilm awaits for further investigation.

Function of the _Veillonella_ haem biosynthesis pathway in _P. gingivalis_ growth

Since it has been documented that haem/haemin is an essential nutrient for the growth of later colonizers such as _P. gingivalis_ and that _Veillonella_ could support _P. gingivalis_ growth in pure saliva where haem is unlikely to be present (Hajishengallis, 2011; Lamont & Jenkinson, 1998), we reasoned that
inactivation of hemG should abolish Veillonella’s ability to support P. gingivalis’ growth in mixed culture in the absence of haemin. Thus, BHIL medium was first used to carry out the co-culture assays. As expected, in BHIL medium without haemin supplementation, OK5 supported the growth of P. gingivalis, although not to the same level as P. gingivalis alone in BHI plus haemin (4.53 × 10⁹ c.f.u. ml⁻¹ vs 8.20 × 10⁹ c.f.u. ml⁻¹ at 48 h) (Fig. 3a). Surprisingly, P. gingivalis growth in co-culture with hemG was similar to that with OK5 wild-type (Fig. 3a). Suspecting that the BHI medium could contain a trace amount of haemin, we did the co-culture experiments again using the semi-CDM medium supplemented with glucose, lactate, veggie peptone and vitamin K (semi-CDMLK). As expected, P. gingivalis did not grow in this medium although it could survive even at 48 h (Fig. 3b). Unexpectedly, the hemG mutant could still support the growth of P. gingivalis in this medium to a level similar to that of the wild-type OK5 (Fig. 3b). Upon further analysis,
we suspected that the hemG homologue in P. gingivalis (Fig. 1) could functionally compensate for the missing hemG in the hemG mutant by converting protoporphyrinogen IX, accumulated and possibly leaked out due to inactivation of hemG in OK5, into haemin, allowing P. gingivalis to grow.

To test this, we inactivated the hemE gene in OK5 by single-crossover inactivation. Because hemE is the first gene in the hemEHG operon (Fig. 1b), its insertional inactivation will knock out transcription of all three genes, hemEHG, due to polar effects. As the hemE homologue is absent in P. gingivalis (Fig. 1a), we expected that this mutation could not be complemented by the function of the hemF and hemG homologues in P. gingivalis (Fig. 1a). As expected, the number of P. gingivalis in co-cultures with V. atypica OK5 hemE mutant was three and four times lower than that with the OK5 wild-type in BHIL broth and semi-CDMLK, respectively (Fig. 3). In addition, the growth rate of the hemE mutant was further reduced compared with the hemG mutant (Fig. 2a), suggesting important roles of the intermediate coproporphyrinogen III in cell growth or possible toxicity of the accumulated uroporphyrinogen III. It is worth noting that adding the same amount (5 µg ml⁻¹) of haemin into the semi-CDMLK medium resulted in complete cell death of P. gingivalis (compare Fig. 3a, b; see Discussion).
The expression of hemEHG operon is up-regulated by P. gingivalis

Having demonstrated that the haem biosynthesis pathway in Veillonella was not essential for the growth of the producer but supported the growth of P. gingivalis, the next logical question was: is the expression of the hem genes induced or up-regulated by P. gingivalis? To answer this question, we made a luciferase gene fusion to the hemEHG operon and measured luciferase activity of the reporter strain OK5-hemG-luc in monocultures and mixed cultures with P. gingivalis. In the OK5-hemG-luc monoculture, operon expression started at early log phase and peaked at

Fig. 4. (a) Luciferase expression pattern of OK5-hemG-luc. The growth was measured every 2 h as OD_{600}, and luciferase expression (relative light units, RLU) was normalized by OD_{600}. (b) Luciferase expression of OK5-hemG-luc in single culture and co-culture with P. gingivalis. The c.f.u. was obtained and used to normalize RLU. The results are shown as mean±SD of at least three independent experiments.

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Fig. 5. Comparison of genomic organization of putative haem biosynthesis genes in oral bacteria. Vei. Veillonella; Pro. Propionibacterium; Agg. Aggregatibacter.
late log phase (Fig. 4a). Based on this expression pattern, we suggest that more than that of the monoculture (Fig. 4b). This result in the mixed culture started to increase at 4 h, and by 8 h, compared with OK5-

Fig. 6. Model depicting function of Veillonella as bridging species. V. atypica employs a surface adhesin Hag1 to mediate co-aggregation with S. gordonii by binding to its surface protein Hsa. Lactic acid produced by S. gordonii supports the growth of Veillonella. The Hag1 adhesin of Veillonella also mediates co-aggregation with P. gingivalis, probably by binding to a yet-to-be identified surface protein. Haemin/haem produced by Veillonella then supports the growth of P. gingivalis under conditions where haemin/haem is not present in the environment. Previous studies also demonstrated co-aggregation between S. gordonii and P. gingivalis (Daep et al., 2011), as well as limited growth of the two species together in saliva (Periasamy & Kolenbrander, 2009); however, the mechanism of this mutualism remains unknown.

Prevalence of the haem biosynthesis pathway in oral bacteria

Using the same strategy as for V. parvula DSM2008, we searched all oral microbial genomes in the KEGG database for the presence of the entire haem biosynthesis pathway. We found that, in addition to the Veillonella genus, all species/strains in the genus Aggregatibacter and most in the genus Propionibacterium (Propionibacterium acnes HL096PA1 lacks hemE and P. acnes 266 lacks hemH) harbour a complete gene set for haem biosynthesis. None of the streptococcal species possesses a complete hem pathway. Interestingly, the genomic organization of the nine genes in the Gram-positive bacterium Propionibacterium is similar to that of Veillonella, a Gram-negative, whilst the Gram-negative genus Aggregatibacter has a completely different genomic organization for these genes (Fig. 5). In the latter genus, instead of forming three gene clusters as for the other two genera, the nine genes are scattered amongst seven operons with other genes not related to haem biosynthesis. Whether these genes have the same function in haem biosynthesis as their counterparts in the other genera has yet to be determined.

DISCUSSION

Two characteristics of the Veillonella species enable them to serve as bridging species in the oral biofilm development. One is their ability to physically co-aggregate with many pioneer, early, middle and late colonizers, as shown by many in vitro and in vivo studies (Kolenbrander et al., 2006, 2010); another is their peculiar metabolic property, i.e. using lactate as major carbon and energy source (Rogosa, 1964). These properties allow them to colonize the early biofilm by physically attaching to the pioneer colonizers such as the streptococci and to utilize lactic acid excreted by the streptococci as carbon and energy source to grow. Growth of veillonellae then offers more attachment sites for later colonizers such as P. gingivalis. Our previous study showed that attachment of V. atypica OK5 tooral streptococci is mediated by a surface adhesin Hag1 (Zhou et al., 2015c), and the binding receptor on Streptococcus gordonii surface is a previously characterized sialic acid binding protein Hsa (Zhou et al., 2015b). We further showed that the same Hag1 is also responsible for binding to P. gingivalis, thus identifying Hag1 as the physical bridge connecting the pioneer colonizer S. gordonii with the later colonizer P. gingivalis [P. gingivalis could also bind to S. gordonii directly (Stinson et al., 1991, 1992)]. In this study, we demonstrate that Veillonella not only offers physical attachment sites for P. gingivalis but also provides an essential nutrient for the latter to grow. Based on these findings, a model is proposed (Fig. 6). In this model, Veillonella species function not only as a physical bridge connecting pioneer and later colonizers through cell-cell co-aggregation but also as a middle player in a relay of nutrient flow from pioneer to later colonizers. It is important to note that we believe this finding can be applied to all species in the genus Veillonella due to the presence of the highly conserved haem biosynthesis pathway amongst all members of this genus although only V. atypica OK5 was used in this study due to its transformability.

The oral biofilm plays a pivotal role in human health and disease. Numerous epidemiological studies have suggested that homeostasis of the biofilm community is associated with oral health, whilst dysbiosis of the community leads to development of diseases (Hajishengallis & Lamont, 2012). Thus, understanding the biological and environmental factors that affect the ecology of the biofilm has significant implications in disease prevention. One of the biological factors in biofilm dysbiosis is the bridging species such as veillonellae. These species not only physically recruit late colonizers such as the periodontopathogen P. gingivalis but also provide essential nutrients for those pathogens to grow (Periasamy & Kolenbrander, 2009, 2010; Zhou et al., 2015c). Thus, the bridging species play a pivotal role at the transition
from a streptococci-dominated supragingival community to a Gram-negative dominated subgingival community. The haem biosynthesis pathway identified in this study could provide a potential target for ecological biofilm modulation towards health. For example, we have shown in this study that inactivation of the haem biosynthesis pathway significantly reduced the number of P. gingivalis in mixed cultures in the absence of externally supplemented haemin.

A few more observations from this study need some discussion. First, monocultures of P. gingivalis in BHIL without haemin could grow to $3.95 \times 10^8$ c.f.u.  ml$^{-1}$ after 48 h but could not grow in semi-CDMLK without haemin (Fig. 3), implying that BHI broth could contain a trace amount of haemin. Second, addition of haemin into the semi-CDMLK medium led to complete cell death of P. gingivalis. It has been shown that haemin/haem is toxic at high concentrations, and Porphyromonas spp. are highly susceptible to haem toxicity (Anzaldi & Skaar, 2010). We speculate that this haemin toxicity could be due to the release of free iron from haemin breakdown in the semi-CDMLK medium, which could result in oxidative stress that killed P. gingivalis. However, in complex medium such as BHI, numerous components could absorb haemin or act as reducing agents to mitigate haemin toxicity.

It is worth noting that, although this study showed hem mutations significantly reduced Veillonella-supported P. gingivalis growth, haemin does not seem to be the only nutritional support that Veillonella gives to P. gingivalis. For example, monocultures of P. gingivalis in semi-CDMLK without haemin could not grow; however, in mixed cultures with hemE mutant in the same medium, it could grow to double the original cell mass after 48 h (Fig. 3b). Identifying the other growth supporting factors would provide a more comprehensive picture for Veillonella’s role as a bridging species.

It is also worth noting that the number of c.f.u. ml$^{-1}$ for P. gingivalis in the mixed culture was $\sim 50\%$ of that of the monoculture in BHI plus 5$\mu$g ml$^{-1}$ of haemin after 48 h incubation although V. atypica OK5 wild-type supported good growth of P. gingivalis in BHIL (Fig. 3a). There could be two explanations for this difference: (1) the haemin produced and secreted into the medium by V. atypica OK5 (Fig. 2b) is not sufficient to support optimal growth of P. gingivalis or (2) there is competition between the two species for other nutrients. Competition between two mutualistic species has been observed. For example, the catalase-positive Actinomyces naeslundii can eliminate H$_2$O$_2$ produced by S. gordonii in co-cultures, thus protecting the latter from auto-oxidation. However, S. gordonii in turn inhibits A. naeslundii growth, apparently by competing for essential nutrients with the latter (Jakubovics et al., 2008). Thus, it would not be surprising that there is a competition between Veillonella and P. gingivalis in addition to the mutualistic relationship shown in this study.

Given the importance of the haem biosynthesis pathway of Veillonella in the growth of P. gingivalis, it is not surprising that expression of the haem biosynthesis genes, represented by the hemEHG operon, was up-regulated by co-culture with P. gingivalis (Fig. 4). Since the two species also coaggregate (Zhou et al., 2015c), this juxtaposition further ensures efficient nutrient flow from Veillonella to P. gingivalis. What benefits Veillonella receives from this relationship remain to be determined. The signal(s) that triggers up-regulation of the hem gene expression also remains to be determined.

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REFERENCES


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