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IDENTIFICATION OF AEROTOLERANCE GENE BATA AND BATB IN SUBGINGIVAL MICROFLORA: *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*, *TANNERELLA FORSYTHIA* AND *PREVOTELLA INTERMEDIA*

H. Parthasarathy¹, R. Gad² and C. A. Jacob^{*3}

Department of Periodontology, SRM Dental College¹, Bharathi Salai, Ramapuram, Chennai - 600089, T. N., India.

Department of Biotechnology, SRM University², Kattankullathur, Chennai - 603203, T. N., India.

Department of Periodontology, Saveetha Dental College³, Chennai- 600077, T. N., India.

Keywords:

Dental plaque, obligate anaerobes, oxidative stress, bacteroides aerotolerance gene.

Correspondence to Author:

C. A. Jacob

Senior Lecturer,
Department of Periodontology,
Saveetha Dental College, No 162,
P.H. Road, Chennai- 600077, T. N.,
India


E-mail: caroline_jacob@ymail.com

ABSTRACT: Subgingival obligate anaerobes present within the biofilm such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* and *Aggregati bacter actinomycetemcomitans* have been implicated in the initiation and progression of periodontal disease. Recent documentation has demonstrated that *P.gingivalis* is capable of maintaining its viability when exposed to oxidative stress by expressing *Bat A* and *Bat B* genes of the aerotolerance operon acting as virulence factors offering protection and further disease progression in the host. The present study aims to determine the presence of bat A and bat B genes in other periodontopathogenic species viz. *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Prevotella intermedia*, and the degree of homology using BLAST analysis.

INTRODUCTION: Periodontitis is a polymicrobial, multifactorial infection that affects the tooth supported structures. The periodontal pocket formed as a result of the disease progression, provides a unique dynamic environment for the interaction and intercellular communication between the microbiota present and the host cells.¹ The subtle shift from a gram positive aerobic microflora to a gram negative one teaming with facultative and obligative anaerobic species^{2, 3} depends on factors such as temperature, pH, GCF flow and redox potential.⁴

Despite the heavy dependence on the aforementioned factors, characteristic bacterial diversity has been seen to exist where certain anaerobic species still manage to thrive in the initial biofilm formation. Li and co-workers⁵ analyzed supragingival plaque over 6 hours of plaque formation. Their findings reported low levels of periodontal pathogens at the initial 4 hours of biofilm formation which included species like *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans*.

These findings were further substantiated by Diaz and co-workers⁶ confirming that anaerobic periodontopathogens can colonize early biofilms. This can be attributed to the apical shift of supragingival plaque as it progresses along the gingival sulcus resulting in ecological changes.⁷ The study of initial subgingival biofilm formation

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<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(2).689-96</p>	

is more challenging due to the lack of models that mimic subgingival colonization or that allow appropriate sampling.⁸ Hence, it can only be hypothesized as to what factors are responsible for the survival of anaerobic periodontal pathogens in initial biofilm formation.

Kolenbrander and co-workers have proposed that coaggregation and close proximity facilitate bacterial mutualism, which in turn influences biofilm development.⁸ Various metabolic interactions can occur at many levels and include nutritional exchange, environmental modification through oxygen detoxification, and small-molecule signal-mediated gene regulation. Though the symbiotic relationship between species might be part of the process that facilitates the survival of anaerobes in plaque, it is likely that there are many situations in which microorganisms are not protected by the overall oxygen metabolism of the microbial community.

In such scenarios, the survival of anaerobes will depend more upon defences against oxidative stress which are specific for each species. Oxidative stress response mechanisms could also be important during the bacterial invasion of host tissues, as a defense against oxygen-dependent killing of neutrophils and macrophages⁹ and for survival in blood during bacteremias. In any of these roles, anti-oxidant defence systems are likely to contribute to the virulence of anaerobic microorganisms. The oxidative stress defences of anaerobes have not been widely studied. In contrast to the vast knowledge on anti-oxidant mechanisms operating in aerobes or facultative species, the mechanisms by which obligate anaerobes tolerate oxygen are poorly understood.

It seems that anaerobes have common anti-oxidant defences with aerobes which are not expressed at high levels in anaerobes or they are not all present simultaneously in the same species. Moreover, the metabolism of anaerobes relies on metabolic schemes built around enzymes that react easily with oxygen such as NADH oxidase^{10, 11} and superoxide dismutase (SOD). *P. gingivalis* also possesses an Oxy R protein⁶, homologous to that present in many facultative and aerobic microorganisms.¹² Anti-oxidant-relevant genes

whose transcription depends on Oxy R have been identified which include genes for SOD, Dps (a nonspecific, protective, DNA-binding protein), tpx (thiol peroxidase), alkylhydroperoxide reductase subunits F and C (ahpC-F operon)¹³, rubrerythrin¹⁴, ferritin¹⁵ a heme layer that acts as an oxidative sink to eliminate the effects of hydrogen peroxide and other reactive oxygen species, thus protecting the cell membrane and other cellular components of the organism from oxidative damage.¹⁶

It seems then that although anaerobes, such as *P. gingivalis*, cannot survive highly oxygenated conditions, their own array of anti-oxidant defenses, plus the interaction with neighbouring facultative or aerobic bacterial species, allows them to adapt to oral biofilms. One proposed mechanism is that of aerotolerance, which allows anaerobes to survive in conditions of oxidative stress, although it does not imply that cell replication can occur under aerated conditions. An extensively studied example is that of *P. gingivalis*, which is able to survive under oxidative stress for several hours until the environment reverts to being anaerobic and cell division and growth are re-initiated.¹⁵

In *P. gingivalis*, the Aerotolerance gene A cluster of genes (PG1582 to PG1586) have been identified, bearing a very similar likeness to the previously described aerotolerance operon of *Bacterioides fragilis*.¹⁷ This operon includes : PG1582 – Bat A, PG1583 – Bat B, PG1584 – Bat C, PG1585 – Bat D and PG1586 – Bat E which promote the survival of *B. fragilis* upon exposure to oxygen. Their presence in *P. gingivalis* suggests that this system may also ensure tolerance to oxygen in the oral cavity.¹⁸ Research based on this operon appears to substantiate the role of Bat A and Bat B proteins in promoting the survival of *P. gingivalis* during oxidative stress. Therefore, it can be postulated that a similar set of genes may be present amongst other periodontopathogenic anaerobes present in mature plaque biofilm.

As the role of *Prevotella intermedia* have been well established in chronic periodontitis^{5, 6} and the literature search revealed no data on the presence and influence of the *Bat A* and *Bat B* genes in *P. intermedia*, the present study was carried out as an attempt to investigate the presence of

aerotolerance genes *Bat A* and *Bat B* in *P. intermedia*.

MATERIALS AND METHODS:

Sample Collection:

25 systemically healthy patients between 18 –35 years with different stages of periodontitis who attended the Department of Periodontics, SRM Dental College, Ramapuram, Chennai, India for various dental concerns were evaluated. Inclusion criteria for subjects with periodontitis were 20 or more natural teeth with 4 or more sites having ≥ 4 mm probing pocket depth and clinical attachment loss. Subgingival plaque samples were collected from the deepest periodontal pockets of each subject with sterile curettes and immediately transferred to 1.5ml of reduced transport fluid for cultivation under anaerobic conditions within 4 hours of sampling.

Bacterial Culture:

Samples were vortexed for 2 minutes and split. 100 μ l each of the plaque sample was used for culture by digest buffer solution and PCR. 100 μ l dilutions were placed on anaerobic blood agar plates (Hi-Media, India) supplemented with sheep blood (5% vol/vol), hemin (5 mg/liter), and menadione (1mg/liter) and incubated in 80% N₂ – 10% H₂ –10% CO₂ at 37°C for 7 to 14 days. Different colonies developed were isolated and subcultured under standard anaerobic conditions as mentioned above for 1 week. *Prevotella intermedia* was grown in Todd Hewitt Broth; supplemented with beef heart, peptic digest of animal tissue, dextrose, sodium chloride, disodium phosphate, sodium carbonate for pH = 7.8(+/- 0.2).

PCR detection of the 16S rRNA gene:

16S rRNA genes of the periodontal pathogens *P.gingivalis* and *P.intermedia* were identified in plaque samples by PCR using gene specific primers.

Extraction of total genomic DNA from plaque samples:

Extraction of total genomic DNA from plaque samples was performed based on the technique elaborated by Sambrook et al 1989. The pellet was washed in 70% ethanol, air-dried and resuspended in TE-buffer and stored at -20°C for further use.

The purity of DNA was determined by UV spectrophotometric analysis at 260nm-280nm and separated by agarose gel electrophoresis.

PCR of Plaque Sample:

The PCR temperature profile included an initial denaturation step at 94°C for 5 minutes, followed for 30 cycles of a denaturation step at a 94°C for 1 minute; a primer-annealing step at 50°C for 2 mins; an extension step at 72°C for 3 minutes, and a final step at 72°C for 7 minutes, temperature at hold is 4°C. The amplicons were stored at -20°C. Amplicons were analysed by 1% agarose gel Electrophoresis performed in TAE buffer.

DNA Isolation:

DNA from bacterial pellets were isolated using Medox Easy® genomic DNA preparation kit (Medox Biotech India Pvt, Ltd.) according to manufacturer's instructions and DNA was run in 0.7% agarose gel electrophoresis and viewed by UV-transilluminator.

RESULTS:

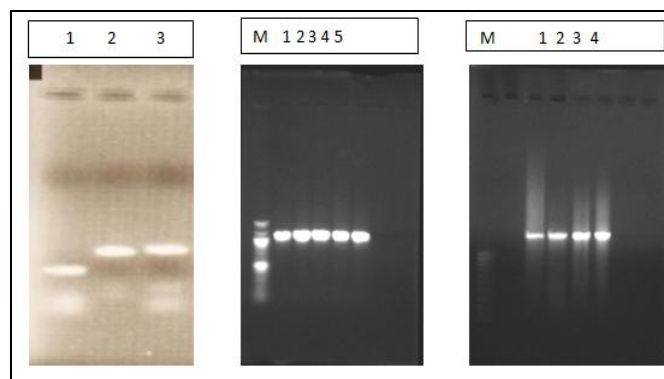


FIG. 1.A PCR amplification of 16S rRNA genes: Lane 1: *P. gingivalis*, Lane 2: *A.actinomycetemcomitans*, Lane 3 : *P. intermedia*. **FIG 1.B)** 1% Agarose gel electrophoresis analysis of PCR amplified Bat A gene from the genomic DNA of *A. actinomycetemcomitans* and *P.intermedia* : Lane M - 1000 bp DNA ladder (molecular marker), Lanes 1 - PCR amplified products of Bat A gene approximately 1050 bp for DNA from *P. gingivalis*. (Positive control), Lane 2 to 3 - PCR amplified products of Bat A gene approximately 1050 bp for DNA from *A. actinomycetemcomitans*, Lane 4 to 5- PCR amplified products of Bat A gene approximately 1050 bp for DNA from *P. intermedia*. **FIG 1.C)** 1% agarose gel electrophoresis analysis of PCR amplified Bat B gene from the genomic DNA of *P. intermedia* and *A.a*. Lane M – 500 bp DNA ladder (molecular marker), Lanes 1- PCR amplified products of Bat B gene approximately 880 bp for DNA from *P. gingivalis*. (Positive control), Lane 2 to 3 - PCR amplified products of Bat B gene approximately 880 bp for DNA from *Aac*, Lane 4 - PCR amplified products of Bat B gene approximately 880 bp for DNA from *P. intermedia*

In Silico Technique:

Legend for links to other resources: [U](#) UniGene [E](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer [P](#) PubChem BioAssay

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NP_905713.1	batA protein [Porphyromonas gingivalis W83] >gb AAQ66612.1 b	460	595	99%	4e-161	100%	G
YP_001928645.1	aerotolerance-related membrane protein BatA [Porphyromonas gin	457	593	99%	9e-159	99%	G
YP_004509448.1	aerotolerance-related membrane protein BatA [Porphyromonas gin	455	590	99%	8e-158	99%	G
ZP_03476585.1	hypothetical protein PRABACTJOHN_02256 [Parabacteroides johns	301	371	88%	2e-97	63%	
ZP_02031887.1	hypothetical protein PARMER_01895 [Parabacteroides merdae ATC	301	370	88%	3e-97	63%	
ZP_09483466.1	hypothetical protein AnHS1_06994 [Anaerophaga sp. HS1]	297	357	94%	9e-96	63%	
ZP_08458730.1	von Willebrand factor type A [Bacteroides coprosuis DSM 18011]	296	366	88%	2e-95	60%	
ZP_06075044.1	conserved hypothetical protein [Bacteroides sp. 2_1_33B] >ref Zf	293	365	88%	3e-94	64%	
ZP_06995229.1	BatA protein [Bacteroides sp. 1_1_14] >ref ZP_09943263.1 hypo	293	358	88%	4e-94	62%	
YP_004161837.1	von Willebrand factor A [Bacteroides helcogenes P 36-108] >gb A	293	357	88%	4e-94	64%	G
NP_809820.1	aerotolerance protein BatA [Bacteroides thetaiotaomicron VPI-546	293	358	88%	4e-94	62%	G
YP_001302338.1	hypothetical protein BDI_0948 [Parabacteroides distasonis ATCC 6	293	365	88%	5e-94	64%	G
ZP_09860235.1	aerotolerance protein BatA [Bacteroides faecis MAJ27]	292	357	88%	6e-94	62%	
ZP_05417555.1	BatA protein [Bacteroides finegoldii DSM 17565] >gb EEX43180.1	291	354	88%	2e-93	62%	
ZP_09642860.1	N-terminal double-transmembrane domain-containing protein [Odo	291	368	89%	3e-93	61%	
YP_005015542.1	von Willebrand factor type A domain-containing protein [Tannerell	290	365	87%	7e-93	60%	G
ZP_03680538.1	hypothetical protein BACCELL_04911 [Bacteroides cellulosityticus (289	354	88%	1e-92	62%	
ZP_03459930.1	hypothetical protein BACEGG_02731 [Bacteroides eggertii DSM 21	289	354	88%	2e-92	62%	
ZP_03014408.1	hypothetical protein BACINT_01981 [Bacteroides intestinalis DSM 21	288	354	88%	2e-92	62%	
ZP_10894441.1	double-transmembrane N-terminal domain protein [Porphyromonas	288	374	88%	2e-92	61%	
ZP_01958959.1	hypothetical protein BACCAC_00547 [Bacteroides caccae ATCC 43	288	352	88%	4e-92	62%	
ZP_06084651.1	conserved hypothetical protein [Bacteroides sp. 2_1_22] >ref Zf	288	350	88%	4e-92	62%	
ZP_08301060.1	von Willebrand factor type A domain protein [Bacteroides fluxus Y	286	350	88%	2e-91	62%	
YP_004252414.1	von Willebrand factor type A [Odonibacter splanchnicus DSM 2071	283	361	89%	9e-91	60%	G
ZP_03010122.1	hypothetical protein BACCOP_01987 [Bacteroides coprocola DSM]	284	354	94%	1e-90	59%	

FIG.2: A) NUCLEOTIDE SEQUENCES OF THE BAT A GENE IN PREVOTELLA INTERMEDIA:

5' atactctgaattgcttggctgctgatctgctgcttctgatagcacttggtatctctcaagcagctgaagacttcggcaacgatgaccatattctcattgaagcccttgaag
 gaagtcgcccagggtttgaggtctatctgcgcattcgttgcgatattgagagctcttccgttcggtttctcattattgcttggctgctcgcgagaataccaatagctggcagaa
 agattcgatcgagggcatagatattatgtggccatggatgtgctcaggcagatgagggcattcaagccgaatcgctggaggcagccaaagatgtagcattctctt
 atcaacaatcgccgaacgacaatatcgggatggtacattcgccggagagagcttactcaatgcccattgactaccgacataccgattgcttaatatggtacaggacttgc
 gatgggtgttctggatgatgttacgctatcggaatgggattggctacggcgtcaatcgactgaaggacgaaagcgaagagccgcgttggttattctgctgacggacggatc
 aaacaatcgggcgacatcacaccccgaatggcagccgatattgcgaacgctcggatattcgtgtgtacacagtcgggtgaggcacagaggagaagctcttccccattca
 gacggaattcggagttggattcagaatgtcctgtcgtatagacgaaccacattggacgggaatgcccagggtatcgggtggaaaatacttcagggtctcgtataatgaaacg
 ctgaatgaaatctacaaggaaatcgacaaatgggagaagacgagacttatgaccaagagctcaaggttatgaggaaaaatacttctgtatgccttattgcttctctgtgtg
 ctgacagagtttttcttcgcaataact 3'

BLASTX

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
NP_905714.1	batB protein [Porphyromonas gingivalis W83] >gb AAQ66613.1 b	270	618	95%	1e-154	100%	G
YP_001928644.1	aerotolerance-related exported protein BatB [Porphyromonas gin	268	613	95%	2e-153	100%	G
YP_004509447.1	aerotolerance-related exported protein BatB [Porphyromonas gin	267	613	95%	4e-153	100%	G
ZP_10894445.1	von Willebrand factor type A domain protein [Porphyromonas sp. c	140	311	88%	2e-69	59%	
ZP_04389503.1	BatB protein [Porphyromonas endodontalis ATCC 35406] >gb EENR	132	310	84%	4e-69	67%	
ZP_02031886.1	hypothetical protein PARMER_01894 [Parabacteroides merdae ATC	129	280	90%	3e-60	63%	
ZP_03476586.1	hypothetical protein PRABACTJOHN_02257 [Parabacteroides johns	129	279	95%	4e-60	63%	
YP_005015541.1	von Willebrand factor type A domain-containing protein [Tannerell	120	262	82%	7e-55	56%	G
ZP_06985499.1	BatB protein [Bacteroides sp. 3_1_19] >gb EF109698.1 BatB prot	128	258	89%	9e-54	64%	
YP_001302339.1	hypothetical protein BDI_0949 [Parabacteroides distasonis ATCC 6	128	257	89%	2e-53	62%	G
ZP_06075043.1	conserved hypothetical protein [Bacteroides sp. 2_1_33B] >ref Zf	128	256	89%	3e-53	62%	
ZP_02435242.1	hypothetical protein BACSTE_01484 [Bacteroides stercoris ATCC 4	116	256	81%	3e-53	55%	
ZP_05545292.1	conserved hypothetical protein [Parabacteroides sp. D13] >gb EE	128	255	95%	5e-53	62%	
ZP_08301061.1	von Willebrand factor type A domain protein [Bacteroides fluxus Y	117	254	81%	2e-52	56%	
YP_004161838.1	von Willebrand factor A [Bacteroides helcogenes P 36-108] >gb A	119	253	85%	3e-52	57%	G
ZP_08458729.1	von Willebrand factor type A [Bacteroides coprosuis DSM 18011] ;	118	252	86%	7e-52	57%	
YP_212124.1	aerotolerance-related membrane protein [Bacteroides fragilis NCT	124	250	85%	3e-51	59%	G
YP_099701.1	hypothetical protein BF2418 [Bacteroides fragilis YCH46] >ref ZP	124	250	85%	3e-51	59%	G
ZP_07809358.1	conserved hypothetical protein [Bacteroides fragilis 3_1_12] >gb	122	246	85%	5e-50	58%	
ZP_07060562.1	BatB protein [Prevotella bryantii B14] >gb EF172278.1 BatB prot	115	246	89%	5e-50	58%	
ZP_07033623.1	BatB protein [Prevotella oris C735] >gb EF149319.1 BatB protein	105	244	87%	1e-49	55%	
ZP_06254188.1	BatB protein [Prevotella oris F0302] >gb EFB33442.1 BatB protei	105	244	87%	1e-49	55%	
ZP_07365026.1	aerotolerance protein BatB [Prevotella marshii DSM 16973] >gb EF	119	244	87%	2e-49	49%	
YP_004252415.1	von Willebrand factor type A [Bacteroides salanitronis DSM 18170]	112	244	90%	2e-49	48%	G
ZP_07322318.1	von Willebrand factor type A domain protein [Prevotella disiens FB	107	243	90%	2e-49	52%	
ZP_03207175.1	hypothetical protein BACPLE_00795 [Bacteroides plebeius DSM 17	108	243	81%	4e-49	49%	
ZP_08471594.1	hypothetical protein HMPREF9456_03189 [Dysgonomonas mossii D	107	242	86%	5e-49	50%	
ZP_11021195.1	hypothetical protein HMPREF9448_01621 [Barnesiella intestinhomi	120	242	73%	6e-49	50%	
YP_003574119.1	BatB/BatC protein [Prevotella ruminicola 23] >gb ADEB1340.1 p	105	241	78%	1e-48	48%	G
ZP_02070562.1	hypothetical protein BACUNI_01988 [Bacteroides uniformis ATCC 6	117	241	80%	1e-48	50%	
ZP_06288369.1	von Willebrand factor type A domain protein [Prevotella timonensis	110	241	86%	2e-48	49%	

FIG.2: B) NUCLEOTIDE SEQUENCE OF THE BAT B GENE IN PREVOTELLA INTERMEDIA

5' attcaccgaatacttatacttactgctgctgctcccattgcttggtagggctctatgcttatcgcaaaagacgggcgcaagaagacggttgcgcaactccgctgctaa
 agctctcaaacgggaagcaagtagcaaacgctggatctggcgaacggattcttcttctccatcgctttcttgatcgggatgcttccgctccgcaaaatagattgtgtgga
 tgtgctcaaacgggagaaaggtatcgaggtatgattgtttggatattccaactccatgctctgtaagatgtcaagcccaatcgtttgagtttgcgaagcaaggtgtgggaa
 agctctttgacggcttcgacaacgacaaggttggattggttattcgcaggtaattatcgcaaaatccgattactacggatttctcagccaaacagtttcttcagatataa
 gtccaatattggttacgccaaggaacggctatcgagcggctatcgaaacttgccecaaatctttccgataataagaaatcggttaagactatcattgtcctgacggatgga
 gaaatcatgaaggaaatgccatcgaaagcggcagcaagccatgaagcaggcatcgggtcaatgtattgtgtcggaaacagccttggggggcacctatacctattgaag
 aaggatctcaaatagatgaacgggcaatctgtctgtaccaaactgcgaaaaagatgtgcgctgacattgctcagcaggagaggggtacttctttccggacagaggtcttc
 tgcgctggttgggctatagagacgagctggacaactccccaaagcgtacttctctcttctccggctatcgcgaagtgtacggatggttcggacttgcggcttggat
 atgtttattgttggaaatcctgattcaggagcgggaagagtaggttcttcagttagat3'

Clustal W:

Using the BLAST results, the highly similar sequences were grouped together and the family of proteins, to which the query protein belongs to, was determined using Clustal W. (Sequence data not included). Based on the dendrograms obtained from the Clustal W result, the family of the Bat A

and Bat B genes were predicted and the related sequences were analysed. The relationship of the Bat A and Bat B genes with other species predicts the family, sub family and the function of the protein sequence that can be obtained from the gene.

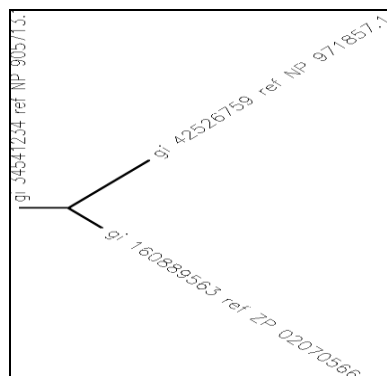


FIG 3.A

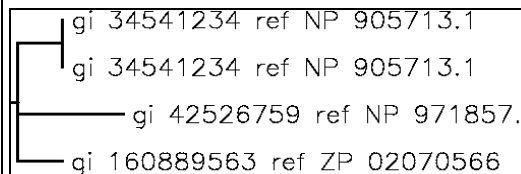


FIG 3.B

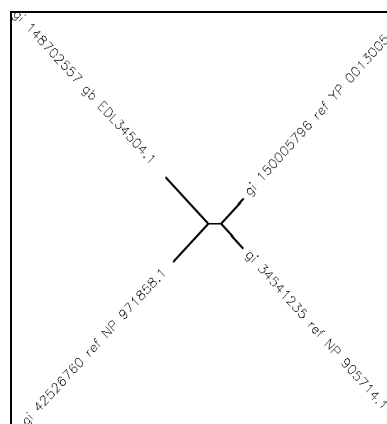


FIG 3.C

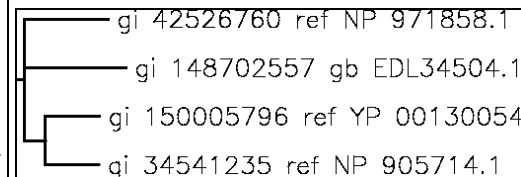
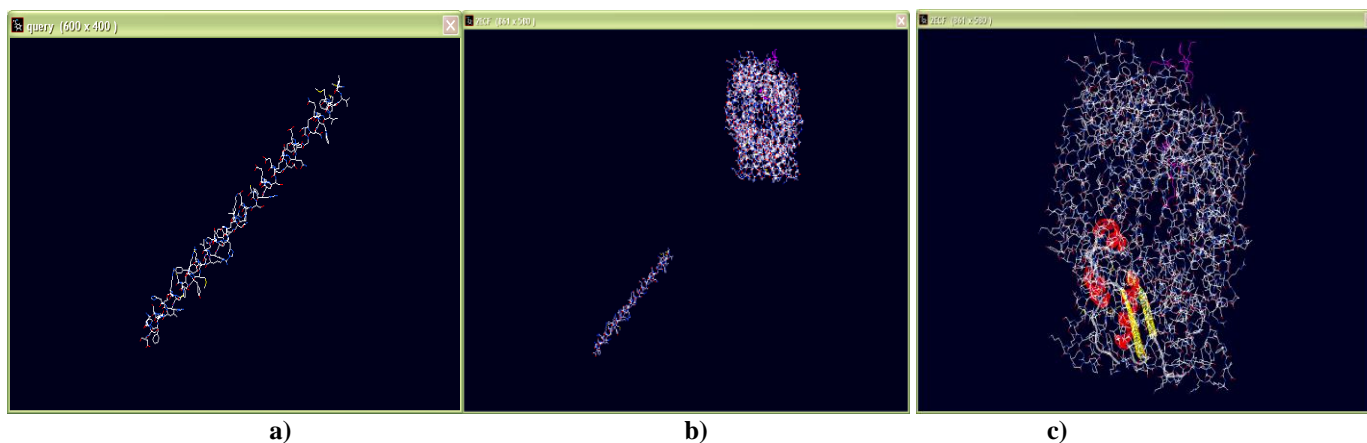


FIG 3.D

FIG 3.A): CLUSTAL W DENDROGRAM FOR BAT A. UNROOTED TREE (GENERATED BY PHYLIP'S DRAWTREE) B) ROOTED TREE (GENERATED BY PHYLIP'S DRAWGRAM FOR BAT A). C) CLUSTAL W DENDROGRAM FOR BAT B. UNROOTED TREE (GENERATED BY PHYLIP'S DRAWTREE) D) ROOTED TREE (GENERATED BY PHYLIP'S DRAWGRAM FOR BAT B).

Homology Modeling:



a)

b)

c)

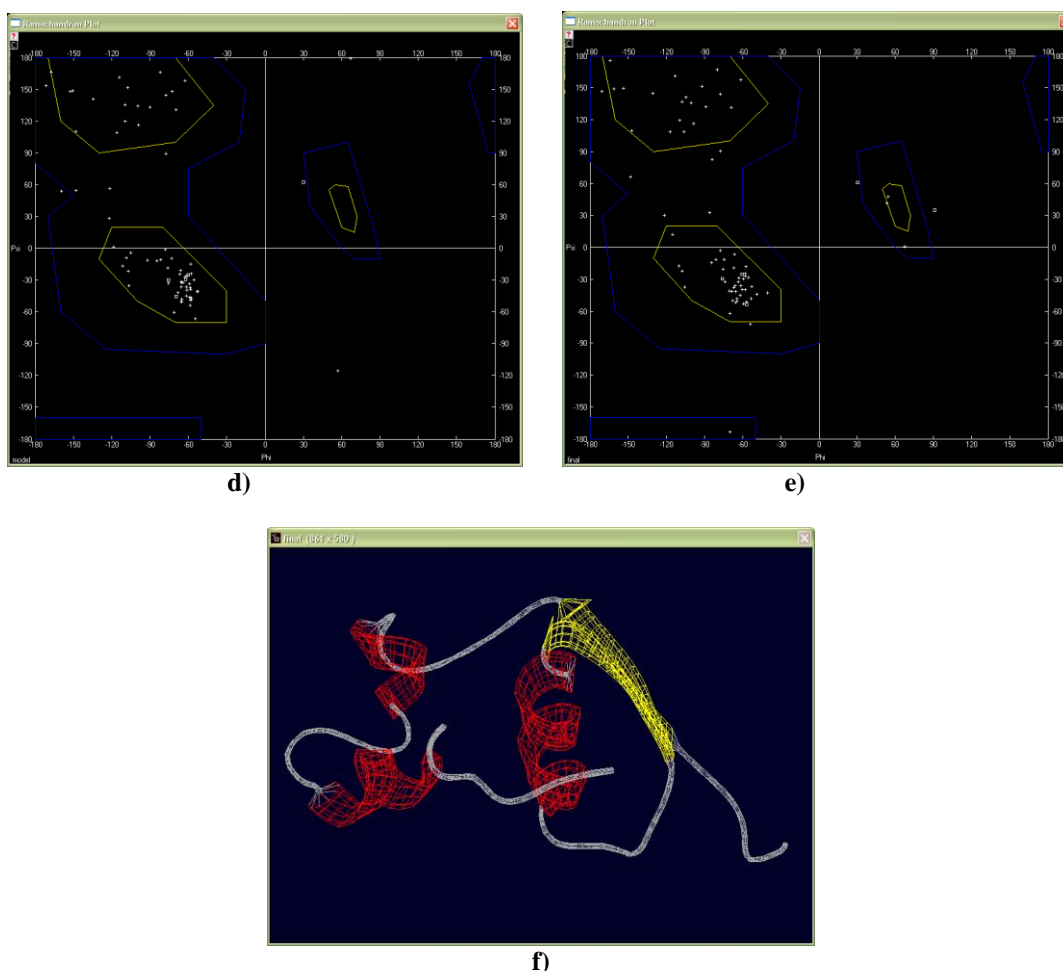


FIG 4.A) LOADING OF RAW SEQUENCE (QUERY), b) LOADING OF TEMPLATE SEQUENCE (PDB TEMPLATE), c) FITTING OF THE RAW SEQUENCE WITH THE TEMPLATE STRUCTURE, d) RC PLOT BEFORE LOOP BUILDING: NON-PROLINE/ NON-GLYCINE RESIDUES PRESENT IN THE FORBIDDEN REGION: GLN53, LYS24, ASN11, ENERGY BEFORE EM = -15371.639, e) RC PLOT AFTER LOOP BUILDING: Energy after EM = -16322.033, f) SECONDARY STRUCTURE OF THE MODELED QUERY PROTEIN

DISCUSSION: Amongst the obligate anaerobic periodontal pathogens that have been implicated in the initiation and progression of periodontal disease, *Porphromonas gingivalis* has been documented in detail.^{5, 6} Although strictly anaerobic, *P.gingivalis* is able to survive in the presence of atmospheric oxygen.³⁰ The ability of anaerobes to survive oxidative stress is referred to as aerotolerance.⁸

A variety of genes that are expressed during oxidative stress including those regulated by the Oxy R operon: *sod*, *ahp C* and *ahp F*; *dps* and *usp A* have been documented in the literature.^{31, 32} With regard to the detail in which the aforementioned genes have been studied, few studies have documented the role of the Bat I operon.^{18, 32, 33} Furthermore, in comparison to the extensive research performed on the aerotolerance

mechanisms maintained by *Porphromonas gingivalis*, data pertaining to the regulation of oxidative stress control in *Prevotella intermedia*, another member of the Bacteroides species, appear to be limited. Extensive documentation has implicated *Prevotella intermedia* in the severity and progression of periodontal disease.³⁴ However, the mechanisms to overcome oxidative stress in these microorganisms are poorly understood.

This study was aimed at identifying the presence of the bacteroides aerotolerance genes Bat A and Bat B in *Prevotella intermedia* and evaluating the degree of homology between these genes if present with that of previously documented Bat A and Bat B genes of *P.gingivalis*. The Bat A and Bat B genes were chosen from among the Bat I operon as they were the most frequently identified genes in the literature.^{17, 18, 21} The present study identified the

Bat A and Bat B genes responsible for the unlimited growth of *Prevotella intermedia* in aerobic conditions by adapting the standardized PCR protocol. The amplified products of PCR were sequenced by di-deoxynucleotide technique while the *in silico* study was carried out with the sequence obtained. Different bioinformatics tools were used to carry out the functional analysis of the gene.

The sequence was analysed using the BLAST X tool and the protein hits obtained were used for multiple sequence alignment to trace out the phylogenetic relationships between the sequences by CLUSTALW tool. The BLAST X results for the Bat A and Bat B query sequence had 152 and 127 protein sequence hits respectively when compared with various protein databases. The first 4 hits in both queries showed much similarity to the query sequence, and thus studied to identify phylogenetic relationships.

The results obtained from the *in silico* studies revealed that the *Bat A* and *Bat B* genes translated into the bat A and bat B protein respectively; where bat A was seen to be involved in transcription regulation and bat B in the immune response activity. The structure of the bat A was also predicted using a homology modelling technique with the beta sheet denoted in yellow and the alpha helix of the protein denoted in red. The above mentioned results therefore, demonstrate the existence of the Bat A and Bat B genes in *Prevotella intermedia*, thus contributing to its survival in conditions of oxidative stress as an inherent virulence factor.

CONCLUSION: From the results of the present study, it may be proposed that the identification of the Bat A and Bat B genes in *Prevotella intermedia* conferring protection solely or in combination with other aerotolerant mechanisms for this bacteria to survive within the periodontal pocket when exposed to varying amounts of oxygen and during the host inflammatory response. Future directions could incorporate the use of the hypothetical protein model obtained to developing a treatment strategy against periodontal disease by host modulation or recombinant therapy. However,

further documentation is warranted on the role these genes play and their clinical significance.

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