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EFFECT OF PH ON THE TRANSCRIPTIONAL PROFILE OF
***PORPHYROMONAS GINGIVALIS* W83**

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

GCF- gingival crevicular fluid

BHI- brain heart infusion

OD- optical density

PCR- polymerase chain reaction

ORF- open reading frame

BSA- bovine serum albumin

BROP- Bioinformatics Resource for Oral Pathogens (www.brop.org)

SOAPMD- Significance Analysis for Oral Pathogen Microarrays

LIMMA- Linear Models for Microarray Data

JCVI/TIGR- J. Craig Venter Institute (formerly The Institute for Genomic Research)

qRT-PCR- quantitative real-time polymerase chain reaction

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Director: Janina P. Lewis, Ph.D.
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Abstract

Porphyromonas gingivalis, a gram-negative, anaerobic bacterium, is widely recognized as a causative agent for periodontal disease. Despite sequencing of the complete genome, limited research exists examining transcriptional response of *P. gingivalis* to shifts in pH. Previous studies have shown that *P. gingivalis* is capable of surviving in the variety of micro-environmental niches found within the oral cavity, including basic and acidic pH conditions; However, the underlying mechanisms of this survival are not well understood. This study examined *P. gingivalis* by comparing bacteria grown at two different acidic and one neutral pH (5.5, 6.5 and 7.0) to bacteria cultured at pH 8.5. Using microarray to examine global transcriptional regulation, differences in RNA abundance were identified in all conditions, with total genes differentially regulated ranging from 30 to almost 500 genes. Among these genes found to up-regulated are those for complete metabolic pathways leading to production of ammonia and weak acids which may serve to shift the net proton balance and moderate pH outside of the cell.

Introduction

Periodontal disease is a range of illness associated with the invasion of bacteria into the subgingival periodontal pockets. Ranging from mild inflammation of the gums to severe soft tissue damage, periodontal disease has the potential to be a serious health threat. Prolonged neglect may result in the loss of alveolar bone and weakening of the periodontal ligament, eventually causing tooth loss. Recent studies have demonstrated potential linkages between periodontal disease and increased incidence of diabetes (Grossi et. al., 1997), rheumatoid arthritis (Mercado et. al. 1999) cardiovascular disease (Beck et. al., 1996) and even low birth weight in babies of mothers with the disease (Offenbacher et. al., 1996). Knowing the strain that periodontal diseases cause on our health care system and the large segment of society at risk, exploration of disease mechanism and new treatments is essential to the health of society.

Porphyromonas gingivalis is a known causative agent of periodontal disease. It is an anaerobic, black pigmented, gram-negative bacterium. *P. gingivalis* is known to constitute up to 5% of the oral bacteria population in healthy individuals, but as periodontal disease progresses, a shift is seen towards a greater percentage of *P. gingivalis* (Teanpaisan et. al., 1995).

P. gingivalis exhibits a variety of adhesins for attachment within the oral cavity including, fimbriae, hemagglutinins and proteinases. Fimbriae, small finger like projections, are key players in adhesion to host and other bacterial cells. For example, *P. gingivalis* is unable to invade epithelial cells in

bacterial mutants that lack fimbriae (Ezzo and Cutler, 2003). Along with fimbriae, hemagglutinins aid in adhesion by causing agglutination of red blood cells, allowing *P. gingivalis* to bind erythrocytes (Lamont and Jenkinson, 2000). Both of these methods mediate attachment of *P. gingivalis* to oral biofilm residents, such as *Streptococcus oralis*, salivary proline-rich proteins, erythrocytes, and sites within the subgingival cavities (Paster et. al., 2001).

P. gingivalis is unable to use carbohydrates for growth (Lamont and Jenkinson, 1998). Instead, *P. gingivalis* must rely on acquisition of peptides in order to provide essential nutrients, such as carbon and nitrogen. Once located inside the oral cavity, hemagglutinins and proteinases are secreted to aid in acquisition of peptides, destruction of host proteins, invasion of host cells and modulation of host immune response (Lamont and Jenkinson, 2000). Hemagglutinins work cooperatively with the antigen molecule lipopolysaccharide to aid in peptide and heme acquisition (Lamont and Jenkinson, 1998, Ezzo and Cutler, 2003). The main sources of peptide acquisition in *P. gingivalis* are proteinases; a complete genome sequence revealed a total of 42 putative proteinases in the genome of *P. gingivalis* (Nelson et. al., 2003). The most widely examined of these are arginine and lysine specific trypsin-like cysteine proteinases, known as gingipains. Without gingipains *P. gingivalis* shows markedly decreased levels of growth (Grenier et. al. 2001), highlighting the importance of peptides for growth. Additionally, *P. gingivalis* has the limited ability to ferment free amino acids,

although this is not thought to be a primary method of energy generation (Takahashi et. al., 1997).

The oral cavity contains many distinct micro-environmental compartments (Aframian et. al., 2006), which vary in pH and oxygen content. *P. gingivalis* must be able adapt to the wide range of conditions it is presented with as it moves through the oral cavity. In the oral cavity, which includes the palate, tongue, buccal mucosa, floor of the mouth and surfaces of the teeth, pH ranges from 6.5 -7.5 (Aframian et. al., 2006), but saliva and diet can cause large, short-term shifts in pH (Figure 1). Additionally, the environment can vary within the oral biofilm, causing distinct environmental differences between the biofilm and the oral cavity (Marsh et. al., 1997). For example, the pH of the oral biofilm may drop as low as 4.5 when acidic fermenting bacteria such as *Streptococcus mutans* are prevalent.

The pH in the subgingival pockets, where *P. gingivalis* preferentially colonizes, ranges from 7.5 – 8.0 range (Bickel et. al., 1985) (Figure1). Gingival crevicular fluid, or GCF, is the main controller of pH in this area; as *P. gingivalis* colonizes the subgingival spaces, it induces inflammation, which is thought to stimulate the release of GCF. This increased release of GCF may further increase alkalinity. It is not well understood whether the increase in GCF causes a change in alkalinity or if *P. gingivalis* plays a role in this pH change (Bickel and Cimasoni, 1985).

The underlying mechanism by which *P. gingivalis* is able to inhabit both acidic and alkaline conditions and overcome shifts in pH has not been specifically examined. Earlier research has shown that proteinase activity in

P. gingivalis differs significantly between acidic and basic growth conditions (McDermid et. al., 1988; Takahashi et. al., 1990). These studies indicate a preference for *P. gingivalis* to exhibit greater proteinase activity under basic conditions, such as those in the subgingival pockets, but do not indicate a mechanism for survival under acidic stress. More recent work shows the ability of *P. gingivalis* to alter culture medium pH by fermenting amino acids into ammonia and weak acids such as butyric acid, acetic acid, propionic acid and succinic acid (Takahashi, 2003). Nelson et al (2003) back this discovery through examination of the complete *P. gingivalis* genome sequence, finding multiple amino acid catabolic pathways ultimately leading to the production of ammonia. Base creation, specifically synthesis of ammonia, appears to be the response *P. gingivalis* enacts to overcome acidic pH stress.

Research has shown that bacteria have developed a variety of mechanisms for acidic pH response. Examination of pH stress response through microarray analysis of *S. mutans*, reveals up-regulation in ATPase activity which may help maintain a proton gradient. Also, up regulated in *S. mutans* are functional categories for transport proteins and energy metabolism (Gong et. al., 2009). Microarray studies in other bacteria including *Mycobacterium tuberculosis* also showed increased expression of genes for intermediary and fatty acid metabolism in response to acidic pH shift (Fisher et. al., 2002). In the stomach, *Helicobacter pylori* exhibit a novel system of ammonia synthesis through production of urease (Merrell et. al., 2002). Additionally, oral bacteria including *Neisseria* sp., *Prevotella intermedia*, *Actinomyces naeslundii*, *Lactobacillus casei*, *Streptococcus*

sanguis and *Streptococcus faecalis* have shown the ability to survive short term acidic shock conditions, although the mechanism has not been characterized (Svensater et al, 1997).

Sequencing of the complete genome has allowed researchers to examine whole-genome transcriptional response in *P. gingivalis*. This provides an overall view of how the organism responds to different conditions such as oxygen stress (Lewis et. al., 2009), host stress (Hosogi and Ducnan, 2005), or exposure to oral biofilm inhabitants (Lo et. al., 2009). To better understand the mechanisms by which *P. gingivalis* is able to survive in the dynamic environment of the oral cavity and subgingival spaces, *P. gingivalis* was grown in six pH conditions; extracted RNA was then used to identify differential RNA expression between acidic, neutral and basic conditions using microarrays. Six pH conditions, 5.5, 6.5, 7.0, 7.5, 8.0 and 8.5, were chosen because they represent the majority of pH conditions *P. gingivalis* is subject to within the oral cavity. These pH conditions were tested at anaerobic and microaerophilic conditions to test both oxygen conditions found within the oral cavity. Figure 1 illustrates the different environmental conditions in the oral cavity.

Increased knowledge of *P. gingivalis* response to change in pH growth conditions, specifically the comprehensive mechanisms of response identified through the use of whole-genome comparisons, provide a greater depth of knowledge about *P. gingivalis*, as well as other Bacteroidetes species that may exhibit a similar response. Additionally, pH stress could become a target for treatment of periodontal disease. Disruption of processes related

to acidic pH response could interfere with or help prevent *P. gingivalis* infection, because the bacteria must first survive these conditions before it is able to colonize alkaline, subgingival pockets and cause infection. Similarly, targeting mechanisms responsible for survival in basic conditions could help treat already established disease. Before any of this can be further examined, we must examine the response of *P. gingivalis* to pH modification at a global level.

Materials and Methods

1. Growth of *Porphyromonas gingivalis* W83

P. gingivalis was grown in brain heart infusion (BHI) broth (pH 6.9) (Difco Laboratories, Detroit, MI) with yeast extract (5mg/ml), cysteine (1%), hemin (5µg/ml; Sigma, St. Louis, MO) and vitamin K3 (0.5µg/ml; Sigma, St. Louis, MO) added. Cultures were grown overnight in either anaerobic conditions (10% H₂, 10% CO₂ and 80% N₂ at 37°C) or microaerophilic conditions (7.2% H₂, 7.2% CO₂, 79.7% N₂, 6% O₂). When the optical density (OD₆₆₀, 660nm, measured with Spectrophotometer DU® 640) of cultures reached approx 1.5, the cultures were divided into BHI media with pH modified using the addition of NaOH or HCl. Final pHs were verified with a pH meter. The cultures were diluted to an OD₆₆₀ of 0.2. Cultures were grown for 3-12 hours, in anaerobic or 6% oxygen, until an OD₆₆₀ of 0.5-0.8 was reached. Cultures were then harvested.

2. Harvesting of cultures and isolation of RNA

Cultures were aliquoted into 15ml tubes and centrifuged at 8,000rpm for 15 minutes at 4°C. The supernatant was then removed and the tubes saved at -80°C until ready for RNA isolation. RNA was prepared using the QIAGEN RNeasy Mini Kit. The isolation followed the QIAGEN RNeasy Mini kit protocol, with some modifications. First, 1mL of RLT buffer (QIAGEN RNeasy Mini kit) was added to the thawed pellet. The mixture was then transferred to FastPrep-24 tubes (MP Biomedicals). Then, 500µl of acid phenol-chloroform

was added to the tubes. Cells were lysed with a FASTPREP-24 Instrument (MP Biomedicals), at a speed of 6m/s for 45 seconds. Purification was then completed according to the QIAGEN protocol. RNase free DNase (QIAGEN) was added to eliminate any remaining DNA. The concentration of RNA was measured using a NanoDrop spectrophotometer ND-1000.

3. cDNA Generation

First, 10µg of total RNA was resuspended in RNase Free Water (QIAGEN) to a total volume of 12µl. Then, 3µl of Random Primers (Stratagene FairPlay III Microarray Labeling Kit) was added and the samples were incubated at 70°C for 10 minutes, after the addition to each sample of 2.4µl 10x Affinity Script RT Buffer, 1µl 20x dNTP mix, 1.5µl 0.1M DTT, 0.5µl RNase Block (40 U/µl), 2µl of Affinity Script HC/RT (all from Stratagene FairPlay III Microarray Labeling Kit) and 2µl of Superscript III Reverse Transcriptase (Invitrogen). The reaction was then incubated overnight at 42°C. The cDNA generation was stopped with 10µl of 1N NaOH and incubated at 70°C for 10 minutes. Samples were then neutralized with 10µl of 1M HCl. cDNA was purified according the QIAGEN MinElute PCR Purification Kit protocol with the exception of two steps. NaHCO₃ was used in place of EB Buffer to elute the cDNA, and TIGR protocol phosphate wash buffer (125µl 1M KPO₄, 3.8ml dH₂O, 21ml EtOH) was used in place of PE Buffer. cDNA concentration was then measured with a NanoDrop spectrophotometer ND-1000. Samples were then immediately labeled.

4. cDNA Labeling

Samples were labeled with 5µl of Cy-3 or Cy-5 dye (GE Healthcare) and allowed to stand in the dark for 1.5 hours at room temperature. In order to stop the reaction, 5µl of 4M hydroxylamine was added and the samples were left at room temperature, in the dark, for 15 minutes. Samples were then purified using the QIAGEN MinElute PCR Purification Kit protocol, eluting twice with 10µl EB buffer, for a total volume of 20µl. If samples were not to be hybridized immediately, 1µl of 0.1M DTT (Stratagene FairPlay III Microarray Labeling Kit) was added to each sample, which was then stored at -20°C.

5. Microarray Pre-Hybridization and Hybridization

Prior to microarray hybridization, Cy3- and Cy5-labeled cDNA were mixed together and purified using a QIAGEN MinElute PCR Purification Kit and protocol, eluting twice with 10µl EB buffer, for a total volume of 20µl. If the eluate exhibited the expected color of the dye, then pre-hybridization was started. Version 1 *Porphyromonas gingivalis* microarray slides from the Institute for Genomic Research were used; these slides contained 70-mer oligonucleotide probes for all 1907 predicted ORFs in *P. gingivalis*. First, slides were marked and incubated with prehybridization buffer (350ml total volume: 87.5ml of 20x SSC (Invitrogen) 3.5ml of 10% SDS (GIBCO Invitrogen), 3.5 g of BSA powder (Fisher Biotech) and 255.5ml of dH₂O) for 2 hours at 42°C. Slides were then washed with three different buffers (Table

1). During washes, labeled cDNA was pelleted, using a SpeedVac centrifuge, until completely dry. After washes were completed the slides were dried with a slide centrifuge and mSeries Lifterslip Cover Slips (Thermo Scientific) were affixed to the slides. The slides were placed in a Hybex Microsample Incubator at 42°C until ready for hybridization.

For hybridization, cDNA pellets were resuspended in 80µl of hybridization buffer (400µl formamide, 250µl 20x SSC, 10µl 10% SDS, 1µl 0.1M DTT, 339µl dH₂O and 60µl of Salmon Sperm DNA (Ambion)). Then, they were heated at 95°C for 5 minutes, vortexed and centrifuged for 30 seconds at 13,000rpm. The cDNA was then heated again, 95°C for 5 minutes and centrifuged at 13,000rpm for 30 seconds. Next, 75µl of the sample was loaded onto the washed microarray slides. Slides were incubated 12-14 hours at 42°C in a Hybex Microsample Incubator. After incubation, the slides were washed again (Table 1). The slides were then dried with a slide centrifuge and coated with DyeSaver 2 (Genisphere Inc.) to preserve them.

6. Microarray Scanning and Analysis

For each condition examined, one biological replicate and four technical replicates were present. An Axon 4200A microarray scanner and GenePix v6.0 software was used to scan the microarray slides and create images. The images were then analyzed using the GenePix v6.0 software, quantifying the fluorescence of each microarray feature. Raw data was normalized using the Lowess normalization. Significant statistical differences were assessed using the Significance Analysis for Oral Pathogen Microarrays

(SAOPMD) tool available at the Bioinformatics Resource for Oral Pathogens (BROP) at The Forsyth Institute (www.brop.org). This software uses Linear Models for Microarray Data (LIMMA) to fit a linear model to expression data for each gene on a microarray slide. Empirical Bayes and other shrinkage methods are to borrow information across genes (Smyth, 2005).

7. Determination of Significance

For one slide comparisons, significance of regulation was determined by choosing those genes which showed four replicates and were up-regulated or down-regulated at least 1.5 fold, with an adjusted p-value less than 0.05. Genes were additionally sorted into JCVI functional role categories (Table 14). Also, all raw data was examined together (pH 5.5, 6.5 and 7.0, microaerophilic and anaerobic) against pH 8.5. Genes that showed 24 spots, indicating four replicates for each of the six conditions with an adj. p-value < 0.05 were chosen to be significant, in order to examine general regulation. A metabolic map for general gene expression (great or less than 1 fold, absolute value, log scale) in pH 5.5 versus pH 8.5 conditions was created using the Omics Viewer available in the Pathway Tools version 14.0 software available from www.biocyc.org.

Results

1. Microbial growth in various pH conditions

P. gingivalis W83 was grown under six pH conditions (5.5, 6.5, 7.0, 7.5, 8.0, 8.5) in both anaerobic and microaerophilic, 6% O₂, conditions. Growth time required to reach OD₆₆₀ of 0.5, which corresponds to mid-logarithmic phase, varied greatly between different conditions from between 3 hours to 8 hours (data not shown). Example growth curves are shown for each condition in Figure 2.

Due to experimental constraints, no statistical analysis could be conducted; however, there are patterns that should be noted. In both anaerobic and microaerophilic conditions, pH 5.5 took longer to reach mid-log phase than at any other pH condition. For example, when examining the growth of *P. gingivalis* at anaerobic conditions, an approximately 58% reduction in growth rate was noted between bacteria grown at pH 5.5 and all other conditions. *P. gingivalis* growth rate in pH 5.5 microaerophilic conditions was reduced by approximately 61% when compared all other microaerophilic grown cultures.

Under all conditions of oxygen and pH tested, *P. gingivalis* grew successfully to mid-logarithmic phase, at which point the cultures were harvested for RNA isolation. At mid-logarithmic phase (OD₆₆₀ - 0.5), the number of bacterial cells present should be close to equivalent under all conditions; this is corroborated because near equal amounts of RNA were isolated from all conditions (data not shown).

2. Microarray Analysis

RNA was isolated and cDNA was generated from all samples; however, microarray analysis was only conducted comparing the three lowest pH conditions (5.5, 6.5, and 7.0) to the most alkaline pH condition (8.5). This comparison was chosen in order to examine the conditions found in the oral cavity (acidic-neutral) vs. the pH conditions *P. gingivalis* is exposed to in the subgingival pockets (basic)(Figure 1). Figures 3 and 4 summarize the data showing the percent of genes that are regulated in each condition. Each condition compared to pH 8.5 was composed of one biological replicate slide, with four technical replicates on each.

Genes were also grouped according to the functional roles of their encoded proteins. Table 14 shows one set of categories as grouped by JCVI. Nineteen different functional role categories were examined, encompassing the majority of the genes of *P. gingivalis*. Figure 4 shows functional role category comparisons for all conditions studied.

2.1 Gene expression in pH 5.5

In both anaerobic and microaerophilic conditions, pH 5.5 compared to pH 8.5 showed the greatest amount of change in RNA abundance (Table 2). Under anaerobic conditions, 210 genes showed changed expression: 150 genes were up-regulated (Table 3) and 60 genes were down-regulated (Table 4). Similarly, under microaerophilic conditions, 492 genes were regulated: 332 up-regulated (Table 8) and 160 down-regulated (Table 9). Of these

regulated genes functional role categories for energy metabolism, central intermediary metabolism and synthesis of nucleotides, nucleosides, purines and pyrimidines showed significant up-regulation (Figure 5). Categories showing down-regulation included fatty acid and phospholipid metabolism and disrupted reading frame (Figure 5) although in general more up-regulation was present than down-regulation.

Through examination of general metabolic regulation (Figure 6) functional categories were examined for specific pathway regulation. Amino acid break down pathways were up-regulated in the pH 5.5 conditions over pH 8.5. Several complete pathways were found including the following: serine degradation (PG0084), methionine degradation (PG0343), glutamate degradation (PG1232), and mixed acid fermentation (PG1082, PG1576, PG1081) (Table 22). A nitrate reduction pathway leading towards ammonia synthesis was also identified (PG1821) as well as a formate-nitrite transporter (PG0209) (Tables 3 and 8). Other pathways found to show up-regulation included the pentose phosphate pathway, purine and pyrimidine *de novo* biosynthesis, FormylTHF biosynthesis and also folate transformations (PG0042, PG1321, PG1116, PG1559). Down-regulated metabolism included genes for thiamin biosynthesis and fatty acid and lipid biosynthesis including fatty acid elongation and palmitate biosynthesis (Figure 5).

2.2 Gene expression in pH 6.5

The least amount of change in RNA abundance, both under anaerobic and microaerophilic conditions, was seen when comparing pH 6.5 and pH

8.5. Under anaerobic conditions, 36 genes were found to be significantly up-regulated (Table 5), while no genes were found to be down-regulated significantly. This lack of regulation when comparing pH 6.5 to pH 8.5 is due at least in part to poor RNA hybridization in this slide, making it different from all others. Under microaerophilic conditions, 98 genes showed changes in abundance: 77 up-regulated (Table 10) and 21 down-regulated (Table 11). Complete metabolic pathways were not identified to be significantly up-regulated in 6.5 vs. 8.5 conditions (Table 5 and 10).

Functional role categories that were up-regulated included energy metabolism and central intermediary metabolism, while down-regulation was less clear because of the poor slide quality in one of the pH 6.5 versus pH 8.5 slides. Pathways up-regulated in other conditions, such as fermentation, amino acid degradation and synthesis of nucleotides were not seen in these conditions. Nitrate reduction was up-regulated, including cytochrome C nitrite reductase and a formate-nitrite transporter (PG0209) (Table 10). Also up-regulated were pathways for FormylTHF biosynthesis and folate transformations, which involve many of the same genes (PG0042, PG1321, PG1116, PG1559).

2.3 Gene expression in pH 7.0

A significant amount of change in abundance can be observed when comparing pH 7.0 to pH 8.5 under both anaerobic and microaerophilic conditions (Table 2). Under anaerobic conditions, 157 genes were found to be regulated: 129 genes were up-regulated (Table 6) and 28 genes were

down-regulated (Table 7). Under microaerophilic conditions, 233 genes were regulated: 167 up-regulated (Table 12) and 66 down-regulated (Table 13).

Similar patterns of RNA abundance to other comparisons were not found, including lack of complete metabolic pathways. Abundance of RNA for pathways including fermentation and amino acid degradation were not found to be significantly up-regulated. Instead, increased expression was noted in nitrate reduction (PG1821), nucleotide biosynthesis (PG1260, PG0953, PG1129) and some parts of fatty acid and lipid synthesis (PG1416, PG0046, PG1249) that were down-regulated in pH 5.5 versus pH 8.5 conditions. Down-regulation was seen in the thiamin biosynthesis pathway (PG2109, PG2217, PG2108).

2.4 Important regulation under basic conditions

Pathways found to be up-regulated under basic conditions, include fatty acid and lipid metabolism, specifically saturated fatty acid elongation (PG1416, PG0046, PG1249). Also important at basic conditions was biosynthesis of cofactors, prosthetic groups and carriers, including thiamin biosynthesis (PG2109, PG2217, PG2108).

2.5 Comparisons between pH conditions

In order to characterize how gene expression may differ over the gradient of pH conditions examined, comparisons were conducted looking for similarities in RNA abundance between all pH and oxygen conditions (pH 5.5, pH 6.5 and pH 7.0, both anaerobic and microaerophilic) when compared to

pH 8.5. If similar patterns of regulation between pH conditions are observed, it indicates that a similar genetic response is enacted when *P. gingivalis* is exposed to these different pH conditions.

Comparisons were first conducted examining like pH conditions to different oxygen conditions, for example comparing pH 5.5 conditions, both anaerobic and microaerophilic. When both pH 5.5 conditions (anaerobic and microaerophilic) were compared to pH 8.5, similar patterns of RNA abundance were observed. There were 95 genes that showed the same expression, with only 4 genes being up-regulated in one condition while down-regulated in the other (Table 14). When comparing pH 6.5 conditions against pH 8.5, five genes were found to be regulated in the same manner between conditions, with one having opposite regulation (Table 15). The absence of down-regulated genes at pH 6.5 vs. pH 8.5 in anaerobic conditions, limited analysis of these conditions. At pH 7.0 (anaerobic and microaerophilic), compared to pH 8.5, 12 genes were found to show differential RNA abundance in the same manner, while 7 show opposite abundance profiles (Table 16).

Similarity between different pH conditions was also examined. When pH 5.5 conditions were combined with pH 6.5 conditions and compared against pH 8.5, 24 genes were found to show similar patterns of expression, while none were found to have opposite expression (Table 17). Similarities in pathway regulation were not prevalent between pH 5.5 and pH 6.5 versus pH 8.5, only the nitrate reduction pathway (PG1821) was found equally expressed in both. When comparing pH 6.5 and pH 7.0 conditions to

pH 8.5, 9 genes were found to be differently expressed in a similar manner, with none had opposing expression profiles (Table 18). With so few genes equally expressed between the two, no pathway expression inferences could be found. Finally, pH 5.5 and pH 7.0 conditions compared to pH 8.5. Here only two genes were found to have similarities in their differential expression (Table 19). This indicates that the response of *P. gingivalis* is more similar in the pH 5.5 and pH 6.5 vs. pH 8.5 conditions, and seems to indicate that a different response is occurring in pH 7.0 vs. pH 8.5.

Raw data for all pH and oxygen conditions, pH 5.5, pH 6.5 and pH 7.0 both microaerophilic and anaerobic were analyzed concurrently to examine all acidic and neutral conditions, against the most basic condition, pH 8.5. This resulted in 128 genes having RNA abundances that were significantly different. Of these 128 genes, 40 genes were found down-regulated, while 88 were found up-regulated (Table 21). After more in depth examination several genes involved in metabolic pathways also found in the single slide comparisons showed up-regulation. Genes in pathways for serine, glutamate and methionine degradation (PG0084, PG0343, 1232) were up-regulated in a similar manner to that seen in pH 5.5 vs. pH 8.5 conditions. Additional up-regulation was seen in FormylTHF biosynthesis and folate transformation pathways.

Discussion

In order for *P. gingivalis* to survive inside the oral cavity and subgingival pockets, it must adapt to a variety of microenvironments. Previous studies have examined *P. gingivalis* under pH stress conditions, but no study has looked at the underlying transcriptional regulation during modification of growth medium pH. This study set out to examine RNA abundance under different pH conditions on a global transcriptional level. It was found that there is a great amount of RNA abundance differences, both up and down, when comparing growth under pH condition changes. The two acidic pH conditions examined, pH 5.5 and pH 6.5 versus pH 8.5, exhibit similar genetic responses to change in pH, while pH 7.0 versus 8.5 seems to differ more from the acidic response. We know that *P. gingivalis* is very capable of living at pH 7.0, whereas prolonged exposure to acidic pH conditions do not favor growth.

Metabolic response of P. gingivalis

Previous studies have illustrated that weak acid and base production may be an important way for the organism to overcome exposure to acidic pH conditions (Takahashi et. al., 2003). *P. gingivalis* may excrete alkaline metabolites in order to create an area of moderated pH around itself when exposed to unfavorable conditions (Takahashi et. al., 2003). Takahashi and his colleagues further proposed that weak acid synthesis from amino acids, such as production of acetic acid from L-aspartate and L-glutamate, served

to lower the net H^+ ion concentration available, thus lowering pH around the cell.

In the two most acidic conditions tested, pH 5.5 anaerobic and pH 5.5 microaerophilic, multiple metabolic pathways leading to synthesis of ammonia, acetate, butyrate, propionate and ammonia were largely up-regulated (Figure 6). Pathways found to be up-regulated include: pyruvate fermentation to acetate (PG0429, PG1082, PG1081), serine degradation (PG0084), glutamate degradation (PG1232), and methionine degradation (PG0343). These patterns were not found in pH 6.5 or pH 7.0 compared to pH 8.5 single slide comparisons. One reason for this response could be that the less extreme acidic and neutral conditions may exhibit decreased need to modify pH.

Another potential source of ammonia production may be the reduction of nitrite by cytochrome C nitrite reductase (PG1821). This enzyme was found to be significantly up-regulated in both pH 5.5 and pH 6.5 vs. pH 8.5 conditions. Additionally, increased uptake of nitrite was evident based on up-regulation of a formate-nitrite transporter (PG0209) in both pH 5.5 and pH 6.5 vs. pH 8.5 conditions. As metabolic byproducts of the early inhabitants in the oral biofilm, formate and nitrite are two substrates widely available for uptake by *P. gingivalis* (Takahashi and Yamada, 1999).

Comparisons between all pH and oxygen conditions vs. pH 8.5 corroborate individual slide analyses, showing evidence for ammonia and weak base creation pathway up-regulation. Through examination of general regulation patterns in this data, many of the genes found to be up-regulated

in single slides (Table 22) are also up-regulation in overall comparisons. Those genes for metabolic pathways shown to have higher RNA abundance include PG0084, PG0343, PG1232 and PG1082, which correspond to various amino acid break down pathways (Table 21). The fermentation pathways up-regulated in pH 5.5 versus pH 8.5 conditions were not found to show the same regulation when comparing all conditions versus pH 8.5.

Other regulation

Other functional role categories also showed regulation, although no specific link between pH has been deduced. Transport and binding proteins, protein synthesis, generation of cell envelope components, transcription, purine and pyrimidine synthesis, and even proteins with unknown function were all shown to have regulation between pH conditions (Figure 5). In each of these cases few complete pathways were found to show significant regulation, making it difficult to make inferences about their role in pH stress conditions. This tells us that there is still much to contemplate on the subject of pH stress in *P. gingivalis*.

Few similarities were found between the response of *P. gingivalis* to acid stress in this study and other microarray studies comparing acidic response in *M. tuberculosis*, *S. mutans* and *H. pylori*. In each case these bacteria exhibited unique responses to acidic pH (Fisher et. al. 2002; Gong et. al., 2009; Merrel and Camilli, 2002) that differ from *P. gingivalis*. *H. pylori*, a bacteria found in the acidic spaces of the gastrointestinal tract of humans, synthesizes ammonia in order to moderate pH (Merrel and Camilli,

2002). Instead of fermentation of amino acids, it breaks down urea to form this ammonia. Fisher et. al. (2002) show up-regulation of lipid metabolism at acidic conditions in *M. tuberculosis*. No significant up-regulation of fatty acid or lipid metabolism is indicated in *P. gingivalis* at similar acidic conditions (Figure 5). In fact, opposite regulation was seen, with higher fatty acid metabolism at basic pH when compared to acidic conditions. In this case, methods of pH tolerance reflect the incredible diversity present between bacterial species.

Conclusions

When *P. gingivalis* is exposed to acidic conditions degradation of amino acids and fermentation products may lead to production of ammonia and some weak acids. Whether this results in moderation of external pH, as indicated by previous research (Takahashi et. al. 2003), is unclear, as no examination of growth medium after pH stress was undertaken. To know if this is the case, potential creation of base and weak acid should be confirmed by quantifying presence of these substances of in media after pH stress. Verification of culture medium pH and quantification of metabolites is crucial to determine if this is contributing to pH stress response. Also important would be determining the extent to which these metabolites are created by examining them across a gradient, similar to the conditions used in this experiment, to determine if a larger pH difference incurs a larger metabolic response. Limiting free substrate, such as a certain amino acid, in the media could help determine if there were a limiting point at which *P. gingivalis* could

no longer mount a pH stress response. Another potential confounding factor is the absence of other oral inhabitants in this experiment. *P. gingivalis* is one member of an incredibly diverse oral ecosystem, other bacteria present in the biofilm may aid or inhibit the ability of *P. gingivalis* to survive acidic stress.

It is also important to recognize that increase or decrease of RNA quantity between pH conditions does not necessarily translate to differences in protein synthesis or activity. Differences in RNA abundance should be confirmed with qRT-PCR. Specific primers for genes of interest, such as those in the ammonia creation pathways should be designed, and qRT-PCR conducted. After confirmation of RNA differences, further proteomic analysis, using mass spectrometry or western blotting would be helpful for discovering whether increased RNA abundance is actually shifting protein expression. Combining these methods would help identify targets that could be further characterized through the creation of a genetic mutant. *P. gingivalis* mutants unable to survive acidic or basic shifts in pH would provide more insight into what mechanisms are important to the pH stress response. By targeting genes required for survival in acidic or basic pH conditions, novel treatments for infection of *P. gingivalis* could arise.

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Table 1- Microarray wash protocol reagents

Pre-Hybridization	First Wash	Second Wash
<u>Wash I</u> 880ml dH ₂ O 100ml 20x SSC 20ml 10x SDS	20 minutes 500ml	20 minutes 500ml
<u>Wash II</u> 900ml dH ₂ O 100ml 20x SSC	15 minutes 500ml	15 minutes 500ml
<u>Wash III</u> 990ml dH ₂ O 10ml 20x SSC 25µl 1M DTT	10 minutes 500ml	10 minutes 500ml
Post-Hybridization		
<u>Wash I</u> 880ml dH ₂ O 100ml 20x SSC 20ml 10x SDS 25µl 1M DTT	20 minutes 500ml	20 minutes 500ml
<u>Wash II</u> 900ml dH ₂ O 100ml 20x SSC 25µl 1M DTT	10 minutes 500ml	10 minutes 500ml
<u>Wash III</u> 990ml dH ₂ O 10ml 20x SSC	5 minutes 500ml	5 minutes 500ml

Table 2- Total number of regulated genes in all conditions.

Anaerobic pH	Up	Down	Total
5.5/8.5	150	60	210
6.5/8.5	36	0	36
7.0/8.5	129	28	157
Microaerophilic pH	Up	Down	Total
5.5/8.5	332	160	492
6.5/8.5	77	21	98
7.0/8.5	167	66	233

Fold change = 1.5 fold up or down
Adj. P-value < 0.05

Table 3- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0269	exodeoxyribonuclease III	2.632279	6.200045	PG0350	internalin-related protein	1.260139	2.395189
PG0539	efflux transporter, MFP component, RND family	2.603031	6.075616	PG1171	oxidoreductase, putative	1.258420	2.392336
PG0538	outer membrane efflux protein	2.459847	5.501583	PG1704	thiol:disulfide interchange protein dsbD, putativ	1.257580	2.390943
PG0555	DNA-binding protein, histone-like family	2.349213	5.095463	PG1091	DHH subfamily 1 protein	1.255442	2.387402
PG0271	single-stranded binding protein	2.337888	5.055619	PG1386	DNA gyrase, A subunit	1.243283	2.367366
PG0593	htrA protein	2.226931	4.681370	PG0296	phosphoribosylformylglycinamide synthase	1.232012	2.348944
PG1327	aminotransferase, putative	2.166174	4.488317	PG1878	cysteinyl-tRNA synthetase	1.231412	2.347966
PG1060	carboxyl-terminal protease	2.003578	4.009933	PG0311	glycosyl transferase, group 2 family protein	1.224769	2.337180
PG0540	AcrB-AcrD-AcrF family protein	1.954766	3.876530	PG0674	indolepyruvate ferredoxin oxidoreductase, beta	1.224499	2.336743
PG1605	aminopeptidase C	1.909138	3.755845	PG2015	CRISPR-associated protein Cas4	1.218994	2.327844
PG1213	ribonuclease H	1.878740	3.677537	PG0715	transporter	1.207907	2.310023
PG1151	alcohol dehydrogenase, iron-containing	1.876887	3.672817	PG0945	ABC transporter, permease protein, putative	1.207011	2.308589
PG0932	DNA polymerase III, delta prime subunit, putativ	1.858883	3.627268	PG1677	phosphoglycerate kinase	1.203272	2.302612
PG1330	large conductance mechanosensitive channel γ	1.827384	3.548931	PG2215	mannose-1-phosphate guanylyltransferase	1.196216	2.291379
PG1682	glycosyl transferase, group 1 family protein	1.789705	3.457442	PG0795	membrane protein, putative	1.180932	2.267232
PG0669	heme-binding protein FetB	1.774840	3.422002	PG1975	hemagglutinin protein HagC	1.178253	2.263026
PG1497	DNA-binding protein, histone-like family	1.712158	3.276505	PG1289	GDP-fucose synthetase	1.177597	2.261997
PG0263	tyrosyl-tRNA synthetase	1.710566	3.272892	PG1826	conserved domain protein	1.161169	2.236385
PG0058	nicotinate (nicotinamide) nucleotide adenyllyltra	1.701542	3.252483	PG0151	signal recognition particle-docking protein FtsY	1.152082	2.222344
PG0293	secretion activator protein, putative	1.696991	3.242240	PG0121	DNA-binding protein HU	1.151758	2.221845
PG0279	NADP-dependent malic enzyme	1.694962	3.237683	PG1249	1-acyl-sn-glycerol-3-phosphate acetyltransferase	1.147459	2.215235
PG0134	magnesium transporter	1.683830	3.212797	PG1885	polyphosphate kinase	1.123958	2.179440
PG1241	GTP-binding protein Lepa	1.677147	3.197950	PG0589	GMP synthase	1.121882	2.176307
PG0288	lipoprotein, putative	1.628802	3.092561	PG2068	glycerol-3-phosphate cytidyltransferase	1.096477	2.138320
PG1385	TPR domain protein	1.622718	3.079548	PG2067	pyridoxal phosphate biosynthetic protein PdxA	1.081222	2.115827
PG1586	batE protein	1.593211	3.017202	PG1345	glycosyl transferase, group 1 family protein	1.076565	2.109009
PG0022	sulfate permease family protein	1.590200	3.010911	PG1821	cytochrome c nitrite reductase, small subunit N	1.075751	2.107820
PG0690	4-hydroxybutyrate CoA-transferase	1.567175	2.963239	PG1947	TPR domain protein	1.064977	2.092137
PG0677	saccharopine dehydrogenase	1.557860	2.944168	PG2221	MiaB-like tRNA modifying enzyme	1.061466	2.087051
PG0714	copper homeostasis protein CutC	1.542078	2.912137	PG0977	ubiquinone-menaquinone biosynthesis methyltr	1.060739	2.086000
PG1173	YkgG family protein	1.539992	2.907928	PG1566	glutamyl-tRNA synthetase	1.060512	2.085671
PG1551	hmuY protein	1.516601	2.861161	PG2069	oxidoreductase, short chain dehydrogenase-re	1.047231	2.066560
PG2150	LysM domain protein	1.500887	2.830167	PG0629	ATP-NAD kinase	1.036221	2.050849
PG1949	malate dehydrogenase	1.499812	2.828058	PG1232	glutamate dehydrogenase, NAD-specific	1.015677	2.021852
PG2047	helicase, putative	1.463236	2.757262	PG0020	transcriptional regulator, MarR family	1.009387	2.013056
PG0691	NifU-related protein	1.447921	2.728146	PG0138	malonyl CoA-acyl carrier protein transacylase	1.000045	2.000062
PG0978	shikimate 5-dehydrogenase	1.439868	2.712960	PG1748	transketolase	0.997217	1.996145
PG1408	heavy metal efflux pump, CzcD family	1.437046	2.707660	PG1346	glycosyl transferase, group 1 family protein	0.977478	1.969020
PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosphate sy	1.435554	2.704860	PG1140	glycosyl transferase, group 2 family protein	0.971065	1.960288
PG0639	signal peptide peptidase SppA, 67K type	1.401799	2.642309	PG0692	4-hydroxybutyryl-CoA dehydratase	0.965250	1.952401
PG2218	potassium uptake protein TrkA	1.373224	2.590488	PG1604	immunoreactive 84 kDa antigen PG93	0.960641	1.946174
PG1072	MutS family protein	1.366950	2.579247	PG1589	dihydropteroate synthase	0.947751	1.928863
PG0249	oxaloacetate decarboxylase, putative	1.307025	2.474308	PG0363	conserved domain protein	0.919632	1.891633
PG0192	cationic outer membrane protein OmpH	1.304150	2.469382	PG0418	ATP-dependent Clp protease, proteolytic subur	0.913399	1.883477
PG1755	fructose-bisphosphate aldolase, class I	1.303648	2.468523	PG0583	cell division protein FtsA	0.910348	1.879499
PG0468	mannose-6-phosphate isomerase, class I	1.282876	2.433236	PG1792	sodium-hydrogen antiporter	0.909484	1.878373
PG1355	acyltransferase, putative	1.274851	2.419738	PG2023	methionyl-tRNA formyltransferase	0.906119	1.873998
PG1210	peptidase, M24 family	1.264131	2.401824	PG0708	peptidyl-prolyl cis-trans isomerase, FKBP-type	0.902529	1.869340

Table 3- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG1127	transcriptional regulator, AsnC Family	0.902009	1.868666	PG0360	lemA protein	0.749046	1.680681
PG0449	TPR domain protein	0.897331	1.862618	PG1235	epimerase-reductase, putative	0.740772	1.671070
PG1877	Na ⁺ -H ⁺ antiporter	0.887458	1.849914	PG0709	peptidyl-prolyl cis-trans isomerase FkpA, FKBP	0.738336	1.668251
PG0976	phosphoribosylaminoimidazole-succinocarboxamide	0.876747	1.836230	PG1816	NAD(P)H dehydrogenase, quinone family, putative	0.735899	1.665435
PG1772	endonuclease III	0.872138	1.830374	PG0758	peptidyl-dipeptidase Dcp	0.730668	1.659407
PG1880	glycosyl transferase, group 2 family protein	0.863188	1.819053	PG0754	DNA topoisomerase I	0.723347	1.651008
PG0951	phosphoribosylaminoimidazole carboxylase, P1	0.855385	1.809241	PG1315	peptidyl-prolyl cis-trans isomerase SlyD, FKBP	0.722232	1.649732
PG1097	Mur ligase domain protein-alanine racemase	0.845269	1.796600	PG0030	cytidine deaminase	0.695616	1.619576
PG0321	LAO-AO transport system ATPase	0.844500	1.795642	PG0689	NAD-dependent 4-hydroxybutyrate dehydrogenase	0.695202	1.619111
PG1998	polyprenyl synthetase	0.844140	1.795194	PG0264	glycosyl transferase, group 2 family protein	0.692012	1.615535
PG0357	aspartate carbamoyltransferase, catalytic subunit	0.838913	1.788703	PG1656	methylmalonyl-CoA mutase, small subunit	0.689030	1.612200
PG0223	exonuclease	0.836080	1.785193	PG0634	ThiJ-PfpI family protein	0.684434	1.607071
PG1579	ATPase, MoxR family	0.828322	1.775619	PG0045	heat shock protein HtpG	0.649172	1.568268
PG1576	L-aspartate oxidase	0.824496	1.770916	PG1565	3-deoxy-D-manno-octulosonic-acid transferase	0.644908	1.563639
PG0055	conserved domain protein	0.824413	1.770814	PG2178	NADH:ubiquinone oxidoreductase, Na translocase	0.631278	1.548936
PG2124	glyceraldehyde 3-phosphate dehydrogenase, type 1	0.823649	1.769877	PG2090	cation efflux family protein	0.626718	1.544048
PG1172	iron-sulfur cluster binding protein, putative	0.822379	1.768320	PG1354	hydrolase, carbon-nitrogen family	0.614598	1.531132
PG0500	queuine tRNA-ribosyltransferase	0.821589	1.767351	PG2207	conserved domain protein	0.610175	1.526445
PG2085	tryptophanyl-tRNA synthetase	0.808238	1.751071	PG0135	dimethyladenosine transferase	0.608719	1.524905
PG0070	acyl-(acyl-carrier-protein)-UDP-N-acetylglucosamine 4-epimerase	0.792800	1.732434	PG1720	conserved domain protein	0.607130	1.523226
PG1608	methylmalonyl-CoA decarboxylase, beta subunit	0.789813	1.728850	PG1609	methylmalonyl-CoA decarboxylase, gamma subunit	0.605001	1.520980
PG1174	thioesterase family protein	0.789134	1.728037	PG0209	formate-nitrite transporter	0.602756	1.518615
PG1898	transporter, putative	0.787937	1.726604	PG0270	redox-sensitive transcriptional activator OxyR	0.599542	1.515235
PG1116	methylenetetrahydrofolate dehydrogenase-methylenetetrahydrofolate reductase	0.785955	1.724233	PG0791	adenylate kinase	0.597522	1.513115
PG0477	pantoate--beta-alanine ligase	0.774567	1.710677	PG2177	NADH:ubiquinone oxidoreductase, Na translocase	0.594246	1.509684
PG0658	phosphatase, YrbI family	0.772730	1.708500	PG0660	nitroreductase family protein	0.593965	1.509389
PG2190	cell-division ATP-binding protein	0.771340	1.706854	PG2147	xanthine phosphoribosyltransferase	0.585915	1.500990

* = Gene ID according to JCVI

** = log(spot intensity pH 5.5/pH 8.5)

*** = fold change (ratio of pH 5.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 4- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG1156	S4 domain protein	-1.005727	0.498019	PG1109	mobilization protein	-1.410487	0.376185
PG1592	HDIG domain protein	-1.006737	0.497671	PG0775	acyl-CoA dehydrogenase family protein	-1.411506	0.375919
PG0425	esterase, putative	-1.010851	0.496253	PG0776	electron transfer flavoprotein, alpha subunit	-1.421737	0.373263
PG1564	membrane protein, putative	-1.022407	0.492294	PG1539	tRNA pseudouridine synthase B	-1.437442	0.369221
PG0398	recF protein	-1.060924	0.479325	PG1765	acyl carrier protein	-1.444700	0.367369
PG2001	signal peptidase I	-1.079095	0.473326	PG0037	ribosomal protein L19	-1.460806	0.363290
PG0120	UDP-N-acetylglucosamine 2-epimerase	-1.083911	0.471748	PG0490	membrane protein, putative	-1.485945	0.357015
PG1428	6,7-dimethyl-8-ribityllumazine synthase	-1.113693	0.462110	PG0726	lipoprotein, putative	-1.518908	0.348950
PG0086	ATP-dependent RNA helicase, DEAD-DEAH bc	-1.118223	0.460661	PG2102	immunoreactive 61 kDa antigen PG91	-1.524925	0.347498
PG1543	thioesterase family protein	-1.140262	0.453677	PG0219	conserved domain protein	-1.550493	0.341393
PG1010	ABC transporter, ATP-binding protein	-1.160965	0.447213	PG0777	electron transfer flavoprotein, beta subunit	-1.555688	0.340166
PG1130	TPR domain protein	-1.167169	0.445294	PG0720	DNA-binding response regulator	-1.556160	0.340055
PG0332	transcription termination factor Rho	-1.182220	0.440673	PG1039	integral membrane protein	-1.556707	0.339926
PG0110	glycosyl transferase, group 1 family protein	-1.199812	0.435332	PG1907	ISPg3, transposase, interruption	-1.562048	0.338670
PG0063	outer membrane efflux protein	-1.205135	0.433729	PG0118	glycosyl transferase, group 2 family protein	-1.573822	0.335917
PG1680	ABC transporter, ATP-binding protein, authentic	-1.225414	0.427675	PG1238	ribosomal large subunit pseudouridine synthase	-1.578823	0.334755
PG1418	DNA polymerase III, gamma and tau subunits	-1.231174	0.425971	PG0383	membrane-associated zinc metalloprotease, putative	-1.580787	0.334300
PG0721	NLP-P60 family protein	-1.249839	0.420495	PG1697	type II restriction endonuclease, putative	-1.595447	0.330920
PG0385	ribosomal protein S21	-1.258642	0.417937	PG1180	membrane protein, putative	-1.647838	0.319118
PG1434	4-diphosphocytidyl-2C-methyl-D-erythritol synthase	-1.259752	0.417616	PG1504	NAD dependent protein	-1.648167	0.319045
PG1622	DNA topoisomerase IV, A subunit, putative	-1.262364	0.416860	PG2056	transposase, ISPg2-related, truncation	-1.657884	0.316904
PG1542	collagenase	-1.278611	0.412192	PG1240	transcriptional regulator, tetR family	-1.673625	0.313465
PG1254	acetyltransferase, GNAT family	-1.286723	0.409881	PG2169	ISPg1, transposase, degenerate	-1.706463	0.306410
PG1395	cell shape-determining protein MreC, putative	-1.297244	0.406903	PG0064	heavy metal efflux pump, CzcA family	-1.827620	0.281729
PG1239	3-oxoacyl-(acyl-carrier-protein) reductase	-1.309817	0.403372	PG2213	nitrite reductase-related protein	-1.893407	0.269171
PG1758	ribosomal protein S15	-1.317300	0.401285	PG1144	peptide chain release factor 2, programmed frame shift	-1.914759	0.265216
PG0378	translation elongation factor Ts	-1.333644	0.396765	PG1500	conserved domain protein	-1.983839	0.252816
PG1255	recombination protein RecR	-1.335518	0.396250	PG1959	ribosomal protein L33	-2.052019	0.241146
PG0487	ISPg4, transposase	-1.350223	0.392231	PG0619	alkyl hydroperoxide reductase, F subunit	-2.247086	0.210649
PG1427	thiol protease-hemagglutinin PrtT precursor, putative	-1.373934	0.385838	PG1421	ferredoxin, 4Fe-4S	-2.295074	0.203758
PG0399	lipoprotein, putative	-1.399992	0.378931				

* = Gene ID according to JCVI

** = log(spot intensity pH 5.5/pH 8.5)

*** = fold change (ratio of pH 5.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 5- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 6.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0692	4-hydroxybutyryl-CoA dehydratase	2.068844	4.195504	PG1290	branched-chain amino acid aminotransferase	1.231308	2.347797
PG0555	DNA-binding protein, histone-like family	1.923290	3.792870	PG0310	nitroreductase family protein	1.216518	2.323852
PG2145	polysaccharide deacetylase	1.848046	3.600123	PG1115	signal recognition particle protein	1.201490	2.299771
PG1116	methylenetetrahydrofolate dehydrogenase-met	1.774236	3.420568	PG1656	methylmalonyl-CoA mutase, small subunit	1.113610	2.163864
PG0945	ABC transporter, permease protein, putative	1.691009	3.228826	PG0162	RNA polymerase sigma-70 factor, ECF subfam	1.101844	2.146289
PG0695	immunoreactive 43 kDa antigen PG32	1.632985	3.101540	PG1927	ribosomal protein L24	1.053792	2.075980
PG1912	ribosomal protein S4	1.620473	3.074759	PG1946	ABC 3 transporter family protein	1.023978	2.033518
PG0387	translation elongation factor Tu	1.576683	2.982832	PG1921	ribosomal protein S5	0.973494	1.963590
PG0430	oxidoreductase, putative	1.575246	2.979863	PG1901	ribosome recycling factor	0.973213	1.963208
PG2215	mannose-1-phosphate guanylyltransferase	1.540286	2.908521	PG1812	2-oxoglutarate oxidoreductase, alpha subunit	0.940131	1.918702
PG1922	ribosomal protein L18	1.456689	2.744777	PG1858	flavodoxin	0.919985	1.892095
PG1913	ribosomal protein S11	1.454539	2.740690	PG0304	electron transport complex, RnfABCDGE type,	0.911135	1.880525
PG0708	peptidyl-prolyl cis-trans isomerase, FKBP-type	1.448195	2.728664	PG0906	lipoprotein, putative	0.884651	1.846317
PG1911	DNA-directed RNA polymerase, alpha subunit	1.438258	2.709934	PG0754	DNA topoisomerase I	0.861853	1.817370
PG0377	ribosomal protein S2	1.359775	2.566452	PG0785	tonB protein, putative	0.830250	1.777994
PG1816	NAD(P)H dehydrogenase, quinone family, puta	1.331868	2.517285	PG1421	ferredoxin, 4Fe-4S	0.743694	1.674457
PG0042	serine hydroxymethyltransferase	1.286007	2.438522	PG1933	ribosomal protein L22	0.740460	1.670709
PG1939	ribosomal protein S10	1.234880	2.353618	PG0307	electron transport complex, RnfABCDGE type,	0.622916	1.539985

* = Gene ID according to JCVI

** = log(spot intensity pH 6.5/pH 8.5)

*** = fold change (ratio of pH 6.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 6- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0090	Dps family protein	1.984027	3.955958	PG1949	malate dehydrogenase	0.898195	1.863733
PG1151	alcohol dehydrogenase, iron-containing	1.802643	3.488588	PG1763	ribonuclease III	0.881122	1.841808
PG0978	shikimate 5-dehydrogenase	1.698906	3.246546	PG0430	oxidoreductase, putative	0.862265	1.817890
PG0708	peptidyl-prolyl cis-trans isomerase, FKBP	1.548927	2.925994	PG0935	4-diphosphocytidyl-2C-methyl-D-erythrit	0.850025	1.802532
PG0271	single-stranded binding protein	1.536374	2.900645	PG0935	4-diphosphocytidyl-2C-methyl-D-erythrit	0.850025	1.802532
PG0687	succinate-semialdehyde dehydrogenase	1.486695	2.802463	PG2069	oxidoreductase, short chain dehydrogena	0.847804	1.799759
PG1858	flavodoxin	1.427555	2.689904	PG0597	ribosomal protein L9	0.843710	1.794659
PG1981	CRISPR-associated protein Cas2	1.282916	2.433303	PG0500	queuine tRNA-ribosyltransferase	0.833831	1.782412
PG1982	CRISPR-associated protein Cas1	1.263480	2.400742	PG1364	1-deoxy-D-xylulose 5-phosphate reducto	0.822133	1.768019
PG1950	membrane protein, putative	1.258954	2.393222	PG1364	1-deoxy-D-xylulose 5-phosphate reducto	0.822133	1.768019
PG0691	NifU-related protein	1.250038	2.378477	PG0138	malonyl CoA-acyl carrier protein transacy	0.817970	1.762923
PG0185	ragA protein	1.236753	2.356675	PG0199	TatD family protein	0.815642	1.760082
PG1345	glycosyl transferase, group 1 family prote	1.234736	2.353383	PG2068	glycerol-3-phosphate cytidyltransferase	0.809367	1.752442
PG1633	galactokinase	1.209247	2.312169	PG0363	conserved domain protein	0.807320	1.749957
PG2024	hemagglutinin protein HagE	1.187220	2.277135	PG1232	glutamate dehydrogenase, NAD-specific	0.795796	1.736035
PG1709	ISPg5, transposase Orf1	1.158053	2.231561	PG0387	translation elongation factor Tu	0.783940	1.721826
PG2145	polysaccharide deacetylase	1.156143	2.228608	PG1946	ABC 3 transporter family protein	0.783748	1.721597
PG1998	polyprenyl synthetase	1.147540	2.215358	PG1946	ABC 3 transporter family protein	0.783748	1.721597
PG0555	DNA-binding protein, histone-like family	1.133095	2.193288	PG0618	alkyl hydroperoxide reductase, C subunit	0.781264	1.718636
PG0173	transcriptional regulator, putative	1.132525	2.192421	PG0945	ABC transporter, permease protein, putal	0.771605	1.707168
PG0186	lipoprotein RagB	1.104675	2.150504	PG0945	ABC transporter, permease protein, putal	0.771605	1.707168
PG0635	ribosomal protein L11 methyltransferase	1.102128	2.146712	PG1521	O-succinylbenzoic acid--CoA ligase	0.764707	1.699025
PG0619	alkyl hydroperoxide reductase, F subunit	1.096755	2.138732	PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosph	0.762126	1.695988
PG0309	thiamine biosynthesis lipoprotein ApbE	1.078677	2.112099	PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosph	0.762126	1.695988
PG0311	glycosyl transferase, group 2 family prote	1.074918	2.106602	PG1249	1-acyl-sn-glycerol-3-phosphate acetyltra	0.759560	1.692974
PG0121	DNA-binding protein HU	1.073284	2.104218	PG1249	1-acyl-sn-glycerol-3-phosphate acetyltra	0.759560	1.692974
PG0497	5-methylthioadenosine-S-adenosylhomoc	1.068659	2.097483	PG0462	transporter, putative	0.754918	1.687536
PG0497	5-methylthioadenosine-S-adenosylhomoc	1.068659	2.097483	PG0709	peptidyl-prolyl cis-trans isomerase FkpA,	0.746538	1.677762
PG1346	glycosyl transferase, group 1 family prote	1.067556	2.095880	PG1880	glycosyl transferase, group 2 family prote	0.742152	1.672669
PG1704	thiol:disulfide interchange protein dsbD, I	1.067180	2.095333	PG0307	electron transport complex, RnfABCDGE t	0.741390	1.671786
PG1901	ribosome recycling factor	1.043375	2.061044	PG1664	ABC transporter, permease protein, putal	0.738108	1.667987
PG1338	umuC protein	1.030574	2.042837	PG1664	ABC transporter, permease protein, putal	0.738108	1.667987
PG0932	DNA polymerase III, delta prime subunit,	1.002840	2.003942	PG1754	conserved domain protein	0.734021	1.663268
PG1898	transporter, putative	0.990168	1.986416	PG1132	valyl-tRNA synthetase	0.729276	1.657806
PG0226	transglutaminase-related protein	0.983561	1.977340	PG1847	endoribonuclease L-PSP, putative	0.725491	1.653463
PG1807	v-type ATPase, subunit K	0.982538	1.975938	PG1163	cobyrinic acid a,c-diamide synthase	0.722971	1.650578
PG0977	ubiquinone-menaquinone biosynthesis m	0.977225	1.968674	PG0128	conserved domain protein	0.721215	1.648570
PG1089	DNA-binding response regulator RprY	0.976930	1.968272	PG1097	Mur ligase domain protein-alanine racem	0.718047	1.644954
PG0158	competence protein F-related protein	0.976781	1.968070	PG1084	thioredoxin family protein	0.714322	1.640712
PG0726	lipoprotein, putative	0.969970	1.958799	PG1119	flavodoxin, putative	0.711019	1.636960
PG1984	CRISPR-associated protein, TM1791.1 far	0.962404	1.948554	PG0464	adenylosuccinate synthetase	0.709977	1.635778
PG2015	CRISPR-associated protein Cas4	0.958123	1.942781	PG1082	phosphotransacetylase	0.703850	1.628846
PG0453	conserved domain protein	0.948530	1.929905	PG0951	phosphoribosylaminoimidazole carboxyla	0.702019	1.626780
PG0906	lipoprotein, putative	0.933221	1.909535	PG2033	glutamate synthase, small subunit	0.699404	1.623834
PG0269	exodeoxyribonuclease III	0.918329	1.889926	PG1385	TPR domain protein	0.694571	1.618403
PG0223	exonuclease	0.910055	1.879117	PG0058	nicotinate (nicotinamide) nucleotide aden	0.693347	1.617030
PG1159	cobalamin biosynthesis protein CbiB	0.900800	1.867101	PG0390	ribosomal protein L11	0.690763	1.614137
PG0393	ribosomal protein L7-L12	0.900400	1.866583	PG1884	alpha-L-fucosidase precursor, putative	0.687635	1.610641

Table 6- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0022	sulfate permease family protein	0.684739	1.607411	PG1589	dihydropteroate synthase	0.621229	1.538185
PG0383	membrane-associated zinc metalloprotea	0.683500	1.606032	PG0703	cobalamin (5-phosphate) synthase, putat	0.618616	1.535401
PG0629	ATP-NAD kinase	0.682321	1.604719	PG2000	signal peptidase-related protein	0.618291	1.535056
PG0953	deoxyuridine 5-triphosphate nucleotidohy	0.678916	1.600936	PG0192	cationic outer membrane protein OmpH	0.617812	1.534546
PG1276	DNA-binding protein, histone-like family	0.676037	1.597744	PG1772	endonuclease III	0.617235	1.533933
PG0936	xanthine-uracil permease family protein	0.674880	1.596464	PG1948	lipoprotein, putative	0.616028	1.532650
PG1935	ribosomal protein L2	0.664206	1.584696	PG1162	ATP:cob(I)alamin adenosyltransferase, pi	0.615544	1.532136
PG1934	ribosomal protein S19	0.660879	1.581045	PG0532	conserved domain protein	0.609144	1.525354
PG0324	histidine ammonia-lyase	0.655235	1.574873	PG0900	cytochrome d ubiquinol oxidase, subunit	0.607734	1.523864
PG0628	ABC transporter, ATP-binding protein	0.652550	1.571944	PG0392	ribosomal protein L10	0.605251	1.521243
PG0628	ABC transporter, ATP-binding protein	0.652550	1.571944	PG1609	methylmalonyl-CoA decarboxylase, gamn	0.603910	1.519830
PG1593	shikimate kinase	0.649828	1.568981	PG2071	conserved domain protein	0.598946	1.514610
PG1705	ribosomal large subunit pseudouridine sy	0.637338	1.555457	PG0990	ribosomal protein L35	0.598914	1.514576
PG0134	magnesium transporter	0.635660	1.553648	PG0073	orotidine 5-monophosphate decarboxylas	0.594301	1.509741
PG2147	xanthine phosphoribosyltransferase	0.632828	1.550601	PG0752	uracil phosphoribosyltransferase, putative	0.590939	1.506227
PG0249	oxaloacetate decarboxylase, putative	0.625090	1.542307	PG0148	sigma-54-dependent transcriptional regu	0.588685	1.503876
PG0754	DNA topoisomerase I	0.621324	1.538286				

* = Gene ID according to JCVI

** = log(spot intensity pH 7.0/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 7- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in anaerobic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG2020	CRISPR-associated protein, TM1814 fami	-1.025554	0.491222	PG1286	ferritin	-1.304432	0.404880
PG1638	thioredoxin family protein	-1.038970	0.486675	PG1203	transcriptional regulator, putative	-1.339024	0.395288
PG0214	RNA polymerase sigma-70 factor, ECF su	-1.057230	0.480554	PG0178	cell surface protein, interruption-C	-1.341663	0.394566
PG1258	DNA-binding protein HU	-1.059848	0.479683	PG1144	peptide chain release factor 2, programm	-1.370740	0.386693
PG0461	ISPg7, transposase	-1.116346	0.461260	PG1541	2-amino-4-hydroxy-6-hydroxymethylidih	-1.397298	0.379639
PG0946	ABC transporter, ATP-binding protein	-1.122026	0.459448	PG1541	2-amino-4-hydroxy-6-hydroxymethylidih	-1.397298	0.379639
PG0946	ABC transporter, ATP-binding protein	-1.122026	0.459448	PG2198	hemagglutinin protein, truncation	-1.423285	0.372862
PG0024	DNA-binding protein, putative	-1.126995	0.457868	PG0432	NOL1-NOP2-sun family protein	-1.438634	0.368916
PG2205	2-dehydropantoate 2-reductase	-1.129183	0.457175	PG1642	cation-transporting ATPase, EI-E2 family,	-1.519672	0.348765
PG2205	2-dehydropantoate 2-reductase	-1.129183	0.457175	PG0721	NLP-P60 family protein	-1.553355	0.340717
PG1798	immunoreactive 46 kDa antigen PG99	-1.146757	0.451639	PG1641	phosphotyrosine protein phosphatase	-1.879929	0.271697
PG0010	ATP-dependent Clp protease, ATP-binding	-1.235554	0.424679	PG2213	nitrite reductase-related protein	-2.045035	0.242317
PG0386	site-specific recombinase, phage integras	-1.281636	0.411329	PG0576	UDP-N-acetylmuramoylalanyl-D-glutamyl	-2.852049	0.138499
PG1828	lipoprotein, putative	-1.281771	0.411290	PG1421	ferredoxin, 4Fe-4S	-3.649059	0.079712
PG0006	MATE efflux family protein	-1.291375	0.408561				

* = Gene ID according to JCVI

** = log(spot intensity pH 7.0/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 8- Genes Upregulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0209	formate-nitrite transporter	3.379221	10.405116	PG1762	protein-export membrane protein SecD-p	1.544250	2.916525
PG0754	DNA topoisomerase I	3.069162	8.392860	PG0383	membrane-associated zinc metalloprotea	1.529130	2.886118
PG0595	ribosomal protein S6	2.963050	7.797707	PG1101	sodium:solute symporter family protein	1.510995	2.850065
PG0589	GMP synthase	2.941466	7.681916	PG0977	ubiquinone-menaquinone biosynthesis m	1.480176	2.789827
PG0946	ABC transporter, ATP-binding protein	2.730474	6.636734	PG2033	glutamate synthase, small subunit	1.450417	2.732871
PG1656	methylmalonyl-CoA mutase, small subuni	2.711097	6.548195	PG1855	carboxyl-terminal protease	1.444400	2.721497
PG0196	peptidase, M16 family	2.670012	6.364343	PG1848	RNA methyltransferase, TrmH family	1.440124	2.713442
PG0766	polyribonucleotide nucleotidyltransferase	2.587207	6.009342	PG0395	DNA-directed RNA polymerase, beta subu	1.437058	2.707681
PG1747	ribose 5-phosphate isomerase B, putative	2.578092	5.971494	PG1105	RNA polymerase sigma-54 factor	1.429912	2.694303
PG1801	v-type ATPase, subunit E, putative	2.521577	5.742096	PG0807	NusB family protein	1.419028	2.674052
PG1794	DNA polymerase type I	2.438530	5.420891	PG0530	carbamoyl-phosphate synthase, large sul	1.415661	2.667820
PG0951	phosphoribosylaminoimidazole carboxyla	2.383334	5.217410	PG1918	preprotein translocase, SecY subunit	1.401342	2.641472
PG0072	UDP-3-O-[3-hydroxymyristoyl] glucosam	2.188176	4.557288	PG1160	L-threonine-O-3-phosphate decarboxylas	1.393160	2.626533
PG0691	NifU-related protein	2.103146	4.296452	PG0708	peptidyl-prolyl cis-trans isomerase, FKBP	1.390312	2.621353
PG2096	conserved domain protein	2.078412	4.223420	PG1321	formate--tetrahydrofolate ligase	1.360515	2.567768
PG2068	glycerol-3-phosphate cytidyltransferase	2.066947	4.189991	PG1806	v-type ATPase, subunit I	1.360154	2.567125
PG0173	transcriptional regulator, putative	2.010648	4.029631	PG0584	cell division protein FtsZ	1.356466	2.560573
PG0070	acyl-(acyl-carrier-protein)-UDP-N-acetylgl	1.985245	3.959299	PG0079	abortive infection protein, putative	1.354407	2.556920
PG1842	acetyltransferase, GNAT family	1.877785	3.675104	PG2199	ABC transporter, ATP-binding protein, pu	1.353863	2.555957
PG0707	TonB-dependent receptor, putative	1.874133	3.665812	PG0636	MATE efflux family protein	1.344257	2.538994
PG0991	translation initiation factor IF-3	1.872462	3.661570	PG0690	4-hydroxybutyrate CoA-transferase	1.337310	2.526798
PG0724	prolyl oligopeptidase family protein	1.865996	3.645195	PG1025	conserved domain protein	1.324645	2.504712
PG0936	xanthine-uracil permease family protein	1.840651	3.581715	PG1704	thiol:disulfide interchange protein dsbD, i	1.319643	2.496044
PG0429	pyruvate synthase	1.822971	3.538090	PG0258	ABC transporter, ATP-binding protein	1.318146	2.493455
PG1043	ferrous iron transport protein B	1.805126	3.494597	PG1343	lipoate-protein ligase B	1.312290	2.483353
PG0571	aspartate-semialdehyde dehydrogenase	1.790429	3.459177	PG0503	dipeptidyl aminopeptidase IV	1.299821	2.461983
PG0211	cobalamin biosynthesis protein CbiG-prec	1.774703	3.421676	PG2060	thymidylate synthase	1.299021	2.460619
PG1559	glycine cleavage system T protein	1.768813	3.407735	PG1975	hemagglutinin protein HagC	1.287007	2.440213
PG0962	prolyl-tRNA synthetase	1.763909	3.396170	PG1960	ribosomal protein L28	1.282365	2.432373
PG0796	leucyl-tRNA synthetase	1.756088	3.377810	PG0176	cell surface protein, interruption-N	1.279898	2.428219
PG0106	glycosyl transferase, group 4 family prote	1.732774	3.323662	PG0783	hydrolase, putative	1.270492	2.412438
PG2219	potassium uptake protein TrkH	1.705113	3.260545	PG0307	electron transport complex, RnfABCDGE t	1.258381	2.392272
PG0687	succinate-semialdehyde dehydrogenase	1.692343	3.231812	PG2094	conserved domain protein	1.254871	2.386458
PG0699	maltodextrin phosphorylase	1.689874	3.226284	PG1858	flavodoxin	1.248668	2.376220
PG0054	single-stranded-DNA-specific exonuclease	1.679320	3.202771	PG0305	electron transport complex, RnfABCDGE t	1.248173	2.375405
PG1023	para-aminobenzoate synthase, componer	1.661928	3.164390	PG0035	DNA polymerase III, alpha subunit	1.245440	2.370909
PG1424	peptidylarginine deiminase	1.644583	3.126575	PG0205	peptide chain release factor 3	1.240456	2.362732
PG1428	6,7-dimethyl-8-ribityllumazine synthase	1.643315	3.123827	PG0288	lipoprotein, putative	1.239710	2.361511
PG1346	glycosyl transferase, group 1 family prote	1.635441	3.106824	PG0579	cell division protein FtsW, putative	1.219282	2.328309
PG0300	TPR domain protein	1.608073	3.048445	PG1328	CoA ligase family protein	1.218428	2.326930
PG0086	ATP-dependent RNA helicase, DEAD-DEAI	1.601725	3.035061	PG1565	3-deoxy-D-manno-octulosonic-acid trans	1.212236	2.316964
PG0500	queueine tRNA-ribosyltransferase	1.600592	3.032677	PG1937	ribosomal protein L4	1.205760	2.306587
PG2047	helicase, putative	1.597928	3.027083	PG0553	extracellular protease, putative	1.202217	2.300930
PG1926	ribosomal protein L5	1.587920	3.006155	PG0965	phosphatidylserine decarboxylase-related	1.201085	2.299125
PG0271	single-stranded binding protein	1.574013	2.977318	PG0635	ribosomal protein L11 methyltransferase	1.197880	2.294023
PG0529	carbamoyl-phosphate synthase, small sul	1.561646	2.951904	PG1927	ribosomal protein L24	1.196504	2.291836
PG0159	endopeptidase PepO	1.548157	2.924432	PG1084	thioredoxin family protein	1.193331	2.286801
PG0573	S-adenosyl-methyltransferase MraW	1.547660	2.923425	PG0935	4-diphosphocytidyl-2C-methyl-D-erythrit	1.190111	2.281704

Table 8- Genes Upregulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

Continued from previous page...

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0435	capsular polysaccharide biosynthesis prote	1.185076	2.273754	PG1780	8-amino-7-oxononanoate synthase	0.969609	1.958310
PG0964	CDP-diacylglycerol--serine O-phosphatidy	1.178943	2.264108	PG1374	immunoreactive 47 kDa antigen PG97	0.967164	1.954994
PG2090	cation efflux family protein	1.178605	2.263579	PG1723	ribosomal protein S20	0.966869	1.954594
PG1082	phosphotransacetylase	1.165656	2.243352	PG1912	ribosomal protein S4	0.966792	1.954489
PG1551	hmuY protein	1.155121	2.227029	PG1803	v-type ATPase, subunit A	0.966155	1.953627
PG0795	membrane protein, putative	1.150105	2.219300	PG1176	ABC transporter, ATP-binding protein, pu	0.965636	1.952924
PG1408	heavy metal efflux pump, CzcD family	1.149771	2.218787	PG0594	RNA polymerase sigma-70 factor	0.964608	1.951533
PG0526	membrane protein, putative	1.144358	2.210478	PG0978	shikimate 5-dehydrogenase	0.963372	1.949862
PG0976	phosphoribosylaminoimidazole-succinoca	1.141959	2.206805	PG0762	trigger factor, putative	0.959706	1.944913
PG0329	formiminotransferase-cyclodeaminase-rel	1.129483	2.187804	PG0616	thioredoxin, putative	0.958432	1.943196
PG1595	ribulose-phosphate 3-epimerase	1.126342	2.183045	PG2177	NADH:ubiquinone oxidoreductase, Na tra	0.958021	1.942643
PG0548	pyruvate ferredoxin-flavodoxin oxidoredu	1.122720	2.177572	PG1306	metallo-beta-lactamase family protein	0.946877	1.927695
PG1430	TPR domain protein	1.120296	2.173915	PG1305	glycine cleavage system P protein	0.938643	1.916725
PG1914	ribosomal protein S13	1.104026	2.149537	PG1965	voltage gated chloride channel, authentic	0.936768	1.914235
PG0143	hydrolase, carbon-nitrogen family	1.103802	2.149204	PG0444	oligopeptide transporter, OPT family	0.936604	1.914017
PG2218	potassium uptake protein TrkA	1.102583	2.147388	PG1638	thioredoxin family protein	0.931488	1.907242
PG1928	ribosomal protein L14	1.096404	2.138211	PG0593	htrA protein	0.928099	1.902767
PG0436	capsular polysaccharide transport protein	1.095217	2.136452	PG0091	transporter, putative	0.923257	1.896391
PG0430	oxidoreductase, putative	1.090896	2.130063	PG1899	TonB-dependent receptor, putative	0.922516	1.895418
PG0352	sialidase, putative	1.090856	2.130004	PG0343	methionine gamma-lyase	0.921166	1.893646
PG1936	ribosomal protein L23	1.088667	2.126774	PG1072	MutS family protein	0.916232	1.887179
PG0483	kinase, putative	1.088062	2.125882	PG1081	acetate kinase	0.916149	1.887071
PG1759	adhesion protein, putative	1.060563	2.085745	PG0110	glycosyl transferase, group 1 family prote	0.915587	1.886336
PG1541	2-amino-4-hydroxy-6-hydroxymethylidihy	1.056494	2.079871	PG0084	L-serine dehydratase, iron-sulfur-depend	0.902193	1.868904
PG0664	oxidoreductase, Gfo-Idh-MocA family	1.049825	2.070279	PG1129	ribonucleotide reductase	0.902181	1.868889
PG0279	NADP-dependent malic enzyme	1.049678	2.070068	PG0412	DNA mismatch repair protein MutL	0.901126	1.867523
PG1923	ribosomal protein L6	1.045315	2.063816	PG1155	ADP-heptose--LPS heptosyltransferase, p	0.900849	1.867165
PG1956	4-hydroxybutyrate CoA-transferase	1.037061	2.052043	PG0784	polyprenyl synthetase	0.898542	1.864181
PG0401	KH-HDIG domain protein	1.035771	2.050210	PG2200	TPR domain protein	0.897456	1.862778
PG0989	ribosomal protein L20	1.034767	2.048783	PG1922	ribosomal protein L18	0.895102	1.859741
PG0334	glycosyl transferase, group 2 family prote	1.028019	2.039222	PG0453	conserved domain protein	0.889122	1.852049
PG0709	peptidyl-prolyl cis-trans isomerase FkpA,	1.021473	2.029991	PG1834	glycogen synthase-related protein	0.876357	1.835733
PG1099	glucokinase regulator-related protein	1.021385	2.029867	PG1986	CRISPR-associated protein, TM1793 fami	0.873211	1.831736
PG1138	pigmentation and extracellular proteinase	1.015792	2.022012	PG0475	oxygen-independent coproporphyrinogen	0.871072	1.829022
PG1775	grpE protein	1.009334	2.012982	PG0982	TPR domain protein	0.867844	1.824934
PG1028	TPR domain protein	1.007642	2.010623	PG0394	DNA-directed RNA polymerase, beta subu	0.860643	1.815848
PG0263	tyrosyl-tRNA synthetase	1.005244	2.007284	PG0213	precorrin-3 methylase-precorrin-8X meth	0.860088	1.815148
PG0416	ATP-dependent DNA helicase RecQ	1.004523	2.006280	PG1920	ribosomal protein L30	0.859822	1.814814
PG2146	conserved hypothetical protein	0.998623	1.998092	PG1852	exonuclease	0.859612	1.814550
PG1255	recombination protein RecR	0.993525	1.991044	PG1542	collagenase	0.858523	1.813181
PG0202	uroporphyrinogen-III synthase HemD, pu	0.992177	1.989185	PG0588	3-methyl-2-oxobutanoate hydroxymethyl	0.856138	1.810185
PG1241	GTP-binding protein Lepa	0.988018	1.983458	PG0525	CTP synthase	0.854988	1.808744
PG0134	magnesium transporter	0.986126	1.980859	PG0264	glycosyl transferase, group 2 family prote	0.850823	1.803529
PG0596	ribosomal protein S18	0.985210	1.979602	PG1552	TonB-dependent receptor HmuR	0.847925	1.799910
PG0791	adenylate kinase	0.983103	1.976713	PG0521	chaperonin, 10 kDa	0.846566	1.798216
PG0910	FHA domain protein	0.981960	1.975147	PG1370	lysyl-tRNA synthetase	0.839555	1.789498
PG0377	ribosomal protein S2	0.979625	1.971953	PG2088	peptide methionine sulfoxide reductase	0.839143	1.788987

Table 8- Genes Upregulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG1386	DNA gyrase, A subunit	0.838567	1.788272	PG0523	inosine-5-monophosphate dehydrogenase	0.761349	1.695075
PG1501	transcriptional regulator, tetR family	0.836432	1.785628	PG1941	ribosomal protein S7	0.760985	1.694647
PG0539	efflux transporter, MFP component, RND	0.833860	1.782448	PG0645	conserved domain protein	0.755853	1.688629
PG0733	riboflavin synthase, alpha subunit	0.833466	1.781961	PG2036	ion transporter	0.754990	1.687620
PG0378	translation elongation factor Ts	0.831136	1.779085	PG1950	membrane protein, putative	0.752987	1.685278
PG0045	heat shock protein HtpG	0.828606	1.775969	PG1938	ribosomal protein L3	0.751161	1.683147
PG0956	peptidase, M23-M37 family, putative	0.824942	1.771464	PG2202	conserved hypothetical protein TIGR0025	0.750487	1.682361
PG0046	phosphatidate cytidylyltransferase	0.823820	1.770087	PG1916	translation initiation factor IF-1	0.749905	1.681682
PG1566	glutamyl-tRNA synthetase	0.821354	1.767064	PG0074	peptide chain release factor 1	0.749563	1.681283
PG0597	ribosomal protein L9	0.818353	1.763391	PG1939	ribosomal protein S10	0.748788	1.680380
PG1915	ribosomal protein L36	0.816091	1.760629	PG0324	histidine ammonia-lyase	0.747960	1.679417
PG1142	exopolysaccharide synthesis-related protein	0.814406	1.758574	PG0321	LAO-AO transport system ATPase	0.742782	1.673400
PG0095	DNA mismatch repair protein MutS	0.812997	1.756857	PG1821	cytochrome c nitrite reductase, small sub	0.741864	1.672335
PG1677	phosphoglycerate kinase	0.806816	1.749346	PG2221	MiaB-like tRNA modifying enzyme	0.738714	1.668688
PG0363	conserved domain protein	0.806668	1.749167	PG1748	transketolase	0.738366	1.668286
PG0315	ribosomal protein L27	0.806398	1.748839	PG1959	ribosomal protein L33	0.734731	1.664087
PG0392	ribosomal protein L10	0.806182	1.748578	PG1543	thioesterase family protein	0.732951	1.662036
PG0269	exodeoxyribonuclease III	0.804658	1.746732	PG2165	glycyl-tRNA synthetase	0.729824	1.658437
PG0752	uracil phosphoribosyltransferase, putative	0.803990	1.745923	PG0538	outer membrane efflux protein	0.723267	1.650916
PG0644	TonB-linked receptor Tlr, authentic frame	0.803638	1.745497	PG0437	polysaccharide export protein, BexD-CtrA	0.723164	1.650798
PG2091	dihydroneopterin aldolase	0.802320	1.743903	PG0151	signal recognition particle-docking protein	0.721463	1.648853
PG0058	nicotinate (nicotinamide) nucleotide aden	0.802148	1.743696	PG1885	polyphosphate kinase	0.720527	1.647784
PG0330	DNA-binding protein, histone-like family	0.800001	1.741102	PG0092	transporter, putative	0.720413	1.647654
PG0528	amidophosphoribosyltransferase, putative	0.797757	1.738396	PG2124	glyceraldehyde 3-phosphate dehydrogenase	0.719574	1.646696
PG1326	hemagglutinin, putative	0.797201	1.737727	PG1711	alpha-1,2-mannosidase family protein	0.719492	1.646602
PG1249	1-acyl-sn-glycerol-3-phosphate acetyltransferase	0.795260	1.735391	PG1771	phenylalanyl-tRNA synthetase, alpha sub	0.715372	1.641907
PG1797	DNA-binding response regulator-sensor histone	0.790638	1.729839	PG1536	cell division protein FtsX, putative	0.714902	1.641372
PG1071	conserved hypothetical protein	0.790380	1.729529	PG1115	signal recognition particle protein	0.712019	1.638095
PG1560	dTDP-glucose 4,6-dehydratase	0.789029	1.727911	PG0047	cell division protein FtsH, putative	0.711541	1.637552
PG1345	glycosyl transferase, group 1 family protein	0.784440	1.722423	PG0411	hemagglutinin, putative	0.710317	1.636164
PG1934	ribosomal protein S19	0.783118	1.720846	PG0785	tonB protein, putative	0.709662	1.635421
PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosphate	0.782545	1.720163	PG0804	flavodoxin	0.707508	1.632981
PG2139	hypothetical protein	0.781385	1.718780	PG0714	copper homeostasis protein CutC	0.706392	1.631719
PG0073	orotidine 5-monophosphate decarboxylase	0.780978	1.718295	PG0719	sensor histidine kinase	0.704825	1.629947
PG1856	cytidine-deoxycytidylate deaminase family	0.779149	1.716118	PG1401	beta-eliminating lyase	0.703425	1.628366
PG1641	phosphotyrosine protein phosphatase	0.774805	1.710958	PG1763	ribonuclease III	0.701623	1.626333
PG0333	membrane protein, putative	0.774303	1.710364	PG0232	zinc carboxypeptidase, putative	0.698223	1.622505
PG0758	peptidyl-dipeptidase Dcp	0.770610	1.705991	PG0022	sulfate permease family protein	0.696624	1.620708
PG1940	translation elongation factor G	0.769468	1.704641	PG1013	acetyl-CoA hydrolase-transferase family	0.696574	1.620651
PG0076	N-acetylmuramoyl-L-alanine amidase, family	0.769328	1.704476	PG0778	conserved hypothetical protein	0.696224	1.620258
PG1327	aminotransferase, putative	0.769024	1.704117	PG0477	pantoate--beta-alanine ligase	0.694959	1.618838
PG0582	cell division protein FtsQ, putative	0.767948	1.702846	PG0032	beta-mannosidase, putative	0.692239	1.615789
PG0443	hemagglutinin-related protein	0.767944	1.702841	PG0381	sodium-hydrogen antiporter	0.689742	1.612995
PG1726	PDZ domain protein	0.766372	1.700986	PG0581	UDP-N-acetylmuramate--alanine ligase	0.688854	1.612003
PG1414	hypothetical protein	0.765077	1.699461	PG2044	conserved hypothetical protein	0.688382	1.611475
PG0198	conserved hypothetical protein	0.764275	1.698516	PG0803	glucosamine-6-phosphate isomerase	0.685166	1.607887
PG0990	ribosomal protein L35	0.761765	1.695564	PG0630	pyridoxal phosphate biosynthetic protein	0.684085	1.606683

Table 8- Genes Upregulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0142	SpoOJ regulator protein	0.683059	1.605541	PG0465	ferric uptake transcriptional regulator	0.646694	1.565576
PG0393	ribosomal protein L7-L12	0.681982	1.604342	PG1170	SerB family protein	0.645596	1.564385
PG1040	transcriptional regulator, putative	0.680620	1.602829	PG1247	3-dehydroquinase synthase	0.642110	1.560610
PG2085	tryptophanyl-tRNA synthetase	0.679118	1.601161	PG0651	HDIG domain protein	0.641310	1.559745
PG1134	thioredoxin reductase	0.678650	1.600641	PG1932	ribosomal protein S3	0.638595	1.556812
PG1729	thiol peroxidase	0.677770	1.599665	PG0767	4-alpha-glucanotransferase	0.636320	1.554359
PG1017	pyruvate phosphate dikinase	0.673754	1.595219	PG1925	ribosomal protein S14	0.635210	1.553164
PG1942	ribosomal protein S12	0.671724	1.592975	PG1553	CobN-magnesium chelatase family protei	0.634518	1.552419
PG1696	type II DNA modification methyltransfera	0.671087	1.592272	PG0648	iron compound ABC transporter, periplasi	0.618191	1.534949
PG1544	yaaA protein	0.669010	1.589982	PG1256	ribonuclease, Rne-Rng family	0.616205	1.532838
PG1075	coenzyme A transferase, beta subunit	0.668363	1.589269	PG0890	alkaline phosphatase, putative	0.614402	1.530924
PG1353	orotate phosphoribosyltransferase	0.664393	1.584901	PG1132	valyl-tRNA synthetase	0.613066	1.529506
PG0380	excinuclease ABC, B subunit	0.663151	1.583537	PG1880	glycosyl transferase, group 2 family prote	0.612638	1.529053
PG2201	peptide deformylase	0.660554	1.580690	PG0504	lipoate synthase	0.612575	1.528986
PG1064	dihydroorotate dehydrogenase, putative	0.660446	1.580572	PG1127	transcriptional regulator, AsnC Family	0.612029	1.528408
PG0476	yngK protein	0.659939	1.580015	PG2095	lipoprotein, putative	0.610296	1.526573
PG2206	ABC transporter, ATP-binding protein	0.658552	1.578497	PG1041	K+-dependent Na+-Ca+ exchanger relat	0.609381	1.525605
PG1921	ribosomal protein S5	0.656031	1.575741	PG1467	methylytransferase, UbiE-COQ5 family	0.606679	1.522750
PG0522	tRNA delta(2)-isopentenylpyrophosphate	0.653773	1.573277	PG0543	transcriptional regulator, putative	0.601107	1.516880
PG0328	imidazolonepropionase	0.651784	1.571110	PG1260	anaerobic ribonucleoside-triphosphate re	0.592949	1.508326
PG0376	ribosomal protein S9	0.651155	1.570425	PG2069	oxidoreductase, short chain dehydrogena	0.591194	1.506493
PG0042	serine hydroxymethyltransferase	0.648690	1.567744	PG1917	methionine aminopeptidase, type I	0.589913	1.505156
PG0790	GTP-binding protein Obg	0.648569	1.567612	PG0669	heme-binding protein FetB	0.588748	1.503941
PG1354	hydrolase, carbon-nitrogen family	0.647945	1.566935	PG1725	competence-damage-inducible protein Ci	0.587278	1.502409

* = Gene ID according to JCVI

** = log(spot intensity pH 5.5/pH 8.5)

*** = fold change (ratio of pH 5.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 9- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0992	threonyl-tRNA synthetase	-1.003181	0.498899	PG0351	hypothetical protein	-1.180800	0.441107
PG0661	hypothetical protein	-1.005007	0.498268	PG0502	SsrA-binding protein	-1.181735	0.440821
PG0511	spore maturation protein A-spore maturation pr	-1.005613	0.498059	PG1846	hypothetical protein	-1.184710	0.439913
PG1375	hypothetical protein	-1.007942	0.497255	PG0739	metallo-beta-lactamase family protein	-1.193925	0.437112
PG1734	transporter, putative	-1.010821	0.496264	PG1014	TPR domain protein	-1.200371	0.435163
PG1571	metallo-beta-lactamase superfamily protein	-1.018184	0.493738	PG1251	hypothetical protein	-1.205378	0.433656
PG0626	hypothetical protein	-1.018968	0.493469	PG0248	translation initiation factor SUI1, putative	-1.211185	0.431914
PG2109	hydroxymethylpyrimidine kinase-thiamin-phosp	-1.019812	0.493181	PG1483	conjugative transposon protein TraE	-1.212817	0.431425
PG1308	hypothetical protein	-1.026864	0.490776	PG0063	outer membrane efflux protein	-1.222601	0.428509
PG0996	conserved hypothetical protein TIGR01777	-1.031676	0.489142	PG0354	hypothetical protein	-1.234107	0.425106
PG0053	hypothetical protein	-1.032004	0.489031	PG1735	MutT-nudix family protein	-1.235302	0.424754
PG1015	hypothetical protein	-1.039464	0.486508	PG1876	conserved hypothetical protein	-1.240972	0.423088
PG2102	immunoreactive 61 kDa antigen PG91	-1.044571	0.484789	PG1150	hypothetical protein	-1.241426	0.422954
PG0919	dihydroorotase	-1.048861	0.483350	PG0869	mobilization protein	-1.244525	0.422047
PG0958	YihY family protein	-1.049998	0.482969	PG2161	transcriptional regulator, AraC family	-1.246931	0.421343
PG0894	DNA repair protein RadC	-1.052284	0.482204	PG0033	RmuC domain protein	-1.247544	0.421164
PG0148	sigma-54-dependent transcriptional regulator	-1.052659	0.482079	PG0399	lipoprotein, putative	-1.253026	0.419567
PG0259	conserved hypothetical protein	-1.052764	0.482044	PG0136	hypothetical protein	-1.268062	0.415217
PG1824	enolase	-1.053669	0.481741	PG2014	CRISPR-associated protein Cas1	-1.269922	0.414682
PG1887	rhodanese-like domain protein	-1.058065	0.480276	PG0117	polysaccharide transport protein, putative	-1.273297	0.413713
PG0747	sigma-54 dependent DNA-binding response re	-1.058684	0.480070	PG1837	hemagglutinin protein HagA	-1.279207	0.412022
PG1774	transcription-repair coupling factor	-1.060068	0.479610	PG1085	hypothetical protein	-1.279864	0.411834
PG1522	mandelate racemase-muconate lactonizing enz	-1.076692	0.474115	PG1153	hypothetical protein	-1.280080	0.411773
PG1030	hypothetical protein	-1.077353	0.473897	PG1477	hypothetical protein	-1.286619	0.409910
PG0347	UDP-glucose 4-epimerase	-1.087830	0.470468	PG2210	excinuclease ABC, A subunit	-1.288292	0.409436
PG0787	hypothetical protein	-1.088479	0.470257	PG1445	rteC protein, truncation	-1.289871	0.408988
PG0447	conserved hypothetical protein	-1.088577	0.470225	PG2016	CRISPR-associated helicase Cas3	-1.298074	0.406669
PG0704	phosphoglycerate mutase family protein	-1.090086	0.469734	PG1903	conserved hypothetical protein	-1.299500	0.406267
PG1514	glycerol dehydrogenase-related protein	-1.091653	0.469223	PG0265	hypothetical protein	-1.301547	0.405691
PG0170	methionyl-tRNA synthetase	-1.095338	0.468026	PG2079	hypothetical protein	-1.310301	0.403237
PG0832	hypothetical protein	-1.098946	0.466857	PG1020	hypothetical protein	-1.317114	0.401337
PG1765	acyl carrier protein	-1.101098	0.466161	PG0490	membrane protein, putative	-1.323744	0.399497
PG1112	hypothetical protein	-1.102536	0.465697	PG1208	dnaK protein	-1.326224	0.398811
PG0445	peptidase T	-1.103467	0.465397	PG2185	transporter, putative	-1.331978	0.397223
PG2028	ebsC protein	-1.104007	0.465223	PG1601	biotin--acetyl-CoA-carboxylase ligase	-1.345063	0.393637
PG1743	2-dehydro-3-deoxyphosphooctonate aldolase	-1.106646	0.464372	PG0685	ABC transporter, ATP-binding protein	-1.345216	0.393595
PG1620	carboxyl-terminal protease-related protein	-1.108658	0.463725	PG0842	mobilizable transposon, hypothetical protein, pi	-1.345441	0.393534
PG1018	hypothetical protein	-1.110592	0.463104	PG0899	cytochrome d ubiquinol oxidase, subunit II	-1.350134	0.392256
PG1964	bacterial sugar transferase	-1.112571	0.462469	PG0216	hypothetical protein	-1.351938	0.391765
PG0425	esterase, putative	-1.130979	0.456606	PG1487	hypothetical protein	-1.361253	0.389244
PG0398	recF protein	-1.134814	0.455393	PG2053	dethiobiotin synthase	-1.374567	0.385668
PG1313	dipeptidase-related protein	-1.135302	0.455240	PG2020	CRISPR-associated protein, TM1814 family	-1.376764	0.385082
PG2050	hypothetical protein	-1.139103	0.454042	PG1301	hypothetical protein	-1.392220	0.380978
PG2207	conserved domain protein	-1.144370	0.452387	PG1448	ISPg1, transposase	-1.414357	0.375177
PG1203	transcriptional regulator, putative	-1.144975	0.452197	PG2169	ISPg1, transposase, degenerate	-1.414816	0.375058
PG0339	hypothetical protein	-1.145948	0.451893	PG1659	hypothetical protein	-1.417266	0.374421
PG1547	hypothetical protein	-1.155773	0.448826	PG0057	nicotinate phosphoribosyltransferase	-1.424592	0.372525
PG0021	TIM-barrel protein, putative, NifR3 family	-1.156904	0.448474	PG0458	ISPg5, transposase Orf2	-1.429642	0.371223

Table 9- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 5.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0612	hypothetical protein	-1.433236	0.370299	PG0064	heavy metal efflux pump, CzcA family	-1.719014	0.303756
PG1491	hypothetical protein	-1.433981	0.370108	PG0487	ISPg4, transposase	-1.733057	0.300814
PG1032	ISPg3, transposase	-1.435331	0.369762	PG1270	conserved hypothetical protein	-1.744489	0.298440
PG0349	hydrolase, haloacid dehalogenase-like family	-1.439649	0.368657	PG1766	phosphoribosylglycinamide formyltransferase	-1.744968	0.298340
PG1238	ribosomal large subunit pseudouridine synthase	-1.454030	0.365000	PG2037	hypothetical protein	-1.761097	0.295024
PG2116	hypothetical protein	-1.470568	0.360840	PG1136	conserved hypothetical protein	-1.769532	0.293304
PG0873	mobilizable transposon, tnpC protein	-1.478327	0.358905	PG1795	hypothetical protein	-1.774035	0.292390
PG0274	hypothetical protein	-1.493188	0.355227	PG1532	hypothetical protein	-1.787985	0.289576
PG1294	ferrous iron transport protein B	-1.494499	0.354904	PG1907	ISPg3, transposase, interruption	-1.798731	0.287427
PG1233	hypothetical protein	-1.498767	0.353856	PG1001	conserved hypothetical protein	-1.832121	0.280851
PG1572	membrane protein, putative	-1.503892	0.352601	PG1130	TPR domain protein	-1.832578	0.280762
PG1022	hypothetical protein	-1.510478	0.350995	PG0371	hypothetical protein	-1.846823	0.278004
PG0569	hypothetical protein	-1.513867	0.350171	PG1460	hypothetical protein	-1.865719	0.274386
PG2198	hemagglutinin protein, truncation	-1.530732	0.346102	PG0246	hypothetical protein	-1.866887	0.274164
PG2001	signal peptidase I	-1.543160	0.343133	PG1434	4-diphosphocytidyl-2C-methyl-D-erythritol synthase	-1.872095	0.273176
PG1680	ABC transporter, ATP-binding protein, authentic	-1.550464	0.341400	PG1109	mobilization protein	-1.886673	0.270430
PG0158	competence protein F-related protein	-1.558433	0.339520	PG2038	N-acetylmuramoyl-L-alanine amidase, putative	-1.899716	0.267996
PG2131	60 kDa protein	-1.559179	0.339344	PG0718	conserved hypothetical protein	-2.016781	0.247109
PG0118	glycosyl transferase, group 2 family protein	-1.577248	0.335120	PG1492	hypothetical protein	-2.018876	0.246750
PG1379	ABC transporter, periplasmic substrate-binding	-1.586174	0.333054	PG2063	hypothetical protein	-2.021450	0.246311
PG1555	conserved domain protein	-1.586445	0.332991	PG1384	ISPg1, transposase, authentic frameshift	-2.024373	0.245812
PG1159	cobalamin biosynthesis protein CbiB	-1.589611	0.332261	PG0065	efflux transporter, RND family, MFP subunit	-2.057713	0.240196
PG0459	ISPg5, transposase Orf1	-1.630301	0.323021	PG1303	helicase, putative	-2.073287	0.237618
PG0004	transcriptional regulator, Sir2 family	-1.632875	0.322445	PG0611	lipoprotein, putative	-2.080677	0.236404
PG1546	hypothetical protein	-1.634390	0.322107	PG0219	conserved domain protein	-2.180823	0.220550
PG0375	ribosomal protein L13	-1.645927	0.319541	PG2213	nitrite reductase-related protein	-2.248869	0.210389
PG1441	lysozyme-related protein	-1.649983	0.318644	PG0760	ISPg1, transposase, authentic frameshift	-2.280620	0.205809
PG1984	CRISPR-associated protein, TM1791.1 family	-1.666548	0.315006	PG1670	hypothetical protein	-2.414637	0.187552
PG1418	DNA polymerase III, gamma and tau subunits	-1.672160	0.313783	PG1271	acetylornithine aminotransferase, putative	-2.419373	0.186937
PG2062	histidyl-tRNA synthetase	-1.675115	0.313141	PG1828	lipoprotein, putative	-2.740719	0.149610
PG1671	hypothetical protein	-1.679025	0.312294	PG1421	ferredoxin, 4Fe-4S	-3.216357	0.107592
PG0720	DNA-binding response regulator	-1.696613	0.308510	PG0340	hypothetical protein	-3.311783	0.100706

* = Gene ID according to JCVI

** = log(spot intensity pH 5.5/pH 8.5)

*** = fold change (ratio of pH 5.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 10- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 6.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0636	MATE efflux family protein	1.489952	2.808797	PG0035	DNA polymerase III, alpha subunit	0.736815	1.666492
PG0685	ABC transporter, ATP-binding protein	1.461097	2.753177	PG1755	fructose-bisphosphate aldolase, class I	0.731728	1.660627
PG0684	ABC transporter, permease protein, putal	1.454047	2.739755	PG0090	Dps family protein	0.728256	1.656635
PG2206	ABC transporter, ATP-binding protein	1.345342	2.540904	PG1726	PDZ domain protein	0.727376	1.655625
PG0635	ribosomal protein L11 methyltransferase	1.341700	2.534498	PG2150	LysM domain protein	0.726396	1.654501
PG0555	DNA-binding protein, histone-like family	1.235600	2.354793	PG1151	alcohol dehydrogenase, iron-containing	0.724278	1.652074
PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosph	1.159503	2.233805	PG0121	DNA-binding protein HU	0.721246	1.648606
PG0683	ABC transporter, permease protein, putal	1.107271	2.154377	PG1821	cytochrome c nitrite reductase, small sub	0.693295	1.616973
PG0539	efflux transporter, MFP component, RND	1.082415	2.117577	PG1855	carboxyl-terminal protease	0.692171	1.615713
PG1173	YkgG family protein	1.061354	2.086890	PG0269	exodeoxyribonuclease III	0.683423	1.605945
PG1586	batE protein	0.999587	1.999428	PG1355	acyltransferase, putative	0.682442	1.604854
PG0263	tyrosyl-tRNA synthetase	0.996183	1.994716	PG0369	phosphopantetheine adenyllyltransferase	0.673612	1.595062
PG1950	membrane protein, putative	0.980120	1.972629	PG1702	DNA gyrase, B subunit	0.667671	1.588507
PG0321	LAO-AO transport system ATPase	0.968401	1.956670	PG1868	membrane protein, putative	0.662042	1.582321
PG1600	membrane protein, putative	0.948318	1.929622	PG0540	AcrB-AcrD-AcrF family protein	0.654500	1.574070
PG0538	outer membrane efflux protein	0.938414	1.916420	PG0680	efflux transporter, MFP component, RND	0.653533	1.573016
PG0232	zinc carboxypeptidase, putative	0.918582	1.890256	PG0099	phenylalanyl-tRNA synthetase, beta subu	0.649631	1.568766
PG1232	glutamate dehydrogenase, NAD-specific	0.917968	1.889452	PG1804	v-type ATPase, subunit B	0.648286	1.567305
PG1289	GDP-fucose synthetase	0.908530	1.877132	PG0795	membrane protein, putative	0.637920	1.556084
PG1025	conserved domain protein	0.904938	1.872464	PG0172	exonuclease	0.632220	1.549948
PG1116	methylenetetrahydrofolate dehydrogenas	0.904452	1.871834	PG0476	yngK protein	0.631843	1.549543
PG1305	glycine cleavage system P protein	0.903448	1.870531	PG0209	formate-nitrite transporter	0.630289	1.547875
PG1657	methylmalonyl-CoA mutase, large subuni	0.872023	1.830228	PG1310	exsB protein	0.628251	1.545690
PG0682	ABC transporter, permease protein, putal	0.867230	1.824157	PG0754	DNA topoisomerase I	0.625310	1.542542
PG1654	D-alanyl-D-alanine dipeptidase	0.866476	1.823204	PG1948	lipoprotein, putative	0.623136	1.540220
PG0679	outer membrane efflux protein	0.862955	1.818760	PG0055	conserved domain protein	0.612791	1.529214
PG0279	NADP-dependent malic enzyme	0.852611	1.805767	PG0477	pantoate--beta-alanine ligase	0.611005	1.527323
PG0945	ABC transporter, permease protein, putal	0.846314	1.797902	PG0475	oxygen-independent coproporphyrinogen	0.609698	1.525939
PG0138	malonyl CoA-acyl carrier protein transacy	0.834111	1.782759	PG0134	magnesium transporter	0.608644	1.524825
PG1408	heavy metal efflux pump, CzcD family	0.823118	1.769226	PG0415	peptidyl-prolyl cis-trans isomerase, PPIC-	0.607120	1.523216
PG2124	glyceraldehyde 3-phosphate dehydrogeni	0.817779	1.762690	PG1330	large conductance mechanosensitive chai	0.605047	1.521028
PG1682	glycosyl transferase, group 1 family prote	0.807962	1.750737	PG0978	shikimate 5-dehydrogenase	0.604048	1.519975
PG0430	oxidoreductase, putative	0.796936	1.737407	PG1235	epimerase-reductase, putative	0.603374	1.519265
PG1327	aminotransferase, putative	0.790068	1.729155	PG1386	DNA gyrase, A subunit	0.595597	1.511098
PG0618	alkyl hydroperoxide reductase, C subunit	0.787284	1.725822	PG0296	phosphoribosylformylglycinamide synth	0.593714	1.509127
PG0271	single-stranded binding protein	0.779669	1.716737	PG2088	peptide methionine sulfoxide reductase	0.590918	1.506205
PG0363	conserved domain protein	0.753077	1.685384	PG0381	sodium-hydrogen antiporter	0.589363	1.504583
PG1981	CRISPR-associated protein Cas2	0.750465	1.682335	PG2222	acyltransferase, HtrB-MsbB family	0.585666	1.500732
PG0977	ubiquinone-menaquinone biosynthesis m	0.748616	1.680181				

* = Gene ID according to JCVI

** = log(spot intensity pH 6.5/pH 8.5)

***= fold change (ratio of pH 6.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 11- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 6.5 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0777	electron transfer flavoprotein, beta subunit	-1.027873	0.490433	PG0726	lipoprotein, putative	-1.276122	0.412904
PG1039	integral membrane protein	-1.034746	0.488102	PG0214	RNA polymerase sigma-70 factor, ECF sigma factor	-1.320181	0.400485
PG0776	electron transfer flavoprotein, alpha subunit	-1.050254	0.482883	PG2056	transposase, ISPg2-related, truncation	-1.421893	0.373222
PG1208	dnaK protein	-1.073209	0.475261	PG1203	transcriptional regulator, putative	-1.464716	0.362307
PG1144	peptide chain release factor 2, programmed	-1.090630	0.469556	PG0385	ribosomal protein S21	-1.575940	0.335425
PG2213	nitrite reductase-related protein	-1.123495	0.458980	PG0037	ribosomal protein L19	-1.646838	0.319339
PG1959	ribosomal protein L33	-1.140730	0.453530	PG0521	chaperonin, 10 kDa	-1.666721	0.314968
PG1240	transcriptional regulator, tetR family	-1.153057	0.449671	PG1258	DNA-binding protein HU	-1.677604	0.312601
PG1142	exopolysaccharide synthesis-related protein	-1.188198	0.438851	PG1469	type I restriction-modification system, MspI	-1.947721	0.259225
PG1765	acyl carrier protein	-1.200546	0.435110	PG0386	site-specific recombinase, phage integrase	-2.028834	0.245053
PG0857	transcriptional regulator, putative	-1.237271	0.424174	PG1421	ferredoxin, 4Fe-4S	-2.341417	0.197316

* = Gene ID according to JCVI

** = log(spot intensity pH 6.5/pH 8.5)

*** = fold change (ratio of pH 6.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 12- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG1406	ISPg2, transposase, truncation	2.475352	5.561031	PG1129	ribonucleotide reductase	1.292124	2.448884
PG2088	peptide methionine sulfoxide reductase	2.296013	4.910988	PG1773	PAP2 superfamily protein	1.271612	2.414312
PG0135	dimethyladenosine transferase	2.238597	4.719379	PG1180	membrane protein, putative	1.270817	2.412982
PG1420	ISPg5, transposase Orf2	2.077072	4.219501	PG0377	ribosomal protein S2	1.269480	2.410746
PG1326	hemagglutinin, putative	1.969542	3.916438	PG1632	aldose 1-epimerase	1.257608	2.390989
PG0087	SIS domain protein	1.959984	3.890578	PG1069	alcohol dehydrogenase, zinc-containing, i	1.250176	2.378704
PG1252	membrane protein, putative	1.931467	3.814430	PG1709	ISPg5, transposase Orf1	1.225497	2.338359
PG1312	capA protein, putative	1.901435	3.735846	PG1221	oxidoreductase, short chain dehydrogena	1.186017	2.275237
PG1696	type II DNA modification methyltransfera	1.888168	3.701649	PG0418	ATP-dependent Clp protease, proteolytic	1.185578	2.274545
PG0969	S-adenosylmethionine:tRNA ribosyltransf	1.831660	3.559465	PG0396	transcriptional regulator, Crp-Fnr family	1.168032	2.247050
PG2195	ISPg1, transposase, truncation	1.798557	3.478721	PG0794	penicillin-binding protein 1A, putative	1.128072	2.185664
PG1397	phosphoribosylaminoimidazolecarboxami	1.760386	3.387889	PG1127	transcriptional regulator, AsnC Family	1.124298	2.179955
PG0506	arginine-specific cysteine proteinase	1.715531	3.284175	PG1232	glutamate dehydrogenase, NAD-specific	1.124154	2.179736
PG0106	glycosyl transferase, group 4 family prote	1.706742	3.264229	PG0530	carbamoyl-phosphate synthase, large sut	1.118326	2.170949
PG0163	phosphofructokinase	1.704836	3.259919	PG1732	integrase-recombinase XerD	1.108082	2.155590
PG1573	transcriptional regulator, Crp family	1.703920	3.257849	PG0687	succinate-semialdehyde dehydrogenase	1.107827	2.155208
PG1072	MutS family protein	1.682965	3.210871	PG0760	ISPg1, transposase, authentic frameshift	1.104539	2.150301
PG1095	RNA methyltransferase, TrmA family	1.666136	3.173635	PG1040	transcriptional regulator, putative	1.087541	2.125115
PG1058	OmpA family protein	1.648607	3.135308	PG0690	4-hydroxybutyrate CoA-transferase	1.080625	2.114952
PG1615	fumarate reductase, flavoprotein subunit	1.638377	3.113155	PG1170	SerB family protein	1.071938	2.102256
PG1814	DNA primase	1.632625	3.100767	PG0792	hypoxanthine phosphoribosyltransferase	1.067054	2.095151
PG0196	peptidase, M16 family	1.613481	3.059892	PG1831	ATP-dependent DNA helicase RecQ	1.063406	2.089860
PG2105	lipoprotein, putative	1.585692	3.001518	PG1142	exopolysaccharide synthesis-related prot	1.063355	2.089785
PG1854	5-formyltetrahydrofolate cyclo-ligase fam	1.568507	2.965976	PG0647	iron compound ABC transporter, permeas	1.058028	2.082084
PG1337	umuD protein	1.544141	2.916304	PG1386	DNA gyrase, A subunit	1.046906	2.066094
PG1762	protein-export membrane protein SecD-p	1.541407	2.910783	PG0988	ISPg9, transposase, authentic frameshift	1.033197	2.046554
PG0813	ISPg1, transposase, truncation	1.533384	2.894640	PG1768	magnesium chelatase, subunit D-I family	1.032819	2.046018
PG1105	RNA polymerase sigma-54 factor	1.503791	2.835870	PG0188	lipoprotein, putative	1.028744	2.040247
PG1677	phosphoglycerate kinase	1.496520	2.821612	PG1932	ribosomal protein S3	1.026528	2.037116
PG0575	penicillin-binding protein 2, putative	1.488578	2.806122	PG1720	conserved domain protein	1.021031	2.029369
PG1536	cell division protein FtsX, putative	1.482794	2.794895	PG0350	internalin-related protein	1.016744	2.023348
PG0707	TonB-dependent receptor, putative	1.476442	2.782616	PG1374	immunoreactive 47 kDa antigen PG97	1.012447	2.017330
PG2052	dihydrodipicolinate synthase	1.451909	2.735699	PG1941	ribosomal protein S7	0.991712	1.988543
PG0032	beta-mannosidase, putative	1.418190	2.672500	PG0754	DNA topoisomerase I	0.972246	1.961892
PG0683	ABC transporter, permease protein, putal	1.409299	2.656081	PG1551	hmuY protein	0.957374	1.941772
PG1454	integrase	1.388550	2.618154	PG1416	enoyl-(acyl-carrier-protein) reductase II	0.952505	1.935230
PG1985	CRISPR-associated protein, TM1792 famil	1.380269	2.603170	PG0228	DdaH family protein	0.932114	1.908069
PG1878	cysteinyI-tRNA synthetase	1.374614	2.592985	PG1404	rhomboid family protein	0.928373	1.903128
PG0669	heme-binding protein FetB	1.372114	2.588495	PG2171	D-isomer specific 2-hydroxyacid dehydro	0.928047	1.902698
PG0740	NLP-P60 family protein	1.360317	2.567415	PG0577	phospho-N-acetylmuramoyl-pentapeptide	0.926639	1.900843
PG1261	ISPg4, transposase	1.359688	2.566297	PG2134	lipoprotein, putative	0.914537	1.884964
PG1983	CRISPR-associated protein, TM1791 famil	1.340033	2.531571	PG0458	ISPg5, transposase Orf2	0.912244	1.881971
PG0562	potassium uptake protein TrkA, putative	1.326930	2.508683	PG0052	sensor histidine kinase	0.909815	1.878805
PG1745	phosphoribulokinase family protein	1.312183	2.483170	PG1448	ISPg1, transposase	0.909082	1.877850
PG0692	4-hydroxybutyryl-CoA dehydratase	1.310418	2.480133	PG1915	ribosomal protein L36	0.906719	1.874778
PG0427	ISPg5, transposase Orf1	1.298580	2.459866	PG0378	translation elongation factor Ts	0.902775	1.869659
PG1384	ISPg1, transposase, authentic frameshift	1.293301	2.450882	PG1305	glycine cleavage system P protein	0.900716	1.866993
PG0429	pyruvate synthase	1.292700	2.449862	PG0522	tRNA delta(2)-isopentenylpyrophosphate	0.899564	1.865503

Table 12- Genes up regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

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ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0314	ribosomal protein L21	0.898503	1.864131	PG1088	acetyltransferase, GNAT family	0.736416	1.666032
PG1017	pyruvate phosphate dikinase	0.892997	1.857030	PG0352	sialidase, putative	0.736163	1.665740
PG1138	pigmentation and extracellular proteinase	0.892437	1.856309	PG1836	nucleoside permease NupG	0.735789	1.665308
PG1960	ribosomal protein L28	0.890041	1.853229	PG1023	para-aminobenzoate synthase, componer	0.728971	1.657456
PG0548	pyruvate ferredoxin-flavodoxin oxidoredu	0.885623	1.847563	PG0618	alkyl hydroperoxide reductase, C subunit	0.728869	1.657340
PG1260	anaerobic ribonucleoside-triphosphate rei	0.884207	1.845750	PG1174	thioesterase family protein	0.728574	1.657001
PG0369	phosphopantetheine adenyltransferase	0.872422	1.830734	PG0462	transporter, putative	0.715691	1.642270
PG1155	ADP-heptose--LPS heptosyltransferase, p	0.868023	1.825161	PG0582	cell division protein FtsQ, putative	0.711796	1.637841
PG2221	MiaB-like tRNA modifying enzyme	0.863012	1.818831	PG1780	8-amino-7-oxononanoate synthase	0.710321	1.636169
PG0047	cell division protein FtsH, putative	0.852674	1.805845	PG0003	membrane protein, putative	0.704538	1.629623
PG1587	PAP2 superfamily protein	0.849712	1.802141	PG0579	cell division protein FtsW, putative	0.703208	1.628121
PG0689	NAD-dependent 4-hydroxybutyrate dehy	0.841515	1.791931	PG2047	helicase, putative	0.701338	1.626012
PG1525	isochorismate synthase, putative	0.830618	1.778447	PG1682	glycosyl transferase, group 1 family prote	0.695579	1.619534
PG1931	ribosomal protein L16	0.830490	1.778290	PG0205	peptide chain release factor 3	0.687950	1.610993
PG1235	epimerase-reductase, putative	0.829967	1.777644	PG0475	oxygen-independent coproporphyrinogen	0.679058	1.601093
PG1996	deoxyribose-phosphate aldolase	0.824235	1.770596	PG0449	TPR domain protein	0.677824	1.599725
PG0738	cytidine-deoxycytidylate deaminase famil	0.821959	1.767805	PG2223	glycosyl transferase, group 2 family prote	0.674721	1.596288
PG2224	membrane protein, putative	0.820126	1.765560	PG0766	polyribonucleotide nucleotidyltransferase	0.667325	1.588125
PG0412	DNA mismatch repair protein MutL	0.812988	1.756847	PG1381	ABC transporter, permease protein	0.663904	1.584364
PG0463	folylpolyglutamate synthase	0.812716	1.756515	PG0676	oxidoreductase, short chain dehydrogena	0.663157	1.583544
PG0430	oxidoreductase, putative	0.808963	1.751951	PG0304	electron transport complex, RnfABCDGE t	0.662861	1.583220
PG2124	glyceraldehyde 3-phosphate dehydrogen	0.806841	1.749377	PG1913	ribosomal protein S11	0.659425	1.579453
PG1885	polyphosphate kinase	0.799599	1.740617	PG0581	UDP-N-acetylmuramate--alanine ligase	0.658659	1.578614
PG1278	phosphoserine aminotransferase	0.796080	1.736377	PG0705	glutamate racemase	0.658525	1.578468
PG0719	sensor histidine kinase	0.795358	1.735509	PG1028	TPR domain protein	0.654902	1.574509
PG1920	ribosomal protein L30	0.795044	1.735130	PG0804	flavodoxin	0.649848	1.569003
PG1408	heavy metal efflux pump, CzcD family	0.790971	1.730239	PG1948	lipoprotein, putative	0.648913	1.567987
PG2085	tryptophanyl-tRNA synthetase	0.789223	1.728143	PG1922	ribosomal protein L18	0.623241	1.540332
PG0006	MATE efflux family protein	0.756364	1.689228	PG1755	fructose-bisphosphate aldolase, class I	0.618178	1.534936
PG0186	lipoprotein RagB	0.753439	1.685806	PG0016	sigma-54 dependent DNA-binding respon	0.612176	1.528563
PG2090	cation efflux family protein	0.749531	1.681246	PG2071	conserved domain protein	0.598394	1.514030
PG1821	cytochrome c nitrite reductase, small sub	0.747747	1.679169	PG1914	ribosomal protein S13	0.597535	1.513129
PG1019	lipoprotein, putative	0.744850	1.675800	PG2205	2-dehydropantoate 2-reductase	0.586956	1.502074
PG0897	alpha-amylase family protein	0.742966	1.673613	PG1121	asparaginyl-tRNA synthetase	0.586735	1.501844
PG0046	phosphatidate cytidyltransferase	0.739406	1.669489	PG1099	glucokinase regulator-related protein	0.586420	1.501516
PG0511	spore maturation protein A-spore matura	0.739301	1.669366				

* = Gene ID according to JCVI

** = log(spot intensity pH 7.0/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 13- Genes down regulated in *Porphyromonas gingivalis* W83 grown at pH 7.0 compared to *Porphyromonas gingivalis* W83 grown at pH 8.5, both in microaerophilic conditions.

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0090	Dps family protein	-1.035000	0.488016	PG1238	ribosomal large subunit pseudouridine sy	-1.511962	0.350634
PG2028	ebsC protein	-1.037753	0.487085	PG0267	arginyl-tRNA synthetase	-1.580410	0.334387
PG0128	conserved domain protein	-1.048408	0.483501	PG2185	transporter, putative	-1.584375	0.333469
PG0346	GTP-binding protein	-1.051549	0.482450	PG0720	DNA-binding response regulator	-1.606141	0.328476
PG1205	DNA-binding protein, histone-like family	-1.059687	0.479736	PG0817	integrase, truncation	-1.607809	0.328096
PG0118	glycosyl transferase, group 2 family prote	-1.064354	0.478187	PG1907	ISPg3, transposase, interruption	-1.659830	0.316476
PG1467	methytransferase, UbiE-COQ5 family	-1.064528	0.478129	PG0985	RNA polymerase sigma-70 factor, ECF su	-1.672588	0.313690
PG1435	integrase	-1.065694	0.477743	PG2108	thiG protein	-1.700916	0.307591
PG0846	integrase, truncation	-1.074220	0.474928	PG1303	helicase, putative	-1.709788	0.305705
PG1162	ATP:cob(I)alamin adenosyltransferase, pi	-1.089988	0.469765	PG0869	mobilization protein	-1.754236	0.296430
PG1258	DNA-binding protein HU	-1.107109	0.464223	PG0873	mobilizable transposon, tnpC protein	-1.769304	0.293350
PG0219	conserved domain protein	-1.123506	0.458977	PG2109	hydroxymethylpyrimidine kinase-thiamin	-1.792540	0.288663
PG1441	lysozyme-related protein	-1.126346	0.458074	PG1434	4-diphosphocytidyl-2C-methyl-D-erythrit	-1.846830	0.278003
PG2125	transcriptional regulator, AraC family	-1.128139	0.457506	PG1203	transcriptional regulator, putative	-1.849542	0.277481
PG2008	TonB-dependent receptor, putative	-1.145597	0.452003	PG0064	heavy metal efflux pump, CzcA family	-1.873934	0.272828
PG0148	sigma-54-dependent transcriptional regul	-1.155800	0.448817	PG1681	glycogen debranching enzyme, archaeal	-1.905165	0.266986
PG1143	sugar dehydrogenase, UDP-glucose-GDP-	-1.167015	0.445342	PG1482	conjugative transposon protein TraF	-1.910357	0.266027
PG1622	DNA topoisomerase IV, A subunit, putativ	-1.178392	0.441844	PG1294	ferrous iron transport protein B	-1.946357	0.259471
PG1872	urocanate hydratase	-1.178718	0.441744	PG2014	CRISPR-associated protein Cas1	-1.959936	0.257040
PG1225	ABC transporter, ATP-binding protein	-1.179330	0.441556	PG1651	TPR domain protein	-1.962651	0.256557
PG2213	nitrite reductase-related protein	-1.189893	0.438335	PG1697	type II restriction endonuclease, putative	-2.000954	0.249835
PG1693	HesA-MoeB-ThiF family protein	-1.194013	0.437085	PG1967	TPR domain protein	-2.264668	0.208098
PG0910	FHA domain protein	-1.216369	0.430365	PG1118	clpB protein	-2.294251	0.203874
PG1500	conserved domain protein	-1.230307	0.426227	PG1350	ISPg2, transposase	-2.319292	0.200366
PG1418	DNA polymerase III, gamma and tau sub	-1.233783	0.425201	PG2169	ISPg1, transposase, degenerate	-2.529983	0.173141
PG2217	deoxyxylulose-5-phosphate synthase	-1.247210	0.421262	PG1109	mobilization protein	-2.594391	0.165581
PG1208	dnaK protein	-1.294304	0.407733	PG1130	TPR domain protein	-2.823933	0.141225
PG0227	DNA repair protein Rada	-1.314301	0.402120	PG0456	PHP N-terminal domain protein	-3.100079	0.116623
PG1211	hexapeptide transferase family protein	-1.385645	0.382718	PG1513	phosphoribosyltransferase, putative-phos	-3.210648	0.108019
PG0894	DNA repair protein RadC	-1.386323	0.382538	PG2016	CRISPR-associated helicase Cas3	-3.409180	0.094131
PG1483	conjugative transposon protein TraE	-1.466092	0.361961	PG1503	LytB-related protein	-4.382770	0.047935
PG1592	HDIG domain protein	-1.472684	0.360311	PG1042	glycogen synthase, putative	-4.426126	0.046516
PG0678	pyrazinamidase-nicotinamidase, putative	-1.488117	0.356478	PG1481	conjugative transposon protein TraG	-4.836168	0.035008
PG2102	immunoreactive 61 kDa antigen PG91	-1.494999	0.354781				

* = Gene ID according to JCVI

** = log(spot intensity pH 7.0/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 14- Genes found differentially expressed in *P. gingivalis* grown in both pH 5.5 anaerobic and pH 5.5 microaerophilic conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0022	sulfate permease family protein	3.010911	1.620708	PG1127	transcriptional regulator, AsnC Family	1.868666	1.528408
PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosph	2.704860	1.720163	PG1130	TPR domain protein	0.445294	0.280762
PG0045	heat shock protein HtpG	1.568268	1.775969	PG1238	ribosomal large subunit pseudouridine sy	0.334755	0.365000
PG0058	nicotinate (nicotinamide) nucleotide aden	3.252483	1.743696	PG1241	GTP-binding protein Lepa	3.197950	1.983458
PG0063	outer membrane efflux protein	0.433729	0.428509	PG1249	1-acyl-sn-glycerol-3-phosphate acetyltra	2.215235	1.735391
PG0064	heavy metal efflux pump, CzcA family	0.281729	0.303756	PG1255	recombination protein RecR	0.396250	1.991044
PG0070	acyl-(acyl-carrier-protein)-UDP-N-acetyl	1.732434	3.959299	PG1327	aminotransferase, putative	4.488317	1.704117
PG0118	glycosyl transferase, group 2 family prote	0.335917	0.335120	PG1345	glycosyl transferase, group 1 family prote	2.109009	1.722423
PG0134	magnesium transporter	3.212797	1.980859	PG1346	glycosyl transferase, group 1 family prote	1.969020	3.106824
PG0151	signal recognition particle-docking protei	2.222344	1.648853	PG1354	hydrolase, carbon-nitrogen family	1.531132	1.566935
PG0209	formate-nitrite transporter	1.518615	10.405116	PG1386	DNA gyrase, A subunit	2.367366	1.788272
PG0219	conserved domain protein	0.341393	0.220550	PG1408	heavy metal efflux pump, CzcD family	2.707660	2.218787
PG0263	tyrosyl-tRNA synthetase	3.272892	2.007284	PG1418	DNA polymerase III, gamma and tau sub	0.425971	0.313783
PG0264	glycosyl transferase, group 2 family prote	1.615535	1.803529	PG1421	ferredoxin, 4Fe-4S	0.203758	0.107592
PG0269	exodeoxyribonuclease III	6.200045	1.746732	PG1428	6,7-dimethyl-8-ribityllumazine synthase	0.462110	3.123827
PG0271	single-stranded binding protein	5.055619	2.977318	PG1434	4-diphosphocytidyl-2C-methyl-D-erythrit	0.417616	0.273176
PG0279	NADP-dependent malic enzyme	3.237683	2.070068	PG1542	collagenase	0.412192	1.813181
PG0288	lipoprotein, putative	3.092561	2.361511	PG1543	thioesterase family protein	0.453677	1.662036
PG0321	LAO-AO transport system ATPase	1.795642	1.673400	PG1551	hmuY protein	2.861161	2.227029
PG0363	conserved domain protein	1.891633	1.749167	PG1565	3-deoxy-D-manno-octulosonic-acid trans	1.563639	2.316964
PG0398	recF protein	0.479325	0.455393	PG1566	glutamyl-tRNA synthetase	2.085671	1.767064
PG0399	lipoprotein, putative	0.378931	0.419567	PG1656	methylmalonyl-CoA mutase, small subun	1.612200	6.548195
PG0425	esterase, putative	0.496253	0.456606	PG1677	phosphoglycerate kinase	2.302612	1.749346
PG0477	pantoate--beta-alanine ligase	1.710677	1.618838	PG1680	ABC transporter, ATP-binding protein, au	0.427675	0.341400
PG0487	ISPg4, transposase	0.392231	0.300814	PG1704	thiol:disulfide interchange protein dsbD, i	2.390943	2.496044
PG0490	membrane protein, putative	0.357015	0.399497	PG1748	transketolase	1.996145	1.668286
PG0500	queueine tRNA-ribosyltransferase	1.767351	3.032677	PG1765	acyl carrier protein	0.367369	0.466161
PG0538	outer membrane efflux protein	5.501583	1.650916	PG1821	cytochrome c nitrite reductase, small sub	2.107820	1.672335
PG0539	efflux transporter, MFP component, RND	6.075616	1.782448	PG1880	glycosyl transferase, group 2 family prote	1.819053	1.529053
PG0589	GMP synthase	2.176307	7.681916	PG1885	polyphosphate kinase	2.179440	1.647784
PG0593	htrA protein	4.681370	1.902767	PG1907	ISPg3, transposase, interruption	0.338670	0.287427
PG0669	heme-binding protein FetB	3.422002	1.503941	PG1959	ribosomal protein L33	0.241146	1.664087
PG0690	4-hydroxybutyrate CoA-transferase	2.963239	2.526798	PG1975	hemagglutinin protein HagC	2.263026	2.440213
PG0691	NifU-related protein	2.728146	4.296452	PG2001	signal peptidase I	0.473326	0.343133
PG0708	peptidyl-prolyl cis-trans isomerase, FKBP	1.869340	2.621353	PG2047	helicase, putative	2.757262	3.027083
PG0709	peptidyl-prolyl cis-trans isomerase FkpA,	1.668251	2.029991	PG2068	glycerol-3-phosphate cytidyltransferase	2.138320	4.189991
PG0714	copper homeostasis protein CutC	2.912137	1.631719	PG2069	oxidoreductase, short chain dehydrogena	2.066560	1.506493
PG0720	DNA-binding response regulator	0.340055	0.308510	PG2085	tryptophanyl-tRNA synthetase	1.751071	1.601161
PG0754	DNA topoisomerase I	1.651008	8.392860	PG2090	cation efflux family protein	1.544048	2.263579
PG0758	peptidyl-dipeptidase Dcp	1.659407	1.705991	PG2102	immunoreactive 61 kDa antigen PG91	0.347498	0.484789
PG0791	adenylate kinase	1.513115	1.976713	PG2124	glyceraldehyde 3-phosphate dehydrogen	1.769877	1.646696
PG0795	membrane protein, putative	2.267232	2.219300	PG2169	ISPg1, transposase, degenerate	0.306410	0.375058
PG0951	phosphoribosylaminoimidazole carboxyla	1.809241	5.217410	PG2177	NADH:ubiquinone oxidoreductase, Na tra	1.509684	1.942643
PG0976	phosphoribosylaminoimidazole-succinoca	1.836230	2.206805	PG2207	conserved domain protein	1.526445	0.452387
PG0977	ubiquinone-menaquinone biosynthesis m	2.086000	2.789827	PG2213	nitrite reductase-related protein	0.269171	0.210389
PG0978	shikimate 5-dehydrogenase	2.712960	1.949862	PG2218	potassium uptake protein TrkA	2.590488	2.147388
PG1072	MutS family protein	2.579247	1.887179	PG2221	MiaB-like tRNA modifying enzyme	2.087051	1.668688
PG1109	mobilization protein	0.376185	0.270430	PG0086	ATP-dependent RNA helicase, DEAD-DEA	0.460661	3.035061

Table 14- Genes found differentially expressed in *P. gingivalis* grown in both pH 5.5 anaerobic and pH 5.5 microaerophilic conditions when compared to pH 8.5

continued from previous page...

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0110	glycosyl transferase, group 1 family prote	0.435332	1.886336	PG0383	membrane-associated zinc metalloprotea	0.334300	2.886118
PG0378	translation elongation factor Ts	0.396765	1.779085				

* = Gene ID according to JCVI

** = fold change (ratio of pH 5.5/pH 8.5) anaerobic

*** = fold change (ratio of pH 5.5/pH 8.5) microaerophilic

adj. p-value<0.05, repeats= 4

Table 15- Genes found differentially expressed in *P. gingivalis* grown in both pH 6.5 anaerobic and pH 6.5 microaerophilic conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0430	oxidoreductase, putative	2.979863	1.737407	PG0945	ABC transporter, permease protein, putal	3.228826	1.797902
PG0555	DNA-binding protein, histone-like family	3.792870	2.354793	PG1116	methylenetetrahydrofolate dehydrogenas	3.420568	1.871834
PG0754	DNA topoisomerase I	1.817370	1.542542	PG1421	ferredoxin, 4Fe-4S	1.674457	0.197316

* = Gene ID according to JCVI

** = fold change (ratio of pH 6.5/pH 8.5) anaerobic

*** = fold change (ratio of pH 6.5/pH 8.5) microaerophilic

adj. p-value<0.05, repeats= 4

Table 16- Genes found differentially expressed in *P. gingivalis* grown in both pH 7.0 anaerobic and pH 7.0 microaerophilic conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0006	MATE efflux family protein	0.408561	1.689228	PG1162	ATP:cob(I)alamin adenosyltransferase, pi	1.532136	0.469765
PG0090	Dps family protein	3.955958	0.488016	PG1203	transcriptional regulator, putative	0.395288	0.277481
PG0128	conserved domain protein	1.648570	0.483501	PG1232	glutamate dehydrogenase, NAD-specific	1.736035	2.179736
PG0148	sigma-54-dependent transcriptional regul	1.503876	0.448817	PG1258	DNA-binding protein HU	0.479683	0.464223
PG0186	lipoprotein RagB	2.150504	1.685806	PG1709	ISPg5, transposase Orf1	2.231561	2.338359
PG0430	oxidoreductase, putative	1.817890	1.751951	PG1948	lipoprotein, putative	1.532650	1.567987
PG0462	transporter, putative	1.687536	1.642270	PG2071	conserved domain protein	1.514610	1.514030
PG0618	alkyl hydroperoxide reductase, C subunit	1.718636	1.657340	PG2205	2-dehydropantoate 2-reductase	0.457175	1.502074
PG0687	succinate-semialdehyde dehydrogenase	2.802463	2.155208	PG2213	nitrite reductase-related protein	0.242317	0.438335
PG0754	DNA topoisomerase I	1.538286	1.961892				

* = Gene ID according to JCVI

** = fold change (ratio of pH 7.0/pH 8.5) anaerobic

*** = fold change (ratio of pH 7.0/pH 8.5) microaerophilic

adj. p-value<0.05, repeats= 4

Table 17- Genes found differentially expressed in *P. gingivalis* grown in both pH 5.5 (anaerobic and microaerophilic and pH 6.5 (microaerophilic) conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0028	2C-methyl-D-erythritol 2,4-cyclodiphosph	2.233805	2.704860	PG0754	DNA topoisomerase I	1.542542	1.651008
PG0134	magnesium transporter	1.524825	3.212797	PG0795	membrane protein, putative	1.556084	2.267232
PG0209	formate-nitrite transporter	1.547875	1.518615	PG0977	ubiquinone-menaquinone biosynthesis m	1.680181	2.086000
PG0263	tyrosyl-tRNA synthetase	1.994716	3.272892	PG0978	shikimate 5-dehydrogenase	1.519975	2.712960
PG0269	exodeoxyribonuclease III	1.605945	6.200045	PG1327	aminotransferase, putative	1.729155	4.488317
PG0271	single-stranded binding protein	1.716737	5.055619	PG1386	DNA gyrase, A subunit	1.511098	2.367366
PG0279	NADP-dependent malic enzyme	1.805767	3.237683	PG1408	heavy metal efflux pump, CzcD family	1.769226	2.707660
PG0321	LAO-AO transport system ATPase	1.956670	1.795642	PG1421	ferredoxin, 4Fe-4S	0.197316	0.203758
PG0363	conserved domain protein	1.685384	1.891633	PG1821	cytochrome c nitrite reductase, small sub	1.616973	1.969020
PG0477	pantoate--beta-alanine ligase	1.527323	1.710677	PG1959	ribosomal protein L33	0.453530	0.241146
PG0538	outer membrane efflux protein	1.916420	5.501583	PG2124	glyceraldehyde 3-phosphate dehydrogen	1.762690	1.769877
PG0539	efflux transporter, MFP component, RND	2.117577	6.075616	PG2213	nitrite reductase-related protein	0.458980	0.269171

* = Gene ID according to JCVI

** = fold change (ratio of pH 5.5/pH 8.5)

*** = fold change (ratio of pH 6.5/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 18- Genes found differentially expressed in *P. gingivalis* grown in both pH 7.0 (anaerobic and microaerophilic and pH 6.5 (microaerophilic) conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0090	Dps family protein	3.955958	1.656635	PG1232	glutamate dehydrogenase, NAD-specific	1.736035	1.889452
PG0430	oxidoreductase, putative	1.817890	1.737407	PG1258	DNA-binding protein HU	0.479683	0.312601
PG0618	alkyl hydroperoxide reductase, C subunit	1.718636	1.725822	PG1948	lipoprotein, putative	1.532650	1.540220
PG0754	DNA topoisomerase I	1.538286	1.542542	PG2213	nitrite reductase-related protein	0.242317	0.458980
PG1203	transcriptional regulator, putative	0.395288	0.362307				

* = Gene ID according to JCVI

** = fold change (ratio of pH 6.5/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 19- Genes found differentially expressed in *P. gingivalis* grown in both pH 5.5 (anaerobic and microaerophilic and pH7.0 (anaerobic and microaerophilic) conditions when compared to pH 8.5

ID*	Common Name	Fld1**	Fld2***	ID*	Common Name	Fld1**	Fld2**
PG0754	DNA topoisomerase I	1.651008	1.538286	PG2213	nitrite reductase-related protein	0.269171	0.242317

* = Gene ID according to JCVI

** = fold change (ratio of pH 5.5/pH 8.5)

*** = fold change (ratio of pH 7.0/pH 8.5)

adj. p-value<0.05, repeats= 4

Table 20- Gene expression comparing raw data from pH conditons (pH5.5, 6.5 and 7.0) microaerophilic and anaerobic against pH 8.5

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG0001	chromosomal replication initiator protein	0.285678	1.218983	PG0708	peptidyl-prolyl cis-trans isomerase, FKBP	0.864988	1.821324
PG0021	TIM-barrel protein, putative, NifR3 family	-0.333659	0.793521	PG0709	peptidyl-prolyl cis-trans isomerase FkpA,	0.567759	1.482219
PG0042	serine hydroxymethyltransferase	0.470429	1.385522	PG0713	anthranilate synthase component II	-0.475903	0.719017
PG0057	nicotinate phosphoribosyltransferase	-0.518410	0.698141	PG0721	NLP-P60 family protein	-0.840293	0.558530
PG0070	acyl-(acyl-carrier-protein)-UDP-N-acetylgl	0.517095	1.431071	PG0734	nitroreductase family protein	-0.540866	0.687358
PG0084	L-serine dehydratase, iron-sulfur-depend	-0.372040	0.772689	PG0752	uracil phosphoribosyltransferase, putative	0.444233	1.360591
PG0130	phosphoglycerate mutase	0.343873	1.269159	PG0754	DNA topoisomerase I	1.140139	2.204022
PG0143	hydrolase, carbon-nitrogen family	0.413309	1.331737	PG0758	peptidyl-dipeptidase Dcp	0.582702	1.497651
PG0162	RNA polymerase sigma-70 factor, ECF su	0.320030	1.248357	PG0760	ISPg1, transposase, authentic frameshift	-0.380960	0.767926
PG0223	exonuclease	0.332563	1.259248	PG0777	electron transfer flavoprotein, beta subur	-0.392872	0.761612
PG0235	carboxyl-terminal protease	-0.411354	0.751917	PG0782	MotA-TolQ-ExbB proton channel family pr	0.395057	1.314995
PG0241	lipoprotein, putative	0.492533	1.406913	PG0785	tonB protein, putative	0.433494	1.350500
PG0248	translation initiation factor SUI1, putative	-0.538727	0.688378	PG0906	lipoprotein, putative	0.355627	1.279542
PG0288	lipoprotein, putative	0.799145	1.740069	PG0910	FHA domain protein	-0.377071	0.769999
PG0307	electron transport complex, RnfABCDGE t	0.396748	1.316537	PG0925	thymidine kinase	-0.230719	0.852210
PG0310	nitroreductase family protein	0.539944	1.453916	PG0945	ABC transporter, permease protein, putal	0.832320	1.780546
PG0311	glycosyl transferase, group 2 family prote	0.500249	1.414457	PG0976	phosphoribosylaminoimidazole-succinoca	0.458653	1.374258
PG0315	ribosomal protein L27	0.287988	1.220937	PG0977	ubiquinone-menaquinone biosynthesis m	0.747344	1.678700
PG0343	methionine gamma-lyase	-0.412312	0.751418	PG0985	RNA polymerase sigma-70 factor, ECF su	-0.665584	0.630434
PG0347	UDP-glucose 4-epimerase	-0.533418	0.690916	PG1082	phosphotransacetylase	0.361089	1.284395
PG0376	ribosomal protein S9	0.225079	1.168841	PG1115	signal recognition particle protein	0.432807	1.349858
PG0377	ribosomal protein S2	0.511998	1.426024	PG1116	methylenetetrahydrofolate dehydrogenas	0.651614	1.570925
PG0385	ribosomal protein S21	-0.805161	0.572298	PG1126	uracil permease	0.245290	1.185331
PG0386	site-specific recombinase, phage integras	-0.976395	0.508248	PG1171	oxidoreductase, putative	0.515626	1.429614
PG0387	translation elongation factor Tu	0.513223	1.427235	PG1215	lipoprotein protein, putative	-0.161231	0.894262
PG0399	lipoprotein, putative	-0.584166	0.667035	PG1232	glutamate dehydrogenase, NAD-specific	0.321605	1.249720
PG0430	oxidoreductase, putative	0.878186	1.838062	PG1259	anaerobic ribonucleoside-triphosphate rei	-0.423305	0.745714
PG0468	mannose-6-phosphate isomerase, class I	0.454234	1.370055	PG1290	branched-chain amino acid aminotransfer	0.435601	1.352474
PG0485	preprotein translocase, YajC subunit	-0.439178	0.737555	PG1328	CoA ligase family protein	0.398176	1.317841
PG0502	SsrA-binding protein	-0.565414	0.675761	PG1330	large conductance mechanosensitive chai	0.539777	1.453748
PG0511	spore maturation protein A-spore matura	-0.321084	0.800468	PG1332	NAD(P) transhydrogenase, beta subunit	0.300903	1.231916
PG0518	abortive infection protein family	0.101813	1.073121	PG1361	dipeptidyl aminopeptidase IV, putative	-0.545631	0.685091
PG0539	efflux transporter, MFP component, RND	0.748007	1.679471	PG1421	ferredoxin, 4Fe-4S	-1.843316	0.278681
PG0548	pyruvate ferredoxin-flavodoxin oxidoredu	0.498145	1.412397	PG1540	S-adenosylmethionine:tRNA ribosyltransf	-0.232954	0.850891
PG0555	DNA-binding protein, histone-like family	1.203244	2.302568	PG1559	glycine cleavage system T protein	0.560104	1.474375
PG0573	S-adenosyl-methyltransferase MraW	0.346545	1.271512	PG1562	dTDP-4-dehydrorhamnose 3,5-epimerase	0.289373	1.222109
PG0588	3-methyl-2-oxobutanoate hydroxymethyl	0.346967	1.271884	PG1656	methylmalonyl-CoA mutase, small subun	0.914101	1.884394
PG0589	GMP synthase	0.905740	1.873505	PG1713	lipoprotein, putative	-0.505316	0.704506
PG0595	ribosomal protein S6	0.743535	1.674273	PG1755	fructose-bisphosphate aldolase, class I	0.574483	1.489144
PG0634	ThiJ-PfpI family protein	0.255310	1.193592	PG1758	ribosomal protein S15	-0.787924	0.579177
PG0639	signal peptide peptidase SppA, 67K type	0.428254	1.345605	PG1765	acyl carrier protein	-0.653009	0.635953
PG0658	phosphatase, YrbI family	0.238858	1.180058	PG1771	phenylalanyl-tRNA synthetase, alpha sub	0.495630	1.409937
PG0674	indolepyruvate ferredoxin oxidoreductase	0.549449	1.463527	PG1781	uridine kinase	-0.475240	0.719347
PG0675	indolepyruvate ferredoxin oxidoreductase	-0.281642	0.822654	PG1812	2-oxoglutarate oxidoreductase, alpha sut	0.323629	1.251474
PG0678	pyrazinamidase-nicotinamidase, putative	-0.382603	0.767052	PG1816	NAD(P)H dehydrogenase, quinone family,	0.455437	1.371198
PG0689	NAD-dependent 4-hydroxybutyrate dehyd	0.426618	1.344079	PG1828	lipoprotein, putative	-0.734970	0.600830
PG0691	NifU-related protein	1.167656	2.246465	PG1847	endoribonuclease L-PSP, putative	0.487277	1.401796
PG0692	4-hydroxybutyryl-CoA dehydratase	0.812880	1.756715	PG1852	exonuclease	0.261442	1.198676

Table 20- Gene expression comparing raw data from pH conditons (pH5.5, 6.5 and 7.0) microaerophilic and anaerobic against pH 8.5

Continued from previous page...

ID*	Common Name	M**	Fld***	ID*	Common Name	M**	Fld***
PG1858	flavodoxin	0.479548	1.394307	PG1946	ABC 3 transporter family protein	0.367344	1.289976
PG1887	rhodanese-like domain protein	-0.400513	0.757589	PG1947	TPR domain protein	0.622203	1.539224
PG1913	ribosomal protein S11	0.486674	1.401211	PG1949	malate dehydrogenase	0.501802	1.415981
PG1917	methionine aminopeptidase, type I	0.322602	1.250584	PG1953	YitT family protein	-0.338256	0.790997
PG1920	ribosomal protein L30	0.423855	1.341507	PG1956	4-hydroxybutyrate CoA-transferase	0.362215	1.285398
PG1921	ribosomal protein S5	0.398761	1.318375	PG1984	CRISPR-associated protein, TM1791.1 far	-0.405993	0.754717
PG1922	ribosomal protein L18	0.583663	1.498649	PG2026	phosphoglycerate mutase family protein	-0.413413	0.750845
PG1923	ribosomal protein L6	0.421229	1.339068	PG2028	ebsC protein	-0.540642	0.687465
PG1925	ribosomal protein S14	0.319018	1.247481	PG2121	L-asparaginase	0.397777	1.317476
PG1926	ribosomal protein L5	0.579091	1.493908	PG2140	ribosomal protein L32	0.057112	1.040381
PG1927	ribosomal protein L24	0.560021	1.474291	PG2150	LysM domain protein	0.472218	1.387241
PG1928	ribosomal protein L14	0.531243	1.445174	PG2177	NADH:ubiquinone oxidoreductase, Na tra	0.456714	1.372412
PG1930	ribosomal protein L29	0.320990	1.249188	PG2178	NADH:ubiquinone oxidoreductase, Na tra	0.401911	1.321257
PG1931	ribosomal protein L16	0.303893	1.234471	PG2181	NADH:ubiquinone oxidoreductase, Na tra	-0.248145	0.841978
PG1933	ribosomal protein L22	0.332490	1.259184	PG2198	hemagglutinin protein, truncation	-0.956646	0.515253
PG1934	ribosomal protein S19	0.525027	1.438961	PG2215	mannose-1-phosphate guanylyltransferas	0.670869	1.592032

* = Gene ID according to JCVI

** = log(spot intensity all pH/pH 8.5)

*** = fold change (ratio of all pH/pH 8.5)

adj. p-value<0.05, repeats= 24

Table 21- Genes of interest regulated in pH 5.5 metabolic pathways

Gene ID	Common Name	Pathway Involved
PG0084	L-serine dehydratase	serine degradation
PG0343	methionine gamma lyase	methionine degradation
PG1232	glutamate dehydrogenase	glutamate degradation
PG1081	acetate kinase	mixed acid fermentation
PG1082	phosphotransacetylase	mixed acid fermentation
PG1949	malate dehydrogenase	mixed acid fermentation
PG0429	pyruvate synthase	mixed acid fermentation
PG1576	L-aspartate oxidase	mixed acid fermentation
PG1821	cytochrome C nitrite reductase	nitrate reduction
PG0209	formate-nitrite transporter enoyl-(acyl-carrier-protein) reductase II	nitrite uptake
PG1416	reductase II	fatty acid and lipid biosynthesis
PG0046	phosphatidate cytidyltransferase 1-acyl-sn-glycerol-3-phosphate acetyltransferase	fatty acid and lipid biosynthesis
PG1249	acetyltransferase	fatty acid and lipid biosynthesis
PG2109	hydroxymethylpyrimidine kinase	thiamine biosynthesis I
PG2217	deoxyxylulose-5-phosphate synthase	thiamine biosynthesis I
PG2108	thiG protein	thiamine biosynthesis I
PG1260	anaerobic ribonucleoside- triphosphate reductase	purine/pyrimidine <i>de novo</i> biosynthesis
PG0953	deoxyuridine 5-triphosphate nucleotidohydrolase	purine/pyrimidine <i>de novo</i> biosynthesis
PG1129	ribonucleotide reductase	purine/pyrimidine <i>de novo</i> biosynthesis
PG0042	serine hydroxymethyltransferase	Formyl THF biosynthesis/ folate transformations
PG1321	formate--tetrahydrofolate ligase	Formyl THF biosynthesis/ folate transformations
PG1116	methylendetetrahydrofolate dehydrogenase	Formyl THF biosynthesis/ folate transformations
PG1559	glycine cleavage system T protein	Formyl THF biosynthesis/ folate transformations

Table 22: Summary of each functional role category as defined by JVICI and the associated number of genes

Letter Abbreviation	Functional Role Category	Total Number of Genes
A	Amino acid synthesis	19
B	Biosynthesis cofactors, prosthetic groups, and carriers	85
C	Cell envelope	140
D	Cellular processes	46
E	Central intermediary metabolism	23
F	Disrupted reading frame	44
G	DNA metabolism	81
H	Energy metabolism	129
I	Fatty acid and phospholipid metabolism	16
J	Mobile and extrachromosomal element function	146
K	Protein fate	77
L	Protein synthesis	118
M	Purine, pyrimidines, nucleotides, and nucleosides	44
N	Regulatory functions	43
O	Signal transduction	12
P	Transcription	30
Q	Transport and binding proteins	118
R	Unknown function	201

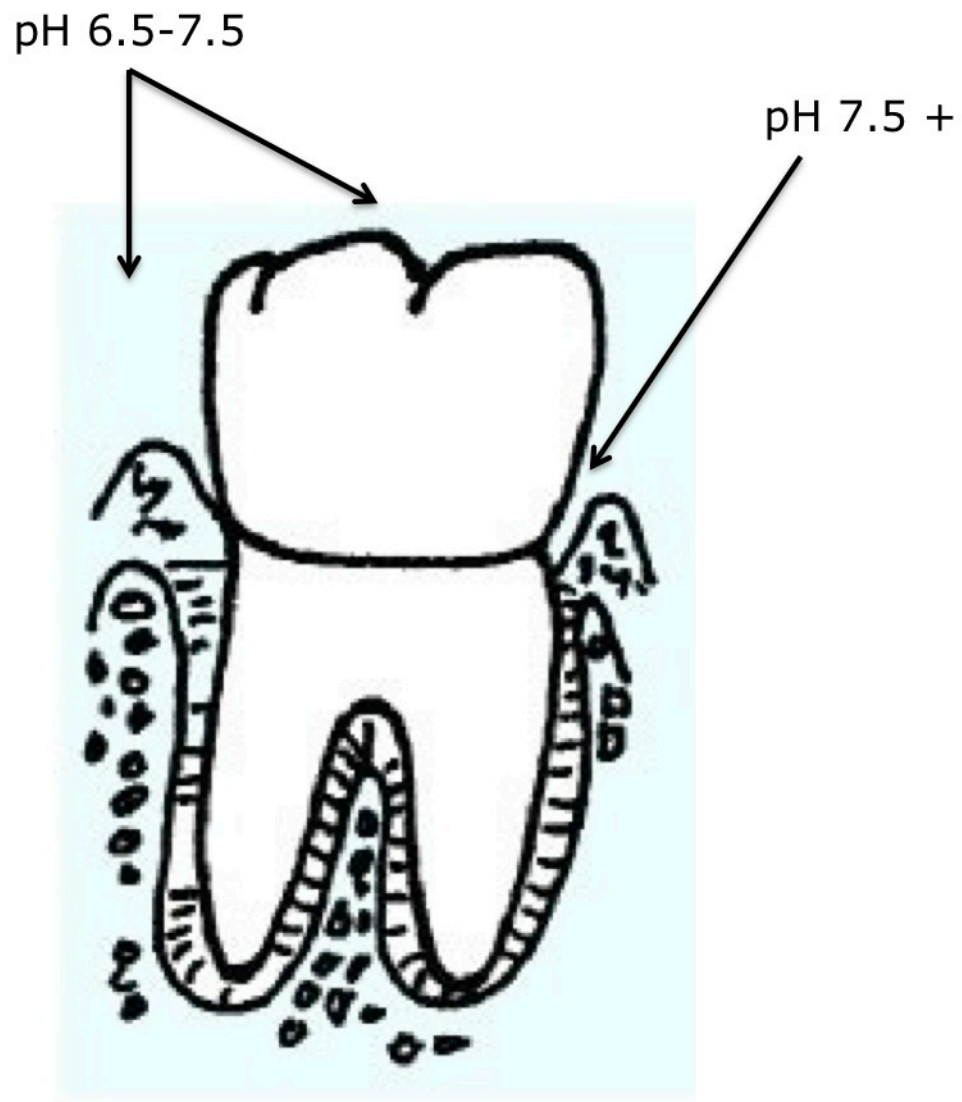


Figure 1: pH conditions vary in the oral cavity. On the surface of the tooth, in the oral cavity and within the biofilm, pH is between 6.5 and 7.5 regularly, becoming more acidic based on diet or oral inhabitants. In the subgingival pockets, pH is 7.5 or greater, becoming more alkaline as disease progresses. Figure adapted from Dye *et. al* (2002).

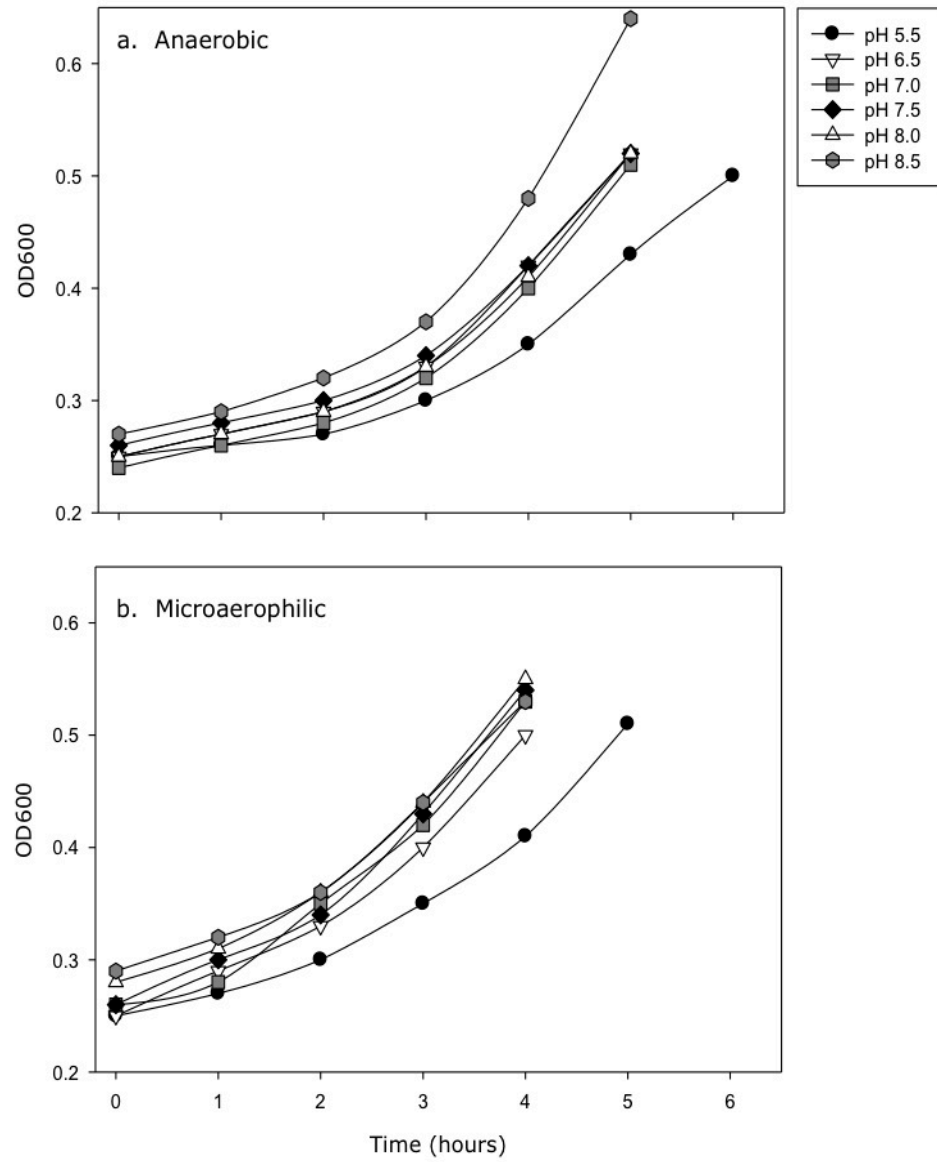


Figure 2- Example growth curves for *Porphyromonas gingivalis* W83 grown in six different pH conditions.

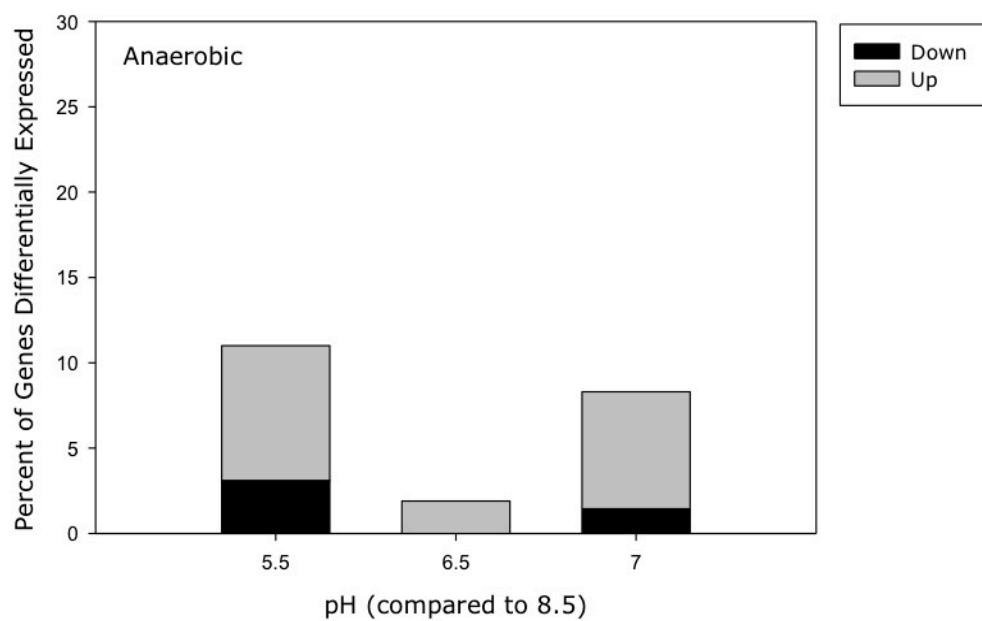


Figure 3- Percentage of genes differentially expressed (1.5 fold up or down) in *Porphyromonas gingivalis* W83 when grown at pH 5.5, 6.5 and 7.0 compared to *P. gingivalis* grown at pH 8.5 in anaerobic conditions

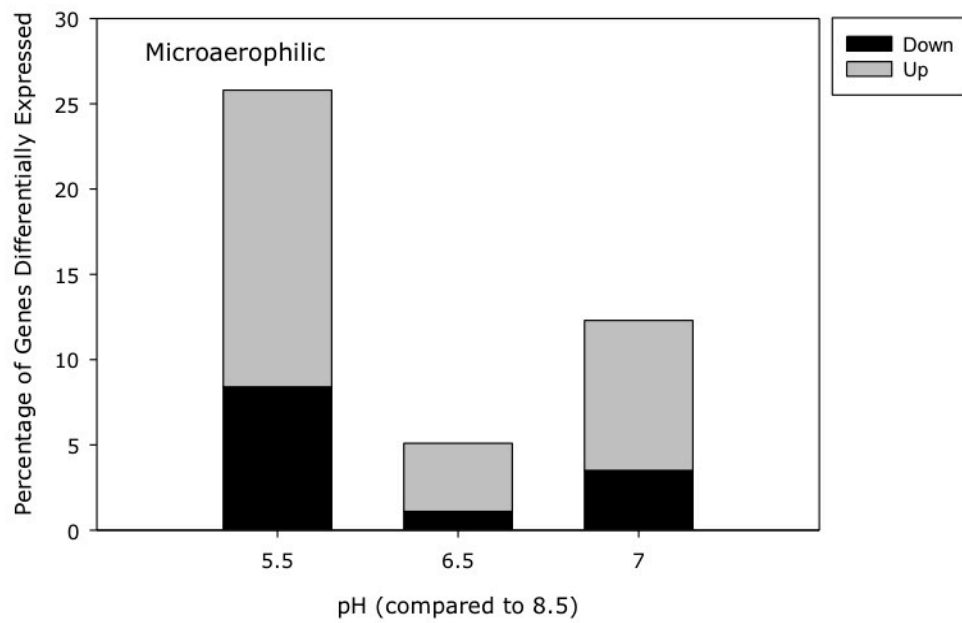


Figure 4- Percentage of genes differentially expressed (1.5 fold up or down) in *Porphyromonas gingivalis* W83 when grown at pH 5.5, 6.5 and 7.0 compared to *P. gingivalis* grown at pH 8.5 in microaerophilic conditions.

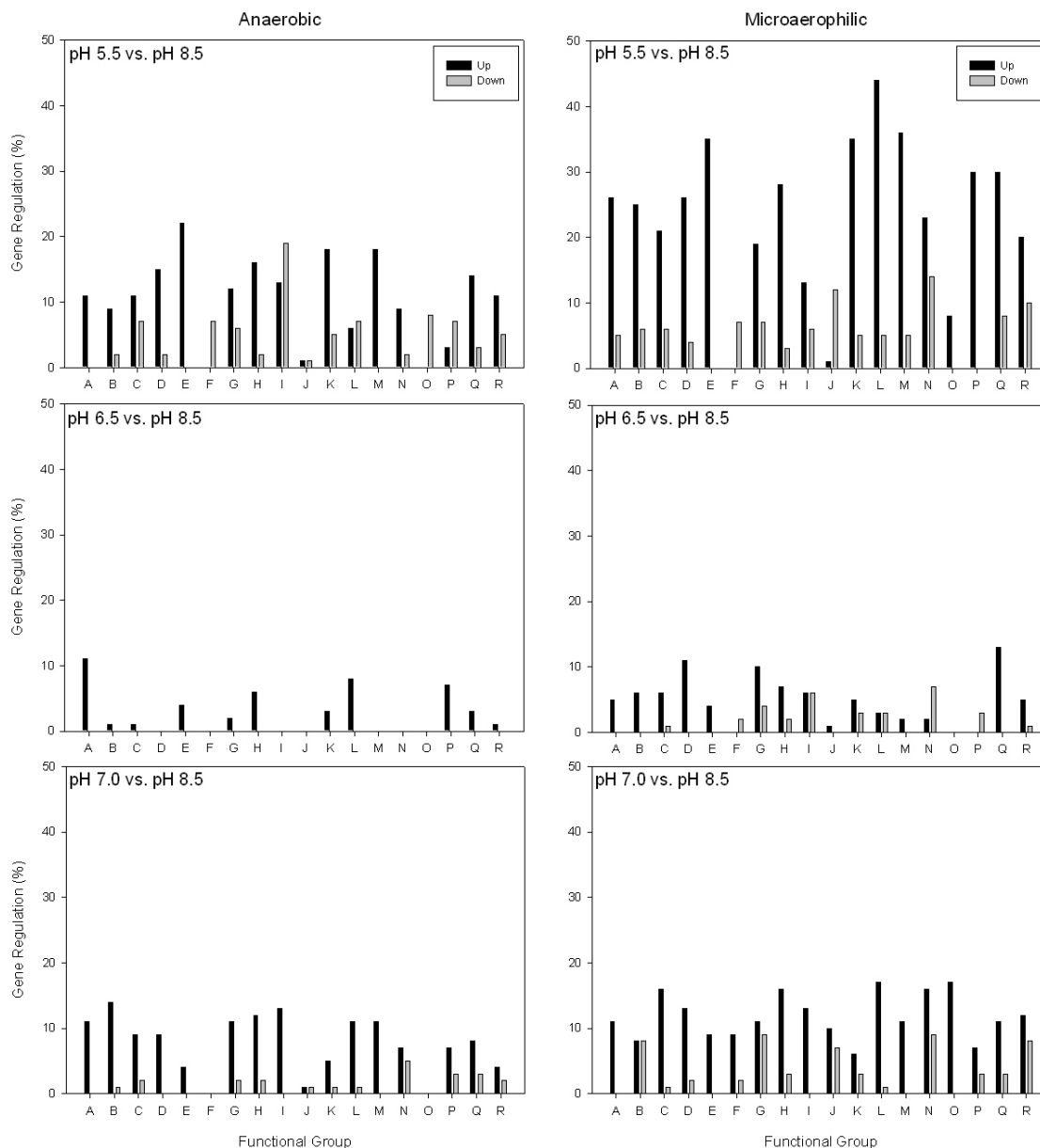


Figure 5- Genes differentially expressed (1.5 fold up or down) in *Porphyromonas gingivalis* W83 grown at pH 5.5, 6.5 or 7.0 versus pH 8.5. Functional role categories by JCVI (formerly TIGR) are: A, Amino acid biosynthesis; B, Biosynthesis cofactors prosthetic groups, and carriers; C, cell envelope; D, Cellular processes; E, Central intermediary metabolism; F, Disrupted reading frame; G, DNA metabolism; H, Energy metabolism; J, Mobile and extrachromosomal element function; K, Protein fate; L, Protein synthesis; M, Purine, pyrimidines, nucleotides, and nucleosides; N, Regulatory functions; O, Signal transduction; P, Transcription; Q, Transport and binding proteins; R, unknown functions.

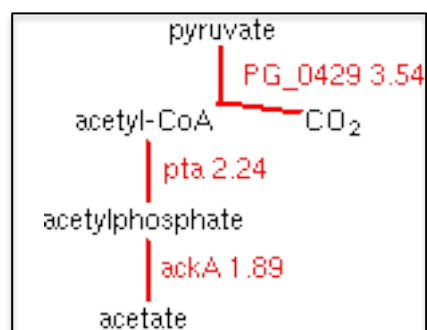
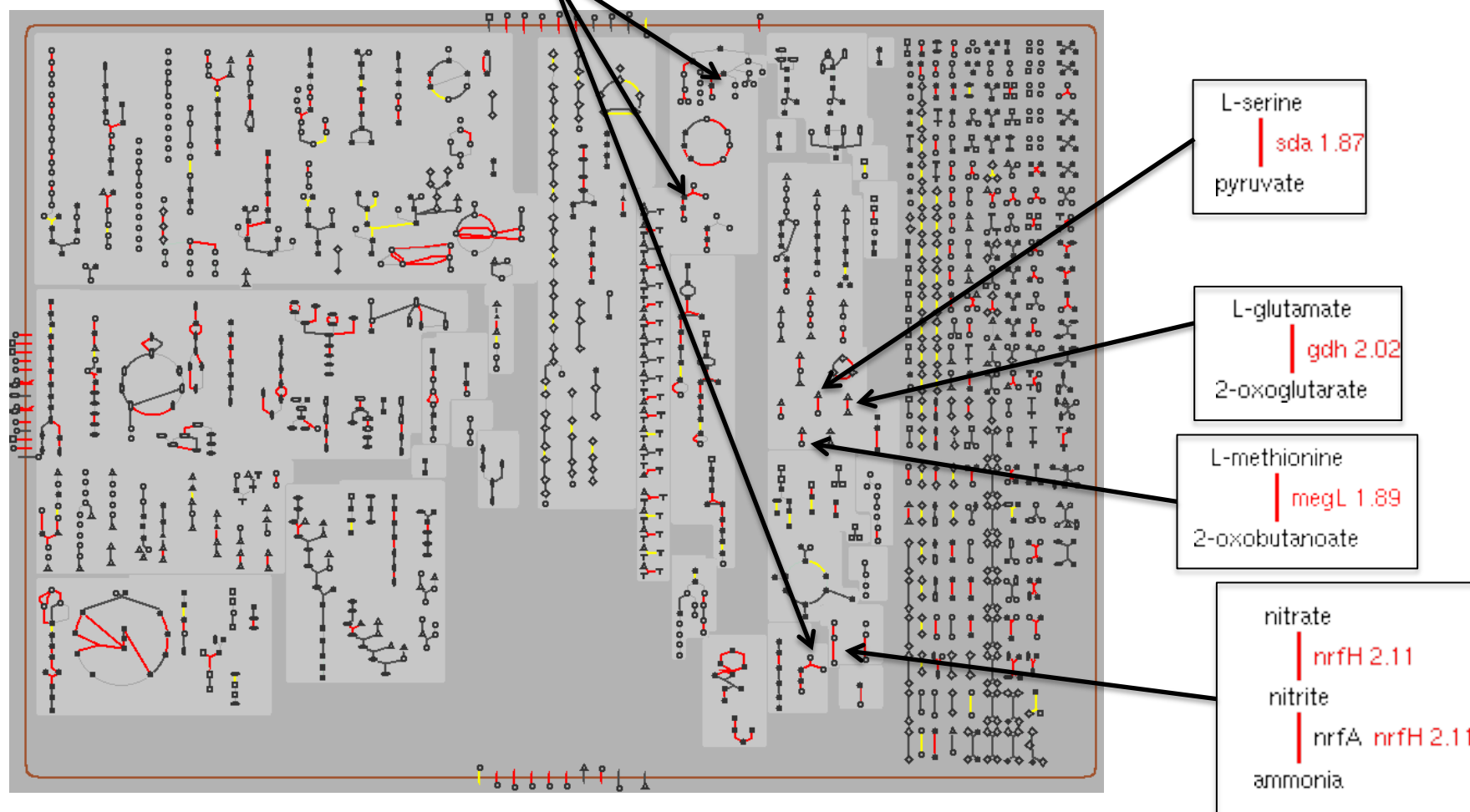


Figure 6- This figure illustrates regulation of metabolism in *P. gingivalis* when comparing general regulation in pH 5.5 anaerobic and microaerophilic vs. pH 8.5. Red lines indicate that RNA abundance for the gene encoded is up-regulated in 5.5 vs. 8.5 (no fold cutoff). Yellow lines indicate down-regulation in 5.5 vs. 8.5. Specific pathway of interest have been highlighted in boxes.



Vita

Nathan Scott Maietta was born on June 4, 1985 in Lake Forest, IL. He lived in California, South Carolina and Sicily before arriving in Virginia Beach where he attended Kellam High School. He received a B.S. in Health Sciences from James Madison University in 2007.