Identification and characterization of a haem biosynthesis locus in *Veillonella*

Peng Zhou,¹ Xiaoli Li¹ and Fengxia Qi¹,²

¹Department of Microbiology and Immunology, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma 73104, USA
²Department of Oral Biology, College of Dentistry, University of Oklahoma Health Sciences Center, Oklahoma 73104, USA

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 & 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. atypicaOK5 and its derivatives were grown in BHIL broth (Difco) with 0.6% sodium lactate (BHIL) or on BHIL agar plates. For transformation, cells were grown in Todd–Heewitt broth (Difco) with 0.6% sodium lactate (THL), and transformants were selected on BHIL 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 33277 was grown in TS medium (trypticase soy broth) supplemented with yeast extract (1 mg ml⁻¹) and haemin (5 µg ml⁻¹) and vitamin K (1 µg 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 SalI 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.

**Determinaton of haemin production in Veillonella.** Overnight cultures of OK5 wild-type, hemG and hemE were diluted 1:100 into fresh BHIL 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 33277 were centrifuged to remove the supernatants, and the cell pellets were washed with BHIL or semi-CDM twice then re-suspended in fresh BHIL or semi-CDML supplemented with 1.2 µM vitamin K (BHILK or semi-CDMLK) to an OD₆₀₀ of 1.0. All cultures were then diluted 1:50 into 2 ml fresh BHILK 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-luc 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-luc integrated cassette using primer pair hemG-luc-up/FI-hemG-luc-down-R. The PCR product was double digested with EcoRI and BsmBI and then ligated with plasmid pBST (Zhou et al., 2015c), which was digested with the same enzymes. The recombinant plasmid pBST-hemG-luc was confirmed by sequencing and then transformed into OK5 (Liu et al., 2012; Zhou et al., 2015a). The transformants were selected on BHIL 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

<table>
<thead>
<tr>
<th>Strains and plasmids</th>
<th>Characteristics</th>
<th>Reference</th>
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<tbody>
<tr>
<td>E. coli DH5a</td>
<td>Cloning strain</td>
<td></td>
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<tr>
<td>V. atypica OK5</td>
<td>Wild-type</td>
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<tr>
<td>hemG</td>
<td>HemG gene insertion mutant</td>
<td>This work</td>
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<tr>
<td>hemE</td>
<td>HemE gene insertion mutant</td>
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<tr>
<td>OK5-hemG-luc</td>
<td>hemEHG operon luciferase reporter</td>
<td>This work</td>
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<tr>
<td>P. gingivalis ATCC 33277</td>
<td>Wild-type</td>
<td>This work</td>
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<tr>
<td>Plasmids</td>
<td></td>
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<tr>
<td>pBST</td>
<td>Suicide vector for V. atypica, Te⁴</td>
<td>Zhou et al. (2015c)</td>
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<tr>
<td>pBST-hemG</td>
<td>Inactivation of hemG gene</td>
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<td>pBST-hemE</td>
<td>Inactivation of hemE gene</td>
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<td>pBST-hemG-luc</td>
<td>Construction of hemG 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) (BHIK). 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 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. × 10$^{9}$ (for mixed species culture).

Statistical analysis. The Student’s $t$ 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/strains 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 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 hemE from 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 precorrin 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 hemG, 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 waits 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, BHI medium was first used to carry out the co-culture assays. As expected, in BHI 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 hemE 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).

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**Fig. 2.** Phenotypes of hemG and hemE mutants. (a) Growth curve of V. atypica OK5 wild-type, hemG and hemE mutants. Overnight cultures of all strains were centrifuged, and the cell pellets were re-suspended in fresh BHIL to an OD\(_{600}\) of 1.0. All cultures were then diluted 1:100 into fresh BHIL and grown under anaerobic condition at 37 °C. Samples were taken every 2 h to measure OD\(_{600}\). The results are shown as mean±SD of three independent experiments. K is the growth rate through the log phase, calculated using the formula \(K = (N_1 - N_0)/(t_1 - t_0)\), where \(N_1\) is the OD\(_{600}\) value at time \(t\) and \(N_0\) is the OD\(_{600}\) value at time \(t_0\). *\(P = 0.047\); **\(P = 0.011\); ***\(P = 0.0006\). (b) Measurement of haemin production in OK5 wild-type, hemG and hemE mutants. The procedure was described in Methods. The results are shown as mean±SD of three independent experiments.

**Fig. 3.** Growth of P. gingivalis in monoculture and co-cultures with OK5 wild-type, hemG and hemE mutants in BHILK (a) and semi-CDMLK (b) medium. Detailed procedure was described in Methods. The results are shown as mean±SD of at least three independent experiments.
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
late log phase (Fig. 4a). Based on this expression pattern, we measured luciferase activity in mixed cultures with V. atypica by binding to its surface protein Hsa. Lactic acid produced by S. gordonii supports the growth of Veillonella. The Hsp1 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 to oral 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 BHILK 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 ~50 % of that of the monoculture in BHI plus 5 µg 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 cat-alase-positive Actinomyces naeslundii can eliminate $\text{H}_2\text{O}_2$ produced by S. gordonii in co-cultures, thus protecting the latter from auto-toxication. 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 co-aggregate (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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