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Expression patterns of genes induced by oxidative stress in *Porphyromonas gingivalis*

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Introduction: *Porphyromonas gingivalis*, a gram-negative anaerobic bacterium, is a major periopathogen whose transmission from host to host involves exposure to atmospheric oxygen. *P. gingivalis* contains genetic factors that function in an oxidative stress response, but their expression has not been analyzed during exposure to atmospheric oxygen. The aim of this study was to obtain a better understanding of atmospheric adaptation of *P. gingivalis*.

Methods: The aerotolerance of wild-type and *oxyR* mutant *P. gingivalis* strains were determined, and quantitative polymerase chain reaction was performed to analyze gene expression patterns in response to exposure to atmospheric oxygen. The analyzed *P. gingivalis* genes encoded proteins involved in oxidative response (*oxyR*, *ahp*C-*F*, *batA*, *dps*, *ftn*, *tpx*) as well as several major virulence factors (*hagA*, *hagB*, *hagE*, *rgpA*, *rgpB*, *hem*).

Results: Our results demonstrated a critical role for the *oxyR* gene in the aerotolerance of *P. gingivalis*. The *ahpC-F*, *batA*, and *hem* genes were slightly overexpressed (between 1.65-fold and 2-fold) after exposure to atmospheric oxygen compared to anaerobic conditions. The level of transcription of *dps*, *ftn*, *tpx*, and *rgpA* genes increased more than 2.5-fold, and the expression of *ahpC-F*, *dps*, *ftn*, and *tpx* was partially or completely OxyR-dependent.

Conclusion: A different transcription pattern of *P. gingivalis* genes was observed, depending on the stimulus of oxidative stress. We present new evidence that the expression of *tpx*, encoding a thiol peroxidase, is partially OxyR-dependent and is induced after atmospheric oxygen exposure.

Key words: gene expression; oxidative stress; *Porphyromonas gingivalis*; *tpx*

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Porphyromonas gingivalis is a gram-negative anaerobic rod bacteria recognized as a major pathogen for aggressive and chronic periodontitis, infection, and inflammation of the ligaments and bones that support the teeth. *P. gingivalis* is a secondary colonizer found in the periodontal biofilm, and is associated with other aero-anaerobic bacteria, which allow its growth and virulence in an anaerobic ecological niche (15). Colonization by *P. gingivalis* is facilitated by many virulence factors, including adhesion proteins such as hemagglutinins, which are critical factors that mediate its interaction with host tissues and commensal bacteria (1, 17, 30). *P. gingivalis* also expresses proteases known as gingipains, which function in tissue infection and destruction; moreover, the gingipain RgpA has several hemagglutinin domains that promote adhesion (23).

Before its adhesion to oral cavity surfaces or to primary-colonizing bacteria, *P. gingivalis* must survive the transit from host to host, during which time it is exposed to atmospheric oxygen, which subjects the bacteria to oxidative stress. Although strictly anaerobic, *P. gingivalis* is able to survive in the presence of atmospheric oxygen (19), likely an essential characteristic for its virulence because the infection sites are aerobic. Superoxide (O_2^-) is the first reactive oxygen species derived from oxygen and triggers the SoxRS regulon in *Escherichia coli* and

Salmonella spp. to respond to the initial oxidative stress (8). *P. gingivalis* do not contain the SoxRS regulon, but instead express a superoxide dismutase (*sod*, PG1545) (21) that converts superoxide to hydrogen peroxide (H_2O_2) (16).

The effect of oxygen on an obligatory anaerobe is likely to be complex. Oxygen usually creates a high redox environment with active metabolic products, such as superoxide, hydrogen peroxide, and hydroxyl radicals, which may convert bacterial enzymes into oxidized, and therefore inactivated, forms. This inactivation may be the source of, or may contribute to, the inability of anaerobes to grow in the presence of oxygen. Moreover, oxidative stress may cause direct damage to bacterial proteins, nucleic acids, and membranes. Several genes have been identified in *P. gingivalis* that are involved in protection against reactive oxygen species during the oxidative stress response. Catalases are known to be involved in detoxification of hydrogen peroxide, but no gene encoding catalase has been found in the *P. gingivalis* genome. However, *P. gingivalis* contains the alkyl hydroperoxide reductase *ahpC-F* operon. AhpC reduces alkyl hydroperoxide in alcohol and is reduced by AhpF (32), so preserving the bacteria from oxidative damage. The *dps* gene encodes a DNA-binding Protein from Starved cells (Dps), which protects cells by its physical association with DNA and nullifies the toxic combination of iron and H_2O_2 or O_2, so avoiding oxidative damage mediated by Fenton chemistry (35). The intracellular iron-storage ferritin protein, encoded by the *ftn* gene, is particularly important for *P. gingivalis* to survive under iron-depleted conditions but it does not appear to be sufficient to protect the bacteria against the oxidative stress caused by peroxides and atmospheric oxygen (26); however, it is able to limit Fenton reactions. *batA* is the first gene of a putative aerotolerance operon, and in *Bacteroides fragilis* it ensures bacterial survival in the early stages of the infection process, when the infected sites are aerobic (33). The Bat complex may be involved in the generation or export of reducing power equivalents to the periplasm to protect bacteria from reactive oxygen species. The mechanism in *P. gingivalis* is possibly similar, if not identical, to this. The *tpx* gene encodes a thiol peroxidase that is involved in the removal of peroxides and H_2O_2 in *E. coli* and *B. fragilis* (3, 31).

Analysis of the genomic DNA sequence of *P. gingivalis* revealed the presence of the peroxide-sensing transcription activator OxyR (PG0270), a relatively unknown regulon in *P. gingivalis*. OxyR is known to regulate only a few genes, including the *ahpC-F* operon and *sod* (24), and *dps* and *ftn* seem to be partially responsive to OxyR regulation (6).

This study simulated the exposure of the bacterium to atmospheric oxygen during transmission to obtain a better understanding of the responsive variation in the expression of genes involved in virulence, and the mechanisms of stress regulation contributing to its atmospheric adaptation. The results show that during exposure to atmospheric oxygen, genes that are generally involved in oxidative stress and virulence are upregulated, and the OxyR transcriptional factor promotes the survival of the bacteria during atmospheric stress, partially by controlling the expression of the *tpx* gene.

Materials and methods

Bacterial strains and growth conditions

P. gingivalis W83 wild-type strain, kindly supplied by C. Mouton (University of Laval, Canada), and the *oxyR* mutant were maintained on Columbia agar plates (AES Chemunex, Combourg, France), and incubated at 37°C in an anaerobic atmosphere of 10% H_2, 10% CO_2, and 80% N_2. The *oxyR* mutant strain was generated in our laboratory by insertion of the *ermF–ermAM* antibiotic-resistance gene cassette into the *oxyR* gene in the W83 strain, as previously described (9).

For experimental analysis, both strains were grown in brain–heart infusion broth (AES Chemunex) enriched with hemin (5 μg/ml; Sigma, Saint Quentin Fallavier, France), vitamin K1 (1 μg/ml; Sigma), and yeast extract (0.5%; AES Chemunex), with shaking (150 r.p.m.) in an anaerobic chamber at 37°C.

Aerotolerance test

For each strain of *P. gingivalis*, two flasks of enriched brain–heart infusion broth were inoculated. When the cultures reached the middle of log-phase growth (optical density at 650 nm $\sim$ 0.4–0.5), one flask of each strain was left in anaerobic conditions and the other was incubated in aerobic conditions: shaking (150 r.p.m.) at 37°C with exposure to atmospheric oxygen (i.e. laboratory air). The aerotolerance was assessed at various times by viable counts on Columbia agar plates after dilution. The bacterial cultures left in the anaerobic chamber were used as controls. Aerotolerance experiments were carried out in duplicate and repeated three times for each strain.

RNA extraction and purification

Samples were taken from W83 and *oxyR* mutant strains just before exposure to atmospheric oxygen and 15 min after incubation at 37°C in atmospheric oxygen. Cells (2×10^9 to 3×10^9) were harvested by centrifugation (10,000 **g**, for 5 min at room temperature) and the pellets were immediately frozen until RNA extraction. For bacterial lysis, pellets were resuspended in TE buffer (10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid, pH 8) supplemented with 20 mg/ml lysozyme, incubated for 20 min at 30°C, and then sonicated (10 W; three pulses of 20 s each, with samples chilled in ice between pulses; VibraCell Sonicator, Fisher Bioblock Scientific, Illkirch, France). Total RNA was purified using the RNeasy kit (Qiagen, Courtaboeuf, France) with the DNase digestion step according to the manufacturer's instructions. Residual DNA was removed by a second DNase treatment using the RQ1 RNase-free DNase 1 (Promega, Charbonnières, France) according to the manufacturer's instructions. The quality of the extracted RNA was confirmed with the Agilent 2100 Bioanalyser (Agilent Technologies, Massy, France). The amount of RNA was estimated by determining the absorbance at 260 nm.

Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (PCR) was used to assess the level of expression of *P. gingivalis* genes whose function has been implicated in the oxidative stress response and in virulence (Table 1). Total RNA (0.5 μg) from each experimental condition and each strain was used in the reverse transcription reaction using the 1st Strand cDNA Synthesis Kit for reverse transcription-PCR (AMV; Roche Diagnostics, Meylan, France) with random hexamer primers according to the manufacturer's recommendations.

PCR amplification was performed on a LightCycler (Roche Diagnostics). A master mix of the following reaction components was prepared with the final concentrations as indicated: $MgCl_2$ (2.5 mM), each primer (0.5 μM), and 1 μl LightCycler Fast Start DNA Master SYBR

Table 1. Genes and specific primers used in the real-time polymerase chain reaction analysis

Locus no.[1]	Gene	Description	Primer sequence 5′ → 3′	Length (bp)[2]
PG0618	*ahpC*	Alkyl hydroperoxide reductase C	ctcactcgcgatctgggtat tgtgcagccttgatcttacg	158
PG0619	*ahpF*	Alkyl hydroperoxide reductase F	cttcctacaacgaggcgaag tccgataccgtcattgttga	203
PG0270	*oxyR*	Redox-sensitive transcriptional activator (putative)	ccacaactgaccgtagagca cctgtctgcaacttgtgcat	188
PG0090	*dps*	Dps family protein (DNA-binding protein from starved cells)	cagggaaaatcgacgaggta ggagaggaaaccgatcatca	245
PG1582	*batA*	batA protein	gaggcagccaaagatgtagc ggctttgctgtccttcagtc	225
PG1286	*ftn*	Ferritin	cggcgaggtgaagatagaag ctcctgagagagacggatcg	244
PG1729	*tpx*	Thiol peroxidase	ccgtttcaatcaggaagcat acgagccaagagacctttca	202
PG1837	*hagA*	Hemagglutinin protein HagA	acagcatcagccgatattcc cgaattcattgccaccttct	208
PG1972	*hagB*	Hemagglutinin protein HagB	tgtcacttgacactgctaccaa attcagagccaaatcctcca	230
PG2024	*hagE*	hemagglutinin protein HagE	gccgagattgttcttgaagc aggagcagcaattgcaaagt	276
PG1427	*rgpA*	Thiol protease/hemagglutinin PrtT precursor (putative)	agtaggagccttgcgaaaca tcggcgagaggagtgttagt	260
PG0506	*rgpB*	Arginine-specific cysteine proteinase	cgctgatgaaacgaacttga cttcgaataccatgcggttt	230
PG1875	*hem*	Hemolysin	acgaagccttgttctcctca caatgaatatgccggtttcc	189
PG16SA	*rRNA 16S*	Ribosomal RNA	tgggtttaaagggtgcgtag caatcggagttcctcgtgat	161

[1]Locus number and identification are according to the NCBI *Porphyromonas gingivalis* genome database.
[2]Reverse transcription polymerase chain reaction product length.

Green I (Roche Diagnostics). The master mix (8 µl) was put into glass capillaries, and 2 µl DNA template, diluted to produce results within the confidence interval of the technique, was added according to the manufacturer's instructions. The PCR conditions were as follows: denaturation for 10 min at 95°C; 45 cycles of denaturation (95°C), annealing (55°C), elongation (72°C), and fluorescence acquisition; and a phase of fusion (55–90°C with a temperature transition 0.2°C/s) with stepwise fluorescence acquisition. Finally, the reactions were cooled to 35°C. Crossing point values were acquired using the second derivative maximum method of the LIGHTCYCLER software 3.5 (Roche Diagnostics). Real-time quantitative PCR was carried out for each gene in duplicate from three independent aerotolerance experiments.

Relative expression analysis

Real-time PCR efficiencies were determined by amplification of a PCR product dilution series, and the slopes were determined using LIGHTCYCLER software 3.5 (Roche Diagnostics). The corresponding efficiencies were then calculated according to the equation: $E = 10^{(-1/\text{slope})}$ (25). For each gene tested, the *E*-value was at least 1.8.

The differences in messenger RNA expression, C_T (cycle threshold) value, were calculated by the $\Delta\Delta C_T$ method in which the amount of target RNA was adjusted to the amount of a reference internal RNA, 16S ribosomal RNA (7), from the same extract. The expression of the 16S ribosomal RNA gene showed no significant changes compared with the anaerobic control at any time after exposure to atmospheric oxygen.

Identification of putative OxyR binding sites

Putative OxyR binding motifs in the promoter sequences of the *tpx*, *ftn*, and *dps* genes of *P. gingivalis* were searched using STAN 1.0 (Suffix Tree Analyser) (22). The consensus OxyR binding sequence in *E. coli* (36) and the consensus −35 and −10 box sequences of the *P. gingivalis* promoter (12) served as templates for the bioinformatic analysis.

Results

Culture growth in anaerobic conditions and after atmospheric exposure

P. gingivalis W83 and the *oxyR* mutant showed similar growth patterns under anaerobic conditions. In contrast, the two strains differed in their responses to growth in the presence of atmospheric oxygen. The *P. gingivalis* W83 strain survived but did not continue to grow in aerobic conditions: the growth remained at about 3.5×10^9 to 5×10^9 colony-forming units (CFU)/ml for 7 h after exposure to oxygen (Fig. 1). Under the same aerobic conditions, the concentration of the *oxyR* mutant strain decreased to 10^7 CFU/ml during the same 7 h. This confirms the sensitivity of the *P. gingivalis oxyR* mutant towards oxygen, and furthermore suggests a role for the *oxyR* gene in the aerotolerance of *P. gingivalis*.

Effect of atmospheric oxygen exposure on the relative expression of genes implicated in oxidative stress in *P. gingivalis*

In the wild-type *P. gingivalis*, the expression of OxyR-dependent genes that are known to be involved in the oxidative stress response, such as *ahpC-F*, slightly increased while *oxyR* was unchanged after 15 min of exposure to laboratory air compared to expression levels under anaerobic conditions (Table 2). Expression of *batA* was also slightly increased both in the wild-type strain and in the *oxyR* mutant strain. Expression of *dps* increased 2.76-fold (C_T value range between 1.21 and 4.41), *ftn* increased 9.29-fold (2.14–19.9), and *tpx* increased

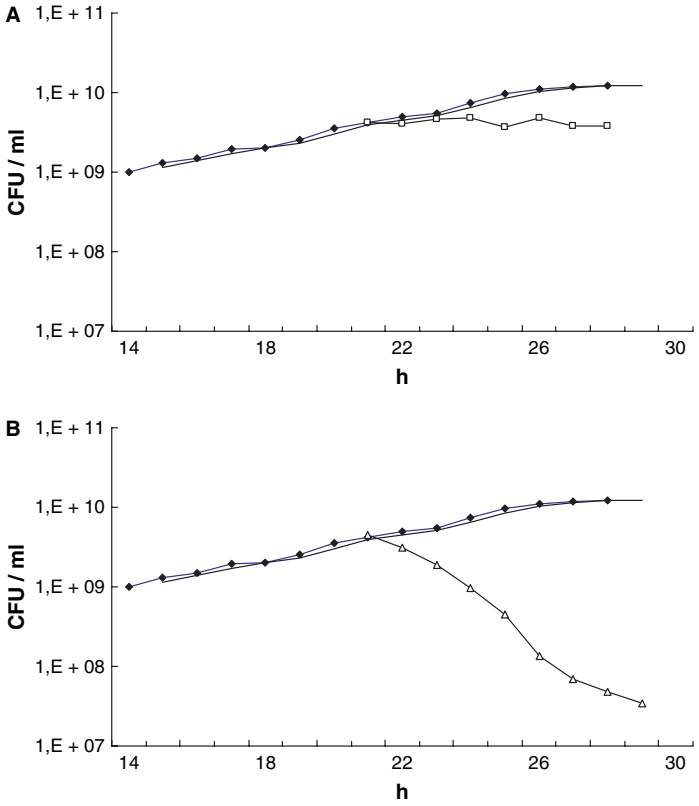

Fig. 1. Growth of *Porphyromonas gingivalis* W83 wild-type and the *oxyR* mutant in anaerobic conditions and after exposure to atmospheric oxygen. (A) *P. gingivalis* W83 wild-type was grown anaerobically to mid-exponential-phase (A_{650} = 0.4–0.5) and then exposed to laboratory air (arrow): anaerobic growth of *P. gingivalis* W83 wild-type (◆) and after exposure to atmospheric oxygen (□). (B) *P. gingivalis oxyR* mutant was grown anaerobically to mid-exponential-phase (A_{650} = 0.4–0.5) and then exposed to laboratory air (arrow): anaerobic growth of *P. gingivalis oxyR* mutant (◆) and after (△) exposure to atmospheric oxygen.

Table 2. Relative quantification by real-time polymerase chain reaction of the expression of genes involved in the oxidative stress response after 15 min of exposure to atmospheric oxygen compared to anaerobic condition

	Relative expression (C_T)	
Genes	*P. gingivalis* wild-type	*P. gingivalis oxyR* mutant
rRNA 16S	1.29 (0.86–1.87)[1]	1.04 (0.86–1.33)
ahpC	1.65 (1.00–2.92)	–[2]
ahpF	1.88 (0.93–3.45)	–[2]
oxyR	1.10 (0.85–1.31)	–[3]
batA	1.70 (1.4–2.25)	1.62 (0.93–2.82)
dps	2.76 (1.24–4.41)	1.52 (0.82–2.81)
ftn	9.29 (2.14–19.90)	3.35 (1.22–7.19)
tpx	7.45 (2.31–15.08)	2.95 (0.89–6.68)

[1]Average C_T value calculated by the $\Delta\Delta C_t$ method of three independent assays. The range of C_t value is indicated in parentheses.
[2]Genes previously confirmed to be OxyR dependent (24).
[3]Gene muted. No transcript was detectable.

7.45-fold (2.31–15.08) after 15 min of oxygen exposure compared to anaerobic conditions in the wild-type strain, while in the *oxyR* mutant strain, the increases in expression were only 1.52-fold (0.82–2.81), 3.35-fold (1.22–7.19), and 2.95-fold (0.89–6.68), respectively, when exposed to atmospheric oxygen (Table 2). The overall trend was a compromised induction of oxidative stress response gene levels when *oxyR* is muted. Furthermore, differences in gene transcription in the *oxyR* mutant strain compared with the wild-type strain were more important during oxygen exposure and decreased 28.36-fold (25.63–31.12) for *dps*, 15.83-fold (8.14–23.57) for *ftn*, and 25.39-fold (19.16–31.89) for *tpx* during oxygen exposure (Table 3). Together, these data indicate a role for OxyR in regulating the transcription of these genes.

The quantitative PCR results were confirmed by bioinformatic analysis. Using the consensus OxyR binding sequence from *E. coli*, we identified putative binding sites upstream of the promoter sequence of the three genes (Fig. 2). Among the four ATAG elements spaced at 9-base-pair intervals comprising the binding sites for *E. coli* OxyR, the numbers of identical bases were 10, 11, and 10 in the *P. gingivalis tpx*, *ftn*, and *dps* promoter sequences, respectively.

Effect of atmospheric oxygen exposure on relative expression of gene implicated in virulence in *P. gingivalis* W83

We then assessed the transcriptomic response to oxidative stress by analyzing the expression levels of virulence genes 15 min after exposure to atmospheric oxygen relative to expression levels in anaerobic conditions (Table 4). Among the genes implicated in adhesion, the hemagglutinin (*hagA*, *hagB*, *hagE*) expression levels were stable during laboratory air exposure. However, the *rgpA* gene encoding a protease that contains many hemagglutinin domains was overexpressed approximately 3.66-fold (0.73–6.84), and the *hem* gene that encodes the hemolytic toxin was overexpressed 1.99 fold (1.14–2.81).

Discussion

P. gingivalis is a strict anaerobic and aerotolerant periopathogen that is confronted with oxidative stress by atmospheric oxygen during interindividual transmission. To understand the mechanism of resistance to oxygen and the functional implication of the hydrogen peroxide-sensing transcription activator OxyR, we analyzed the pattern of gene expression in the wild-type W83 strain in anaerobic and aerobic conditions, and compared these results to those from the *oxyR* mutant strain. The W83 *P. gingivalis* strain was able to survive in response to atmospheric oxygen exposure, at least for 7 h. The inactivation of the *oxyR* gene did not modify anaerobic growth but dramatically decreased its survival upon exposure to atmospheric oxygen. *oxyR* plays an important role in the aerotolerance of this anaerobic bacterium (24). The effect of oxygen on an obligatory anaerobe may be complex, generating a high redox

Table 3. Relative quantification by real-time polymerase chain reaction of the expression of oxidative stress response genes in the *Porphyromonas gingivalis oxyR* mutant compared to *P. gingivalis* W83 wild-type in anaerobic and aerobic condition

Genes	Relative expression (C_T)	
	Anaerobic conditions	After exposure to atmospheric oxygen
ahpC	−195.73 (−124.07 to −272.48)[1]	−216.01 (−128 to −317.37)
ahpF	−164.65 (−108.76 to −223.63)	−171.05 (−136.24 to −207.94)
dps	−15.28 (−8.17 to −23.18)	−28.36 (−25.63 to −31.12)
ftn	−5.62 (−4.86 to −6.66)	−15.83 (−8.14 to −23.67)
tpx	−8.28 (−5.15 to −11.71)	−25.39 (−19.16 to −31.89)

[1]Average C_T value calculated by the $\Delta\Delta C_T$ method of three independent assays. The range of C_T value is reported in parentheses.

the induction of transcription was demonstrated to be OxyR-dependent, consistent with the results from a previous study (24). In contrast, when *P. gingivalis* was exposed to H_2O_2, *ahpC* was downregulated approximately 4.27-fold (2.0–9.1) (6). This differential transcriptional response to two stimuli was also reported for the *dps* gene. Our results showed that the expression of the *dps* gene was partially OxyR-dependent, and *dps* was overexpressed

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Consensus
E. coli    ATAG---------CTAT---------ATAG---------CTATAGATA----------------TATATT---TATATT
                                                    -35 box              -10 box

tpx   -314 AGAACGAACGCTTCGATCTGATTT--ATATGGATGCAGACAAGAGACAATACACCCTTTACTA-TGAAATGATCATAGA -238
           * *          * **         ***          * *

ftn   -273 ATCGAGACTC---CTAAGGGATCTCCATCGAGACTCC--CAAGGGATCTTCATATTATCAAG--TATGGAGGCTTTATG -201
           ** *         ***          ** *         * *

dps   -236 AAAATGAAG----CTTTCTGCTG---ACAGAATTA----GTTTTTATACGCTTCACCGACT---TCTTGTCAGTAAGTC -171
           * *          ** *         * **         * *
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Fig. 2. Comparison of the putative OxyR-binding sequences and putative −35 and −10 boxes in the promoters of *Porphyromonas gingivalis tpx*, *ftn*, and *dps* genes. The consensus sequence of the OxyR-binding motif from *E. coli* used to search the motifs is underlined (36) and the consensus −35 and −10 boxes of *P. gingivalis* promoter are in bold letters in the first line (12). The nucleotides identical to those in the consensus OxyR-binding motif are indicated by asterisks.

Table 4. Real-time polymerase chain reaction, relative quantification of the expression of genes involved in virulence of *Porphyromonas gingivalis* exposed after 15 min of exposure to atmospheric oxygen compared to anaerobic condition

Genes	Relative expression (C_T)
rRNA 16S	1.29 (0.86–1.87)[1]
hagA	0.68 (0.41–1.07)
hagB	0.88 (0.35–1.35)
hagE	0.93 (0.78–1.09)
rgpA	3.66 (0.73–6.84)
rgpB	0.88 (0.62–1.05)
hem	1.99 (1.14–2.81)

[1]Average C_T value calculated by the $\Delta\Delta C_T$ method of three independent assays. The range of C_T value is reported in parentheses.

environment with active metabolic products, such as superoxide, hydrogen peroxide, and hydroxyl radicals, and *P. gingivalis* contains several genes involved in the oxidative stress response to protect against reactive oxygen species.

The transcription of *batA* was slightly increased after 15 min of exposure to atmospheric oxygen in both the wild-type and the *oxyR* mutant strains, indicating that expression of *batA* is OxyR-independent but still sensitive to exposure to atmospheric oxygen. The mechanism of export of reducing power equivalents to the periplasm to protect *B. fragilis* from reactive oxygen could be similar or identical in *P. gingivalis* (33).

After exposure of *P. gingivalis* to atmospheric oxygen, our data showed a slight increase in the expression of *ahpC*, approximately 1.65-fold (1.00–2.92), and after exposure to atmospheric oxygen, in accordance with a previous study by Ueshima et al. (34). However, the induction of oxidative stress by H_2O_2 led to a decrease in *dps* transcription (6). The patterns of transcription of the oxidative response by *ahpCF* and *dps* genes are therefore sensitive to the stimulus used to induce oxidative stress. The difference (Table 5) may be a result of the reactive oxygen species derived from oxygen produced before the synthesis of hydrogen peroxide. The first reactive oxygen species derived from molecular oxygen is superoxide (O_2^-), which should indirectly induce a specific pattern of expression of genes involved in the response of oxidative stress, such the overexpression of *dps*

Table 5. Comparison of reported gene expression in response to different oxidative stress determined by various methods

Genes	Stress and time of exposure	Expression		Methodology	Ref.
		Change in levels[1]	C_T value[2]		
ahpC	O_2, 15 min	Increase	+1.65 (1; 2.92)	qPCR	This study
	H_2O_2, 125 μM, 5 min	Decrease	−4.27 (−2; −9.1)	qPCR	(6)
dps	O_2, 15 min	Increase	+2.76 (1.24; 4.41)	qPCR	This study
	H_2O_2, 125 μM, 5 min	Decrease	−1.25 (−1; −1.5)	qPCR	(6)
	O_2, 20–120 min	Slight increase		LacZ fusion	(34)
ftn	O_2, 15 min	Increase	+9.29 (2.14; 19.9)	qPCR	This study
	H_2O_2, 125 μM, 5 min	Decrease	−2.95 (−2.4; −3.5)	qPCR	(6)
tpx	O_2, 15 min	Increase	+7.45 (2.31; 15.08)	qPCR	This study
rgpA	O_2, 15 min	Increase	+3.66 (0.76; 6.84)	qPCR	This study
	O_2, 30 min	Decrease		Differential display and qPCR	(27)

[1]Variation of gene expression when *Porphyromonas gingivalis* is exposed to various oxidative stress compared to untreated samples.
[2]Average C_T value calculated by the $\Delta\Delta C_T$ method of three independent assays. The range of C_T values is reported in parentheses.
qPCR, quantitative polymerase chain reaction.

and *ahpCF*. In contrast, exposure of *P. gingivalis* to H_2O_2 would induce another mechanism of activation, leading to the repression of the transcription of the *dps* and *ahpCF* genes (Table 5).

These two different mechanisms of activation of genes involved in the oxidative stress response may also exist for the *ftn* gene encoding ferritin, a gene that seems to be involved with the response to atmospheric oxygen. After exposure to atmospheric oxygen, the transcription of *ftn* increased, while incubation with H_2O_2 reportedly resulted in a decrease in its expression (Table 5) (6). In both studies, partial OxyR-dependent expression of the *ftn* gene was shown.

Our study provides the first evidence that the expression of *tpx* increases after exposure to atmospheric oxygen and that this induction is, at least partially, OxyR-dependent. The gene *tpx* encodes a thiol peroxidase, and in *E. coli* and *B. fragilis* this peroxidase is functionally linked to thioredoxin (3, 31). These two enzymes function as an *in vivo* thiol-regenerating system to act as antioxidant, removing peroxides and H_2O_2. The expression of the thiol peroxidase gene is induced by oxygen stress and is not controlled by transcriptional regulators such as OxyR or SoxRS in *E. coli* and *B. fragilis* (11, 14). However, the expression of the gene encoding thioredoxin is partially OxyR-dependent in these bacteria. In *P. gingivalis*, the role of OxyR in the expression of the thioredoxin gene (PG0275) was described previously (6). Therefore, it seems that the expression levels of both enzymes, thiol peroxidase and thioredoxin, are partially regulated by OxyR in *P. gingivalis*, suggesting a common mechanism of regulation in this organism in response to oxidative stress. These preliminary findings provide some insight into the mechanism of transmission through air, and implicate the involvement of Tpx in this process. Analysis of the *tpx* mutant is needed to define more clearly the role of Tpx, and this study is currently underway in our laboratory.

Exposure to atmospheric oxygen does not alter the transcription patterns of the genes encoding major virulence factors such as hemagglutinins – surface proteins that are involved in the adhesion of *P. gingivalis* to other oral bacteria. However, the modification of the expression pattern of virulence factors by oxidative stress has been reported in other bacteria, such as in *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella typhimurium* (2, 4, 13, 18). In *P. gingivalis*, the *de novo* synthesis of virulence factors involved in adhesion should be induced when the bacterium is in favorable growth conditions, such as an anaerobic atmosphere. When the periopathogen is exposed to molecular oxygen, virulence factors involved in protection against oxidative stress are synthesized. This study showed that the transcription levels of genes encoding gingipain (*rgpA*) and hemolysin (*hem*) are increased 3.66-fold and two-fold, respectively, 15 min after a short exposure to atmospheric oxygen, compared to bacteria maintained in an anaerobic atmosphere and compared to a longer exposure as described by Shelburne et al. (27) (Table 5). These two virulence factors are involved in the acquisition of heme; production of hemolysins has been associated with the ability of pathogenic bacteria to obtain heme as a source of iron from lyzed erythrocytes and other host cells (10). RgpA is also implicated in heme acquisition by proteolytic degradation of hemoglobin (5) and in adsorption and heme accumulation (20). Moreover, RgpA also mediates the formation and aggregation of the micro-oxo–bis-haem complex via the HA2 domain (28). The complex acts as a defensive molecule, because its formation from Fe(II) protoporphyrin IX, a monomer derived from hemoglobin, ties up di-oxygen and a toxic oxygen intermediate (superoxide and hydrogen peroxide) (29).

In conclusion, the transcriptional regulator OxyR is involved in the aerotolerance of *P. gingivalis*, a strict anaerobic bacterium. OxyR controls, to varying degrees, the expression of genes such as *ahpCF*, *dps*, and *ftn*. Different patterns and levels of responsiveness of transcription of these genes were observed, according to the stimulus of oxidative stress (Table 5). Our data provide the first evidence that expression of the *tpx* (PG1729) gene, encoding a thiol peroxidase, is partially OxyR dependent and is induced after exposure to atmospheric oxygen. This report offers preliminary findings on *tpx* and further confirmation and analysis are now in progress in our laboratory. In addition to the genes of the oxidative stress response, exposure to atmospheric oxygen also induced the expression of virulence factors involved in heme acquisition, another mechanism developed by *P. gingivalis* to protect against molecular oxygen and the reactive oxygen species.

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