Supplemental Materials

Supplemental Materials and Methods

Construction and complemetantion of *P. gingivalis* deletion mutants

The oligonucleotide primers used are listed in Table S2 in Supplemental material. To disrupt PGN_0300, two fragments were PCR-amplified from the chromosomal DNA of *P. gingivalis* ATCC 33277, using PrimeSTAR® GXL DNA Polymerase (Takara Bio). The upstream fragment was amplified using the primers PGN_0300UF and PGN_0300UR, and contained sequences from the 5′ end of PGN_0300, including the region upstream of the ATG initiation codon. The downstream fragment was amplified using the primers PGN_0300DF plus PGN_0300DR, and contained sequences from the 3′ end of PGN_0300, including the region downstream of its stop codon. The upstream and downstream fragments were double digested with EcoRI-BamHI and BamHI-XbaI, respectively, and ligated together into the EcoRI/XbaI site of pUC19. The *ermF* cassette was PCR-amplified from pKD355 (1) using the primer set ErmF-Fw-ErmF-Rv and inserted into the BamHI site within PGN_0300 of this plasmid. The resulting plasmid pUCPGN0300, in which the direction of *ermF* was same as that of PGN_0300, was linearized by PvuII digestion and introduced into *P. gingivalis* ATCC 33277 and W83 cells by electroporation as described previously (2), resulting in Δ*omp17* and Δ*omp17*-83, respectively. Correct gene replacement, which occurred through double crossover recombination events, was verified by PCR analysis (data not shown).

For complementation of PGN_0300, PGN_0300 was inserted into the PGN_1045 locus of Δ*omp17*. Since PGN_1045 encodes β-galactosidase and *P. gingivalis* does not utilize lactose, disruption of PGN_1045 was not expected to affect cell viability. The

plasmid pCPG0300 was constructed with two DNA fragments that were PCR-amplified from the chromosomal DNA of *P. gingivalis* ATCC 33277. The upstream DNA fragment was amplified using the primers PGN_1045UF and PGN_1045UR and contained sequences from the 5′ end of PGN_1045, including the region upstream of the ATG initiation codon. The downstream DNA fragment was amplified using the primers PGN_1045DF and PGN_1045DR and contained sequences from the 3′ end of PGN_1045, including the region downstream of its stop codon. The upstream DNA fragment was double digested with XhoI-HindIII and inserted into the XhoI/HindIII site of pBluescript II SK(−) (pBSSK), resulting in p1045A. Then, the downstream DNA fragment was double digested with BamHI-NotI and inserted into the BamHI/NotI site of p1045A, resulting in p1045AB. Next, a 3.1-kb BamHI-BglII *tetQ* DNA fragment from pKD375 (3) was inserted into the BamHI site within p1045AB to yield the plasmid p1045TET. The promoter region of *P. gingivalis fimA* was PCR-amplified from *P. gingivalis* ATCC 33277 chromosomal DNA using the primers FimA-PROU and FimA-PROD, digested with StuI-BamHI, and inserted into the SmaI/BamHI region of p1045TET, resulting in pCPG. Finally, the coding region of PGN_0300 was PCR-amplified from *P. gingivalis* ATCC 33277 chromosomal DNA using the primers PGN_0300EXU and PGN_0300EXR, digested with BamHI, and inserted into the BamHI site of pCPG. The resulting plasmid containing the PGN_0300 open reading frame in the same direction as the *fimA* promoter transcript was selected and designated as pCPG0300. The plasmid pCPG0300 was linearized by BssHII digestion and introduced into Δ*omp17* cells by electroporation, resulting in Δ*omp17*/*omp17*$^{+}$. Correct gene replacement, which occurred through double crossover recombination events, was verified by PCR analysis (data not shown).

Construction of *E. coli* expressing a His_6-tagged PorU (Asn^{800} to Asn^{1140})

A His_6-tagged recombinant protein of PorU (Asn^{800} to Asn^{1140}) was overexpressed in *E. coli* BL21(DE3) cells transformed with the expression plasmid. The expression plasmid was constructed as follows: DNA fragment of the *porU* was PCR-amplified with primer set PorUF-PorUR using *P. gingivalis* ATCC 33277 chromosomal DNA as a template. The amplified DNA of *porU* was digested with EcoRV and XhoI and then inserted into EcoRV-XhoI-digested pET-32b(+) (Novagen). *E coli* BL21 (DE3) harboring the resulting plasmid was grown on LB broth at 30°C, and *porU* was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside. His_6-tagged recombinant protein was purified by using a HiTrap Chelating HP column (GE Healthcare Life Sciences).

Protease activity assay

For Kgp and Rgp activity assays, *P. gingivalis* cells were grown anaerobically in enriched BHI medium at 37°C overnight. Bacterial cells and culture supernatants were separated by centrifugation at 10,000 × *g* for 10 min at 4°C. Cells were suspended in the original volume of phosphate-buffered saline (PBS). Kgp and Rgp activities were determined using the synthetic substrates benzyloxycarbonyl-L-histidyl-L-glutamyl-L-lysine-4-methyl-coumaryl-7-amide (Z-His-Glu-Lys-MCA) and benzyloxycarbonyl-L-phenylalanyl-L-arginine-4-methylcoumary-7-amide (Z-Phe-Arg-MCA) in 20 mM sodium phosphate buffer (pH 7.5) containing 5 mM cysteine in a total volume of 1 mL. After incubation at 40 °C for 10 min, the reaction was terminated by adding 1 mL of 10 mM iodoacetamide (pH 5.0), and the released

7-amino-4-methylcoumarin was measured at 460 nm (excitation at 380 nm). One unit of enzyme activity was defined as the amount of enzyme required to release 1 nmol of 7-amino-4-methylcoumarin under these conditions. Kgp and Rgp activities are indicated as units per milliliter of cell suspension or culture supernatant. All cultures had similar cell densities at OD600 of approximately 1.0.

Subcellular fractionation

P. gingivalis cells from a 200-mL culture were harvested by centrifugation at 10,000 × *g* for 30 min at 4 °C. The supernatant was used as the vesicle-containing supernatant. The cell pellets were resuspended using 100 mL of PBS containing 0.1 mM N_{α}-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) (Wako) and 0.1 mM leupeptin (Peptide Institute), and were disrupted by two passes through a French pressure cell at 100 MPa. The remaining bacterial cells were removed by centrifugation at 2,400 × *g* for 10 min, and the supernatant was subjected to ultracentrifugation at 100,000 × *g* for 60 min. The pellets were treated with 1% Triton X-100 in PBS containing 20 mM $MgCl_2$ for 30 min at 20 °C. The outer membrane fraction was recovered as a pellet by ultracentrifugation at 100,000 × *g* for 60 min at 4 °C. The supernatant was obtained as the cytoplasmic membrane fraction.

The particle-free culture supernatant fraction was obtained as described previously (4). *P. gingivalis* cells from a 140-mL culture containing 0.1 mM TLCK and 0.1 mM leupeptin were harvested by centrifugation at 6,000 × *g* for 10 min at 4 °C. The supernatant was ultracentrifuged at 100,000 × *g* for 60 min to remove vesicles. The vesicle free supernatant was precipitated with trichloroacetic acid (TCA) at the concentration of 20%, stored on ice for 2 h, and washed twice with cold acetone. After

drying, the pellet was dissolved in distilled water containing 0.1 mM TLCK and 0.1 mM leupeptin.

Tiling Microarray Analysis

Custom tiling microarrays spanning the whole genome of *P. gingivalis* ATCC 33277 with 25-mer probes each of which was eight-bases shifted on the genome sequence were purchased from Affymetrix. Antisense biotinylated cDNA was prepared from 10 μg of total RNA according to the Affymetrix GeneChip prokaryotic one-cycle target preparation protocol (Affymetrix). In short, reverse transcriptase (Superscript II; Invitrogen,) and random hexamer primers were used to produce DNA complementary to the RNA. The cDNA products were then fragmented by DNase I and labeled at the 3′ termini with terminal transferase and biotinylated Gene Chip DNA labeling reagent (Affymetrix). The fragmented and labeled cDNA was hybridized to the GeneChip at 45°C for 16 h. Staining, washing, and scanning procedures were carried out according to the GeneChip Expression Analysis Technical manual (Affymetrix). Hybridization was performed three times with the labeled cDNAs independently prepared. Signal intensities were quantified with the GeneChip Operating Software (Affymetrix), and further data analyses were performed with a microarray genomic analysis program (Insilico Molecular Cloning Array edition, In Silico Biology) and Microsoft Excel software (Microsoft).

Supplemental References

1. **Ueshima J, Shoji M, Ratnayake DB, Abe K, Yoshida S, Yamamoto K, Nakayama K.** 2003. Purification, gene cloning, gene expression, and mutants of Dps from the

obligate anaerobe *Porphyromonas gingivalis*. Infect Immun **71:**1170-1178.

2. **Tagawa J, Inoue T, Naito M, Sato K, Kuwahara T, Nakayama M, Nakayama K, Yamashiro T, Ohara N.** 2014. Development of a novel plasmid vector pTIO-1 adapted for electrotransformation of *Porphyromonas gingivalis*. J Microbiol Methods **105:**174-179.

3. **Shi Y, Ratnayake DB, Okamoto K, Abe N, Yamamoto K, Nakayama K.** 1999. Genetic analyses of proteolysis, hemoglobin binding, and hemagglutination of *Porphyromonas gingivalis*. Construction of mutants with a combination of *rgpA*, *rgpB*, *kgp*, and *hagA*. J Biol Chem **274:**17955-17960.

4. **Potempa J, Pike R, Travis J.** 1995. The multiple forms of trypsin-like activity present in various strains of *Porphyromonas gingivalis* are due to the presence of either Arg-gingipain or Lys-gingipain. Infect Immun **63:**1176-1182.

Supplemental Tables

Supplementary Table S1. Bacterial strains and plasmids used in this study

Name	Description	Source or reference
E. coli strain		
DH5α	General purpose host strain for cloning	Nippongene
BL21 (DE3)	Host strain for expression vector pET-15b and pET-32b(+)	Nippongene
P.gingivalis strain		
ATCC 33277	Wild type	American Type Culture Collection
Δ*omp17*	ΔPGN_0300::*ermF*; Em^r	This study
Δ*omp17*/*omp17*$^+$	ΔPGN_0300 PGN_1045::[PGN_0300$^+$ *tetQ*], Em^r Tc^r	This study
KDP136	*kgp*::*cat rgpA*::[*ermF ermAM*] *rgpB*::*tetQ*, Cat^r Em^r Tc^r	Shi *et al*. (3)
W83	Wild type	
Δ*omp17*-83	ΔPG0192::*ermF*; Em^r	This study
E. coli plasmid		
pBluescript II SK(-)	Ap^r, cloning vector	Stratagene
pUC19	Ap^r, cloning vector	Takara Bio
pET-32b(+)	Ap^r, expression vector	Novagen
pUCPGN0300	Ap^r Em^r, pUC19-PGN_0300::*ermF*	This study
pCPG	Ap^r Tc^r, pBSSK containing PGN_1045::p-*fimA*-*tetQ*	This study
pCPG0300	Ap^r Tc^r, pBSSK containing PGN_1045::p-*fimA*-PGN_0300-*tetQ*	This study
pKD355	Ap^r Em^r, contains the *ermF ermAM* DNA cassette in pUC19	Ueshima *et al*. (1)
pKD375	Ap^r Tc^r, contains the *tetQ* DNA cassette in pUC19	Shi *et al*. (3)

Supplementary Table S2. Oligonucleotide primers used in this study

Primer	Sequence (5'-3')
PGN_0300UF	GGAATTCCGATCGTTCGCTTCAGATGTTAG
PGN_0300UR	CGGGATCCCGAACCGTTTCATATCGCCATTC
PGN_0300DF	CGGGATCCCGTACTGAGCAAGATGGGCTTT
PGN_0300DR	GCTCTAGAGCCTCTTTCTTGGCAACACTT
PGN_0300EXF	GGGATCCAACTCCGTTTGAGAGGTATGTC
PGN_0300EXR	ACGGATCCAGGAAGAGCCATCAGAAGCA
PGN_1045UF	CCGCTCGAGTAGCGCATTGATGGGAGCAG
PGN_1045UR	CCCAAGCTTACGGGCATCGGGAGAATAAC
PGN_1045DF	CGGGATCCCGTCTTGCGCTTCGACTATATGC
PGN_1045DR	TGGCGGCCGCCATCAGTCATTCAAGGAAG
PorUF	CGGGATATCAACGGGCAGGATCCCGAAGGGCAG
PorUR	GCCCTCGAGGTTCACCACACAACGATAGAGGTA
FimA-PROU	GAAGGCCTTATATGCCTACAGCGAAAAATGG
FimA-PROD	CGGGATCCCATGCTGATGGTGGCATTACCTT
ErmF-Fw	CGGGATCCCCCGATAGCTTCCGCTATT
ErmF-Rv	CGGGATCCTACGAAGGATGAAATTTTTCAG
PGN_0299PF	GGACGAGTTTTTACTCCATTGC
PGN_0299PR	TCGCTGTAGAGAATTGTCAGGA
PGN_0300PF	TGAACGAACAGCTGGAACAG
PGN_0300PR	CTTGCTAAAGCCCATCTTGC
PGN_0301PF	AATGCCGGAACAAGTAGCTG
PGN_0301PR	TGCGGTCAAGTCAATAGCAG

Undrerlined nucleotides indicate recognition sites of restriction enzymes.

Supplementary Table S3. Identification of protein bands shown in Supplemental figure S4.

Strain	Band	PGN number	Score	Protein
KDP136	1	PGN_1261	59	Cobalamin adenosyltransferase
	2	PGN_0335*	122	Hypothetical protein
	3	PGN_0294	92	RagB
	4	PGN_0180	82	FimA
	5	PGN_0123*	70	Hypothetical protein

* CTD-containing proteins

Supplemental Figures

Supplemental figure S1

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PGN_0300 MNGDMKRFLILIGFALAIAFSGFSQKFALVDMEYILKNIPDYEMMNEQLEQVSKKWQNEI 60
PGN_0301 ----MKKFFLMLLMALPLSL--LAQKVAVVNTEEIISKMPEQVAATKQLNELAEKYRLDL 54
             **:*:::: :**.:::  ::**.*:*: * *:.::*:    .:**::::*:: ::

PGN_0300 EALENEAQSMYKKYQSDLVFLSAAQKKAQEEAIVKKEQQASELKRKYFGPEGELYKKRSD 120
PGN_0301 KSMDDEFAKKTEEFVKEKDSLLENIRNRRQQELQDIQ---TRYQQSYQTMQEDLQKRQQQ 111
         :::::*  .  ::: .:   *    :: ::: : . :   :. ::.*   : :* *::.:

PGN_0300 LMKPIQDEIWNAIKEIAKRNNYQMVLDRGTSGIIFASPSIDISDLVLSKMGFSK 174
PGN_0301 LFAPIQQKVADAIKKVGDEENCAYIMEAG-MMLYTGATAIDLTAKVKAKLGIK- 163
         *: ***::: :***::...:*   ::: *   :  .:.:**::  * :*:*:.
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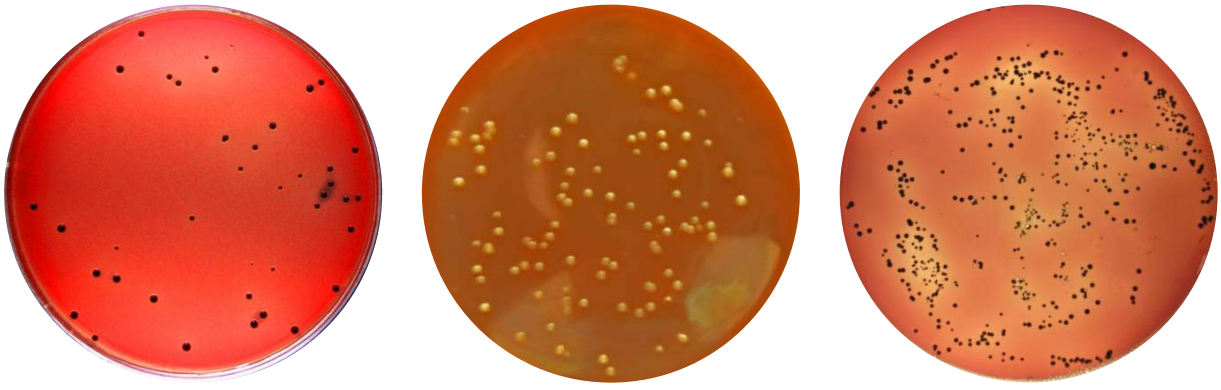
Supplementary figure S1. Multiple sequence alignment of *P. gingivalis* PGN_0300 and PGN_0301. Identical amino acids, conservative substitutions, and semiconservative substitutions are indicated by stars, stacked double dots, and single dots, respectively. Dashes are gaps introduced to maximize alignment.

Supplemental figure S2



Supplemental figure S2. Colonial pigmentation. *P. gingivalis* cells were grown anaerobically on blood agar plates at 37°C.

Supplemental figure S3

Band No. 11 PGN 1728

MRKLLLLIAA SLLGVGLYAQ SAKIKLDAPT TRTTCTNNSF KQFDASFSFN EVELTKVETK
GGTFASVSIP GAFPTGEVGS PEVPAVRKLI AVPVGATPVV RVKSFTEQVY SLNQYGSEKL
MPHQPSMSKS DDPEKVPFVY NAAAYARKGF VGQELTQVEM LGTMRGVRIA ALTINPVQYD
VVANQLKVRN NIEIEVSFQG ADKVATQRLY DASFSPYFET AYKQLFNRDV YTDHGDLYNT
PVRMLVVAGA KFKEALKPWL TWKAQKGFYL DVHYTDEAEV GTTNASIKAF IHKKYNDGLA
ASAAPVFLAL VGDTDVISGE KGKKTKKVTD LYYSAVDGDY FPEMYTFRMS ASSPEELTNI
IDKVLMYEKA TMPDKSYLEK VLLIAGADYS WNSQVGQPTI KYGMQYYYNQ EHGYTDVYNY
LKAPYTGCYS HLNTGVSFAN YTAHGSETAW ADPLLTTSQL KALTNKDKYF LAIGNCCITA
QFDYVQPCFG EVITRVKEKG AYAYIGSSPN SYWGEDYYWS VGANAVFGVQ PTFEGTSMGS
YDATFLEDSY NTVNSIMWAG NLAATHAGNI GNITHIGAHY YWEAYHVLGD GSVMPYRAMP
KTNTYTLPAS LPQNQASYSI QASAGSYVAI SKDGVLYGTG VANASGVATV NMTKQITENG
NYDVVITRSN YLPVIKQIQA GEPSPYQPVS NLTATTQGQK VTLKWDAPSA KKAEGSREVK
RIGDGLFVTI EPANDVRANE AKVVLAADNV WGDNTGYQFL LDADHNTFGS VIPATGPLFT
GTASSDLYSA NFEYLIPANA DPVVTTQNII VTGQGEVVIP GGVYDYCITN PEPASGKMWI
AGDGGNQPAR YDDFTFEAGK KYTFTMRRAG MGDGTDMEVE DDSPASYTYT VYRDGTKIQE
GLTATTFEED GVAAGNHEYC VEVKYTAGVS PKVCKEVTVE GSNEFAPVQN LTGSAVGQKV
TLKWDAPNGT PNPNPNPNPN PGTTTLSESF ENGIPASWKT IDADGDGHGW KPGNAPGIAG
YNSNGCVYSE SFGLGGIGVL TPDNYLITPA LDLPNGGKLT FWVCAQDANY ASEHYAVYAS
STGNDASNFT NALLEETITA KGVRSPEAIR GRIQGTWRQK TVDLPAGTKY VAFRHFQSTD
MFYIDLDEVE IKANGKRADF TETFESSTHG ETPAEWTTID ADGDGQGWLC LSSGQLGWLT
AHGGTNVVAS FSWDGMALNP DNYLISKDVT GATKVKYYYA VNDGFPGDHY AVMISKTGTN
AGDFTVVFEE TPNGINKGGA RFGLSTEANG AKPQSVWIER TVDLPAGTKY VAFRHYNCSD
LNYILLDDIQ FFMGGSPNPT DYTYTVYRDG TKIKEGLTET TFEEDGVATG NHEYCVEVKY
PAGVSPKECV NVTINPTQFN PVQNLTAEQA PNSMDAILKW NAPASKRAEV LNEDFENGIP
SSWKTIDADG DGNNWTTTPP PGGSSFAGHN SDICVSSASY INFEGPQNPD NYLVTPELSL
PGGGTLTFWV CAQDANYASE HYAVYASSTG NDASNFANAL LEEVLTAKTV VTAPEAIRGT
RAQGTWYQKT VQLPAGTKYV AFRHFGCTDF FWINLDDVVI TSGNAPSYTY TIYRNNTQIA
SGVTETTYRD PDLATGFYTY GVKVVYPNGE SAIETTTLNI TSLADVTAQK PYTLTVVGKT
ITVTCQGEAM IYDMNGRRLA AGRNTVVYTA QGGYYAVMVV VDGKSYVEKL AVK

Band No. 12 PGN_1094

MNTNKFENRH IGIAERDLSV MLRTIGVESM DQLIHETIPG DILLPEPLDL PEAMTERELL
EHFMELGSKN KIFTSYIGQG WYDTVTPAPI QRNVLENPAW YTSYTPYQAE VSQGRLEALF
NFQTVITELT GLPLTNCSLL DEATAGAEAA RMFFDTRSRT QIKAGANVLF VDKKVFRSTI
EVIRTRIIPQ GMQIVVDDYK TFNFTPEVFA AIVQFPDAEG SVNDYKSFIE AAHAANAKVA
VATDLLSMTI LTPPGEWGAD IVFGSAQRFG IPMYYGGPSA GYLATSMDYK RNIPGRIIGM
SKDAYGRPAY RLALQTREQH IKREKATSNI CTAQALLATM SSFYAVYHGA DGLRHIAGRV
HAYAGFISEK LSGLGYKLTN KDFFDTIRVE LPAGVTADKV RELAEECSVN FFYPADGNIL
ISLDETTLPS DLGVLLYIFS SVLGKEETIT EDIAIDKRYF DGAFARTSNF LDYDVFNNYH
TETELMRYIK RLERKDISLA HSMIPLGSCT MKLNAASELF NLSMPQFNAI HPYAPKDQTE
GYNEMLESLE RYLAVITGFD ATSLQPNSGA AGEYTGLLTI RGYLNSIGQG HRHLVILPAS
AHGTNPASAI QCGYDTVTVA CDERGNVDME DFMAKTEQHK DEIAAMMITY PSTHGIFETN
IIDLCHRIHE CGGQVYMDGA NMNAQVGLTS PGFIGADVCH LNLHKTFAIP HGGGGPGVGP
ICVAKHLTPF LPSHPLREDY NGLEVSAAPY GSAGVTTITY AYIRLMGYEG LRRATKIAIL
NSNYMANRLK DTFGIVYTGA TGRVGHELIL ECRKIKESGI DENDIAKRLM DFGYHAPTLS
FPVHGTLMIE PTESESLAEL DRFIDVMNCI WEEIQEVARG EQDAIDNVLK NAPHPQYEVT
ANDWSHPYSR EKAAYPLEYL RENKFWLNVA RIDNGYGDRN LVPSLCSACE VFNNQ

Band No. 13 PGN_0335

MKKKNFLLLG IFVALLTFIG SMQAQQAKDY FNFDERGEAY FSFKVSDRAV LQELALIMSI
DEFDPVTNEA IAYASEEEFE AFLRYGLKPT FLTPPSMQRA VEMFDYRSGE KYEWNAYPTY
EAYISMMEEF QTKYPSLCTT SVIGKSVKDR KLIICKLTSS ANTGKKPRVL YTSTMHGDET
TGYVVLLRLI DHLLSNYESD PRIKNILDKT EVWICPLTNP DGAYRAGNHT VQGATRYNAN
NVDLNRNFKD DVAGDHPDGK PWQPEATAFM DLEGNTSFVL GANIHGGTEV VNYPWDNKKE
RHADDEWYKL ISRNYAAACQ SISASYMTSE TNSGIINGSD WYVIRGSRQD NANYFHRLRE
ITLEISNTKL VPASQLPKYW NLNKESLLAL IEESLYGIHG TVTSAANGQP LKCQILIENH
DKRNSDVYSD ATTGYYVRPI KAGTYTVKYK AEGYPEATRT ITIKDKETVI MDIALGNSVP
LPVPDFTASP MTISVGESVQ FQDQTTNNPT NWEWTFEGGQ PAMSTEQNPL VSYSHPGQYD
VTLKVWNASG SNTITKEKFI TVNAVMPVAE FVGTPTEIEE GQTVSFQNQS TNATNYVWIF
DGGTPATSED ENPTVLYSKA GQYDVTLKAI SASGETVKTK EKYITVKKAP VPAPVADFEG
TPRKVKKGET VTFKDLSTNN PTSWLWVFEG GSPATSTEQN PVVTYNETGK YDVQLTATNE
GGSNVKKAED YIEVILDDSV EDIVAQTGIV IRPQNGTKQI LIEANAPIKA IVLYDINGRV
VLKTTPNQLR STVDLSILPE GIYTINIKTE KSARTEKIHI G

Band No. 17 PGN 1476

MKKLFLASVA FLCAWIWSAN AQTMAPNYFH ADPQQFKHRI VKEKSFSSYS NYEYGVDNRL
QRIYSVDESS GEIEHERRFF FNEGGYMIRE EEYDGTVQIP VRKWEFVRDD KGYITHFSRY
SPKDGSQELI EDIRIDFSYD ADMKLIKADI DFFDVMANVW GDLRTTELIY NENGLLKEMI
QTDPGSGQEF NREELTYNNL NKIVAIRFIP GPASTGLNEF ELIYEYDSEG MDIVKAGRDD
FWYYYEYDKE MLASETFFPK PSIADLVYFG LKDYVDFSGL PFKNSYTHVV VKESTNEIEA
IYEPISVYSV VVIQPENGEI KLTADGQPLN SGSTLVAGRR IKIHPIPAEG YEVDKVMVNG
ESIEAPYEFL LEKDTEVTAL MKKSNAVGEV DTKGFHVYPI PTSKDLTIEI PAEMVGKVAS
LIDMNGQIVY RVTLNNIFQQ IDISHLKGVF LLQIGDITER VIVQ

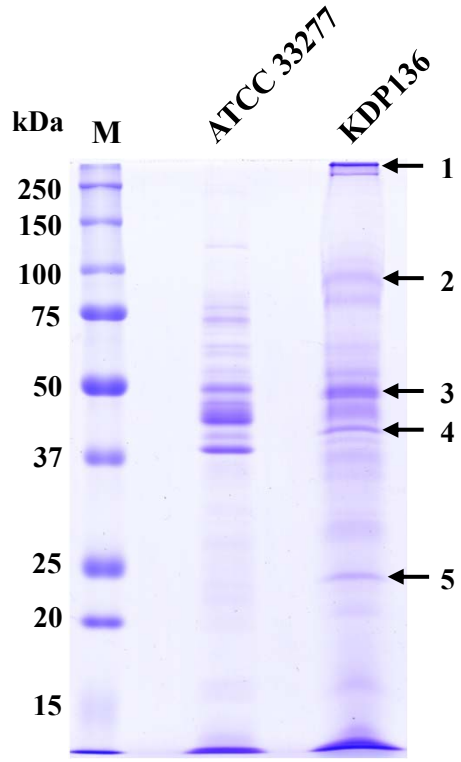
Band No. 22 PGN_0335

MKKKNFLLLG IFVALLTFIG SMQAQQAKDY FNFDERGEAY FSFKVSDRAV LQELALIMSI
DEFDPVTNEA IAYASEEEFE AFLRYGLKPT FLTPPSMQRA VEMFDYRSGE KYEWNAYPTY
EAYISMMEEF QTKYPSLCTT SVIGKSVKDR KLIICKLTSS ANTGKKPRVL YTSTMHGDET
TGYVVLLRLI DHLLSNYESD PRIKNILDKT EVWICPLTNP DGAYRAGNHT VQGATRYNAN
NVDLNRNFKD DVAGDHPDGK PWQPEATAFM DLEGNTSFVL GANIHGGTEV VNYPWDNKKE
RHADDEWYKL ISRNYAAACQ SISASYMTSE TNSGIINGSD WYVIRGSRQD NANYFHRLRE
ITLEISNTKL VPASQLPKYW NLNKESLLAL IEESLYGIHG TVTSAANGQP LKCQILIENH
DKRNSDVYSD ATTGYYVRPI KAGTYTVKYK AEGYPEATRT ITIKDKETVI MDIALGNSVP
LPVPDFTASP MTISVGESVQ FQDQTTNNPT NWEWTFEGGQ PAMSTEQNPL VSYSHPGQYD
VTLKVWNASG SNTITKEKFI TVNAVMPVAE FVGTPTEIEE GQTVSFQNQS TNATNYVWIF
DGGTPATSED ENPTVLYSKA GQYDVTLKAI SASGETVKTK EKYITVKKAP VPAPVADFEG
TPRKVKKGET VTFKDLSTNN PTSWLWVFEG GSPATSTEQN PVVTYNETGK YDVQLTATNE
GGSNVKKAED YIEVILDDSV EDIVAQTGIV IRPQNGTKQI LIEANAPIKA IVLYDINGRV
VLKTTPNQLR STVDLSILPE GIYTINIKTE KSARTEKIHI G

Supplemental figure S3. PMF peptide data of protein bands in Fig. 6. The yellow marks indicate the first amino acid of C-terminal domain peptide which should be cleaved by the peptidase.

Supplemental figure S4

A



B

Band No. 2 PGN_0335

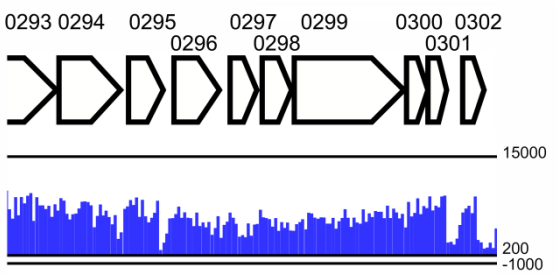
MKKKNFLLLG IFVALLTFIG SMQAQQAKDY FNFDERGEAY FSFKVSDRAV LQELALIMSI
DEFDPVTNEA IAYASEEEFE AFLRYGLKPT FLTPPSMQRA VEMFDYRSGE KYEWNAYPTY
EAYISMMEEF QTKYPSLCTT SVIGKSVKDR KLIICKLTSS ANTGKKPRVL YTSTMHGDET
TGYVVLLRLI DHLLSNYESD PRIKNILDKT EVWICPLTNP DGAYRAGNHT VQGATRYNAN
NVDLNRNFKD DVAGDHPDGK PWQPEATAFM DLEGNTSFVL GANIHGGTEV VNYPWDNKKE
RHADDEWYKL ISRNYAAACQ SISASYMTSE TNSGIINGSD WYVIRGSRQD NANYFHRLRE
ITLEISNTKL VPASQLPKYW NLNKESLLAL IEESLYGIHG TVTSAANGQP LKCQILIENH
DKRNSDVYSD ATTGYYVRPI KAGTYTVKYK AEGYPEATRT ITIKDKETVI MDIALGNSVP
LPVPDFTASP MTISVGESVQ FQDQTTNNPT NWEWTFEGGQ PAMSTEQNPL VSYSHPGQYD
VTLKVWNASG SNTITKEKFI TVNAVMPVAE FVGTPTEIEE GQTVSFQNQS TNATNYVWIF
DGGTPATSED ENPTVLYSKA GQYDVTLKAI SASGETVKTK EKYITVKKAP VPAPVADFEG
TPRKVKKGET VTFKDLSTNN PTSWLWVFEG GSPATSTEQN PVVTYNETGK YDVQLTATNE
GGSNVKKAED YIEVILDDSV EDIVAQTGIV IRPQNGTKQI LIEANAPIKA IVLYDINGRV
VLKTTPNQLR STVDLSILPE GIYTINIKTE KSARTEKIHI G

Band No. 5 PGN_0123

MKKTKRNMRK IFISIALLAG FIATLNAQVV IKVGDAILEN NATVDITAFT TEDGTEEMKF
KGMVINQSAT PINVIGKITK QEMIGDGHFA LCFGQCMLPN VSVSPVVEVG GEGEPLSLRY
TFPVSNEGHT GAFTFSCFPE SGAPGTELAT VNINFKYKGG GTDLTNIGLG RIALIQSGNT
CTLQYNSNGK RLALEVYNLL GVKVFTSQLP AGSGSYTLPV RLQRGVHIFR ITEGGKPAFV
QKYLIK

Supplemental figure S4. Peptide map fingerprinting (PMF) analyses of proteins in the *P. gingivalis* KDP136 culture supernatant. (A) The vesicle-free supernatants of *P. gingivalis* KDP136 were separated by SDS-PAGE, and the resulting gel was stained with Coomassie Brilliant Blue. (B) PMF peptide data of protein bands in A. The yellow marks indicate the first amino acid of C-terminal domain peptide which should be cleaved by the peptidase.

Supplemental figure S5



Supplemental figure S5. Tiling microarray analysis on the vicinity of the genes PGN_0299, *omp17*, and PGN_0301.