

Supplementary Information for:

Involvement of the Wbp pathway in the biosynthesis of *Porphyromonas gingivalis* lipopolysaccharide with anionic polysaccharide

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Supplemental Figure 1 | Comparison of amino acid sequences among WbpB homologs.

Porphyromonas gingivalis ATCC 33277: PGN_0168, *Pseudomonas aeruginosa* PAO1: PA3158, *Helicobacter pylori* NCTC 11637: HP0679 and *Bordetella pertussis* Tohama I: BP0093 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangle indicates the nonsense mutation in *P. gingivalis* strain HG66.

Supplemental Figure 2 | Comparison of amino acid sequences among WbpA homologs.

Porphyromonas gingivalis ATCC 33277: PGN_0613, PGN_1243, *Pseudomonas aeruginosa* PAO1: PA3159 and *Bordetella pertussis* Tohama I: BP1629, BP3150 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site.

Supplemental Figure 3 | Comparison of amino acid sequences among WbpE homologs.

Porphyromonas gingivalis ATCC 33277: PGN_1236, *Pseudomonas aeruginosa* PAO1: PA3155 and *Bordetella pertussis* Tohama I: BP0091 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangle indicates the essential residue for WbpE activity.

Supplemental Figure 4 | Comparison of amino acid sequences among WbpD homologs.

Porphyromonas gingivalis ATCC 33277: PGN_0002, *Pseudomonas aeruginosa* PAO1: PA3156 and *Bordetella pertussis* Tohama I: BP0092 were compared. The alignment was created using the ClustalW and Boxshade programs hosted at the Swiss EMBnet web site. The red triangles indicate the essential residue for WbpD activity.

Supplemental Table 1 | Strains used in this study.

Supplemental Table 2| Plasmids used in this study.

Supplemental Table 3| Primers used in this study.

Supplemental Text 1| Construction of *P. gingivalis* mutant strains.

Construction of *P. gingivalis* mutants

To create *P. gingivalis* PGN_0168 (*wbpB*)::Em^r (KDP220) and PGN_0002::Em^r (KDP222) mutants, the upstream regions were amplified with PGN_0168upFw/PGN_0168upRev and PGN_0002upFw/PGN_0002upRev primers, respectively, using *P. gingivalis* ATCC 33277 as the template, and cloned into the pCR4 vector or pGEM-T Easy vector. Each upstream and downstream region was swapped with the appropriate restriction region of pKD740 ²⁶, generating pKD892 and pKD893. The resulting targeting vectors pKD892 and pKD893 were linearized with SacI and introduced into *P. gingivalis* ATCC 33277 by electroporation followed by selection on blood agar plates containing Em (10 µg/ ml).

To create a *P. gingivalis* PGN_1236 (*porR*)::Tc^r (KDP221) mutant, the upstream region was amplified with PGN_1236upFw/PGN_1236upRev primers using *P. gingivalis* ATCC 33277 as the template and cloned into the pCR4 vector, yielding pCR4-porRup. The downstream region was amplified with PGN_1236dwFw/PGN_1236dwRev primers using *P. gingivalis* ATCC 33277 as the template and cloned into the pCR4 vector, yielding pCR4-porRdw1. To eliminate the EcoRI site that was closest to the NotI site in pCR4-porRdw1, pCR4-porRdw1 was digested with NotI and was then self-ligated, yielding pCR4-porRdw2. The EcoRI-BamHI region of pCR4-porRup was inserted into the same region of pCR4-porRdw2, yielding pCR4-porRupdw2. The *tetQ* gene, which is the BamHI-BglII region of pKD375 ⁵¹, was inserted into the BamHI site of pCR4-porRupdw2, generating pKD894. The resulting targeting vector pKD894, linearized with NotI, was introduced into *P. gingivalis* ATCC 33277 by electroporation and was then selected on blood agar plates containing Tc (0.7 µg/ ml).

Construction of *P. gingivalis* complemented strains

The promoter region of the *Porphyromonas gulae* catalase gene (accession no. AB083039 in GenBank/EMBL/DDBJ databases) was amplified using p6-34-F-KpnI/p6-34-R-SalI from pKD955⁶ and was cloned into the pCR4 vector to yield pCR4-cat pro. A KpnI-EcoRI DNA fragment containing the promoter region of the *Porphyromonas gulae* catalase gene of pCR4-cat pro was swapped into the same region of pKD854⁵ to yield pBSSK-cat pro-rgpBter.

To create complementation plasmids for the *wbpB* (PGN_0168) and PGN_0002 genes, *P. gingivalis* ATCC 33277 was used as the template for amplification with PGN_0168compFw/PGN_0168compRev or PGN_0002compFw/PGN_0002compRev primers, respectively. Each PCR product was cloned into pUC118, yielding pUC118-*wbpB* and pUC118-PGN_0002.

To create a complementation plasmid with the *wbpD* gene from *Pseudomonas aeruginosa*, the coding region of *wbpD* was amplified from *P. aeruginosa* using the *P. a. wbpDFw/ P. a. wbpDRev* primers. The PCR product was cloned into pUC118, yielding pUC118-*P.a. wbpD*. The SalI-XbaI DNA fragment containing the coding region of the *wbpB*, PGN_0002 and *P.a. wbpD* genes from pUC118-*wbpB*, pUC118-PGN_0002 and pUC118-*P. a. wbpD* was inserted into the same region of pBSSK-cat pro-rgpBter to yield pBSSK-cat pro-*wbpB*-rgpBter, pBSSK-cat pro-PGN_0002-rgpBter and pBSSK-cat pro-*P.a. wbpD*-rgpBter. The KpnI-NotI DNA fragment of pBSSK-cat pro-*wbpB*-rgpBter, pBSSK-cat pro-PGN_0002-rgpBter and pBSSK-cat pro-*P.a. wbpD*-rgpBter was inserted into the same region of a pTCB vector⁵² to yield pTCB-*wbpB*, pTCB-PGN_0002 and pTCB-*P.a. wbpD*. The pTCB vector containing *wbpB*, PGN_0002 or *P. a. wbpD* was introduced into *Escherichia coli* S17-1⁵³ by electroporation and was then selected on LB agar plates containing Ap (100 µg/ ml). The

transformant was mated with strain HG66 or the PGN_0002 mutant (KDP222) and was then selected on blood agar plates containing Gm (50 µg/ ml) and Tc (0.7 µg /ml), yielding HG66/pWbpB+, PGN_0002/pPGN_0002+ (KDP223) and PGN_0002/p*P. a.*WbpD+ (KDP224).

Supplemental Text 2| Sequence comparison of the *wbpB* gene in *P. gingivalis* strains.

	10	20	30	40	50
W83	ATGCACAACT	TTGCCATCAT	CGGTGTGGCC	GGCTATATTG	CTCCGCGCCA
TDC60	ATGCACAACT	TTGCCATCAT	CGGTGTGGCC	GGCTATATTG	CTCCGCGCCA
HG66	ATGCACAACT	TTGCCATCAT	CGGTGTGGCC	GGCTATATTG	CTCCGCGCCA
33277	ATGCACAACT	TTGCCATCAT	CGGTGTGGCC	GGCTATATTG	CTCCGCGCCA
Consensus	*****	*****	*****	*****	*****
	60	70	80	90	100
W83	CCTTCGCGCC	ATCAAGGATA	CAGGCAACCG	CTTGGTATCG	GCTATGGATA
TDC60	CCTTCGCGCC	ATCAAAGATA	CAGGCAATCG	CTTGGTATCG	GCTATGGATA
HG66	CCTTCGCGCC	ATCAAGGATA	CAGGCAACCG	CTTGGTATCG	GCTATGGATA
33277	CCTTCGCGCC	ATCAAGGATA	CAGGCAACCG	CTTGGTATCG	GCTATGGATA
Consensus	*****	*****	*****	*****	*****
	110	120	130	140	150
W83	AGTTCGACAG	TGTGGGTATC	ATGGACAGTT	ACTTCCCGAA	AGCTGCCTTC
TDC60	AGTTCGACAG	TGTGGGTATC	ATGGACAGTT	ACTTCCCGAA	AGCTGCCTTC
HG66	AGTTCGACAG	TGTGGGTATC	ATGGACAGTT	ACTTCCCGAA	AGCTGCCTTC
33277	AGTTCGACAG	TGTGGGTATC	ATGGACAGTT	ACTTCCCGAA	AGCTGCCTTC
Consensus	*****	*****	*****	*****	*****
	160	170	180	190	200
W83	TTTACGGAGC	AAGAACTGTT	CGACCGTCAC	AACTCCAAGC	TACGAGGAAC
TDC60	TTTACGGAGC	AAGAACTGTT	CGACCGTCAC	AACTCCAAGC	TACGAGGAAC
HG66	TTTACGGAGC	AAGAACTGTT	CGATCGTCAC	AACTCCAAGC	TACGAGGAAC
33277	TTTACGGAGC	AAGAACTGTT	CGATCGTCAC	AACTCCAAGC	TACGAGGAAC
Consensus	*****	*****	***	*****	*****
	210	220	230	240	250
W83	CGATCAGGCT	ATCGACCACG	TGTCCGTTTG	TACACCGAAC	TATCTGCACG
TDC60	CGATCAGGCT	ATCGACCATG	TGTCCGTTTG	TACACCGAAC	TATCTGCACG
HG66	CGATCAGGCT	ATCGACCACG	TGTCCGTTTG	TACACCGAAC	TATCTGCACG
33277	CGATCAGGCT	ATCGACCACG	TGTCCGTTTG	TACACCGAAC	TATCTGCACG
Consensus	*****	*****	*	*****	*****

	260	270	280	290	300
W83	ATGCTCATTG TCGCTATGGC CT CGACTTG GAGCGGATGT GATATGCGAG				
TDC60	ATGCTCATTG TCGCTATGGC CTACGACTTG GAGCGGATGT GATATGCGAG				
HG66	ATGCTCATTG TCGCTATGGC CT CGACTTG GAGCGGATGT GATATGCGAG				
33277	ATGCTCATTG TCGCTATGGC CT CGACTTG GAGCGGATGT GATATGCGAG				
Consensus	*****	*****	**	*****	*****
	310	320	330	340	350
W83	AAGCCTTGG TGCTCAATCC TTGGAATATA GACGCTTGC AGGAGATCGA				
TDC60	AAGCCTTGG TGCTCAATCC TTGGAATATA GACGCTTGC AGGAGATCGA				
HG66	AAGCCTTGG TGCTCAATCC TTGGAATATA GACGCTTGC AGGAGATCGA				
33277	AAGCCTTGG TGCTCAATCC TTGGAATATA GACGCTTGC AGGAGATCGA				
Consensus	*****	*****	*****	*****	*****
	360	370	380	390	400
W83	GCGAGAGACG GGGCATCGGA TCTATAATAT TCTTCAACTG CGACTCCATG				
TDC60	G CAAGAGACG GGGCATCGGA TCTATAATAT TCTTCAACTG CGACTCCATG				
HG66	G CAAGAGACG GGGCATCGGA TCTATAATAT TCTTCAACTG CGACTCCATG				
33277	G CAAGAGACG GGGCATCGGA TCTATAATAT TCTTCAACTG CGACTCCATG				
Consensus	**	*****	*****	*****	*****
	410	420	430	440	450
W83	AGTCGATCGT GGCCCTGAAG AGAAAGATCG AATCCGGATC GGCGGACAAG				
TDC60	AGT C TATCGT GGCCCTGAAG AGAAAGATCG AATCCGG CC GGCGGACAAG				
HG66	AGT C TATCGT GGCCCTGAAG AGAAAGATCG AATCCGG CC GGCGGACAAG				
33277	AGT C TATCGT GGCCCTGAAG AGAAAGATCG AATCCGG CC GGCGGACAAG				
Consensus	***	*****	*****	*****	*****
	460	470	480	490	500
W83	ATCTATGATG TCACGCTTAC ATATATTACT TCGCGCGGCA ATTGGTACTA				
TDC60	ATCTATGATG TCACGCTGAC G TATATTACT TCGCGCGGCA ATTGGTACTA				
HG66	ATCTATGATG TCACGCTGAC G TATATTACT TCGCGTGGCA A CTGGTACTA				
33277	ATCTATGATG TCACGCTGAC G TATATTACT TCGCGTGGCA A CTGGTACTA				
Consensus	*****	*****	**	*****	*****
	510	520	530	540	550
W83	TACGAGCTGG AAGGGAGACG AGCGTAAGAG CGGTGGCATA GCTACGAACA				
TDC60	TACGAGCTGG AAGGGAGACG AGCGTAAGAG CGGTGGCATA GCTACGAACA				
HG66	TACGAGCTGG AAGGGAGACG AGCGTAAGAG CGG CG GTATA G C AACGAATA				
33277	TACGAGCTGG AAGGGAGACG AGCGTAAGAG CGG CG GTATA G C AACGAATA				
Consensus	*****	*****	*****	**	***

	560	570	580	590	600
W83	TCGGTGTCA	TTCTACGAC	ATGCTGTCAT	GGATTTCGG	CAGTGTACGA
TDC60	TCGGTGTCA	TTCTACGAC	ATGCTGTCAT	GGATTTCGG	CAGTGTACGA
HG66	TCGGTGT CCA	TTCTACGAC	ATGCTG CGT	GGATTTCGG	CAGTGT CCGA
33277	TCGGTGT CCA	TTCTACGAC	ATGCTG CGT	GGATTTCGG	CAGTGT CCGA
Consensus	*****	***	*****	*****	*****
	610	620	630	640	650
W83	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
TDC60	CACAATGTCG	TACATGTGTG	TGTGCATGAC	AGGGCTGCCG	GCTATTTGGA
HG66	CACAATGTCG	TACATGTGT A	TGAGCATGAC	AGGGCTGCCG	GCTAT CTGGA
33277	CACAATGTCG	TACATGTGT A	TGAGCATGAC	AGGGCTGCCG	GCTAT CTGGA
Consensus	*****	*****	**	*****	*****
	660	670	680	690	700
W83	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
TDC60	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	TTCGATCAAT	GAGGAGCTTC
HG66	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	CTCGATCAAT	GA A GAGCTTC
33277	ATTGGAGCGC	GCCCGTGTGC	GTTACTTCCT	CTCGATCAAT	GA A GAGCTTC
Consensus	*****	*****	*****	*****	**
	710	720	730	740	750
W83	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTCG	TACGATAGAA
TDC60	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTCG	TACGATAGAA
HG66	TGCCCGAGAA	TGCCGT A AG	GGAGAGAAGC	GAACCTTCG	TACGATAGAA
33277	TGCCCGAGAA	TGCCGTACAG	GGAGAGAAGC	GAACCTTCG	TACGATAGAA
Consensus	*****	*****	**	*****	*****
	760	770	780	790	800
W83	ATCGACGGCG	AATCATTGCA	GTTCAGTGAA	GGGTTACGG	AGCTGCACAC
TDC60	ATCGACGGCG	AATCATTGCA	GTTCAGTGAA	GGGTTACGG	AGCTGCACAC
HG66	ATCGACGGCG	AATCATTGCA	GTTCAGTGAA	GGGTTACGG	AGCTGCACAC
33277	ATCGACGGCG	AATCATTGCA	GTTCAGTGAA	GGGTTACGG	AGCTGCACAC
Consensus	*****	*****	*****	*****	*****
	810	820	830	840	850
W83	CGAGAGTTAT	CGGCGGATT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
TDC60	CGAGAGTTAT	CGGCGGATT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
HG66	CGAGAGTTAT	CGGCGGATT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
33277	CGAGAGTTAT	CGGCGGATT	TGGCCGGTGA	AGGTTTCGGT	TTGGACGAAG
Consensus	*****	*****	*****	*****	*****

	860	870	880	890	900
W83	CTCGAACAG	CATTCAGATC	GTTCATGACA	TTCGCAATGC	TTCTCCCGTA
TDC60	CTCGAACAG	CATTCAGATC	GTTCATGACA	TTCGCAATGC	TTCTCCCGTA
HG66	CTCGAACAG	CATTCAGATC	GTTCATGACA	TTCGCAATGC	TTCTCCCGTA
33277	CTCGAACAG	CATTCAGATC	GTTCATGACA	TTCGCAATGC	TTCTCCCGTA
Consensus	*****	*****	*****	*****	*****
	910	920	930	940	950
W83	GGACTGAAGG	GCGAATACCA	CCCCTTGCA	CGTTTGC _{CCCC}	TGGCCTCTCA
TDC60	GGACTGAAGG	GCGAATACCA	CCCC _{TTTGCA}	CGTTTGC _{CCCC}	TGGCCTCTCA
HG66	GGACTGAAGG	GCGAATACCA	CCCC _{TTTGCA}	CGTTTGC _{CCCC}	TGGCCTCTCA
33277	GGACTGAAGG	GCGAATACCA	CCCC _{TTTGCA}	CGTTTGC _{CCCC}	TGGCCTCTCA
Consensus	*****	*****	***	*****	*****
	960	970			
W83	TCCTTCGGA	TGGAGACGCT	GA		
TDC60	TCCTTCGGA	TGGAGACGCT	GA		
HG66	TCCTTCGGA	TGGAGACGCT	GA		
33277	TCCTTCGGA	TGGAGACGCT	GA		
Consensus	*****	*****	**		

Yellow color indicates the same residues between HG66 and other strains.

Red color indicates the HG66 nonsense mutation.

Supplement Text 3| Abbreviations

GlcNAc, glucosamine; GlcNAcA, 2-acetamido-2,3-dideoxy-D-glucuronic acid;
3-keto-D-GlcNAcA, 2-acetamido-2-deoxy-D-ribo-hex-3-uluronic acid; GlcNAc3NA,
2-acetamido-3-amino-2,3-dideoxy-D-glucuronic acid; GlcNAc3NAcA,
2,3-diacetamido-2,3-dideoxy-D-glucuronic acid; ManNAc3NAcA,
2,3-diacetamido-2,3-dideoxy-D-mannuronic acid. AcCoA, acetyl-coenzyme A; CoA,
coenzyme A; 2-HG, 2-hydroxyglutarate; α -KG, α -ketoglutarate; NAD⁺, nicotinamide adenine
dinucleotide; NADH, nicotinamide adenine dinucleotide hydrate; PLP, pyridoxal 5'-phosphate;
PMP, pyridoxamine 5'phosphate.

Supplemental Figure1

<i>P. gingivalis</i>	1	MENFAIIIGVAG - YIAPRHLRAIKDTGNPLVSAMDKFDSVGIMDSYFPKAAPFTEQELEDR	
<i>P. aeruginosa</i>	1	MENFAIIIGVAG - YIAPRHLRAIKDTGNPLVSAMDINDSVGIDDSISPOSEFFTEPEEFELD	
<i>H. pylori</i>	1	-MIFAMIGSGG - FIAPIPHLQAIRDTGHFLDCSFDVBDSVGVLDEYFPOSEFFTNIEDEEKK	
<i>B. pertussis</i>	1	MSSLPITDRKIRPGLVGCGRISKNHIGAIAQHGDRABELVEICDTNPPEALQAAEAATGARP	
Consensus	*	
<i>P. gingivalis</i>	60	HNSKLARG - TDQAIDEHSVCTPNYLEDAHCRYGLRLGADVCEKPLVLNPWNIDALQEIEQ	
<i>P. aeruginosa</i>	60	HASNLRDSTAALDYVSIICEPNYLYHPIAAGLRLCCDVICEKPLVPTPEMEDOLAVIER	
<i>H. pylori</i>	59	HLEQSRA - MGKEINYLSVCTPTHTEDHIREGLRNCHMVICEKPLVIDPGEIQEQLKDLLEV	
<i>B. pertussis</i>	61	FSSLSDMLAQGNADALVLATPSGLEPWQAIIEVAQACRHVVSEKPMATRWEDGKRMVKACD	
Consensus	*	
<i>P. gingivalis</i>	119	ETGERIYNILQLRLHESIVALRKKIESGPA -- DEIYDVTLTYITSRGNWYITSWKGDER	
<i>P. aeruginosa</i>	120	ETDKRLYNILQLRHHQAINVALKDKIVAREKS -- PHKYEVDLTYITSRGNWYLSWKGDPR	
<i>H. pylori</i>	119	KHQKRVPSLPLRLHCDTLALKKEKIKSELDKNPDEVFDITLTYSIVQCKWYFSSWRADVN	
<i>B. pertussis</i>	121	EAGVRLEVVVKQERNATLQLVKKAIEQGRFG - RIYMTVNVFWTRPQEYYDAARWRGKWE	
Consensus	*	
<i>P. gingivalis</i>	176	KSGGIATNIGVHFYDMLSWIFGSVVRHNVVHVYEDRAAGYLELERARVRY --	
<i>P. aeruginosa</i>	177	KSEGVATNIGVHFYDMLHFIFGKLQRNVVHFTSEYKTAGYLEYEQARVRY --	
<i>H. pylori</i>	179	RSGGLATQMGVNIEDTLLYIFGGVKDKVINREEPDCVCGILFLEHAKIRE --	
<i>B. pertussis</i>	190	WDGCAFMMQASHEYVDLIDWLVGPVESVYAYTATLAERIEAEEDTGVAALRWRHGAMGSINV	
Consensus	*	
<i>P. gingivalis</i>	226	-PLSINEELLPENAVQGEKETPTRTIEIDG -- ESEFESEGFTELHTESYRFILAGEEGGL	
<i>P. aeruginosa</i>	227	-PLSVDANDLPESVK - GKKPTYRSITVNG -- EEMEFSEGFTDLHTTSYEEILAGRGYGI	
<i>H. pylori</i>	229	-EFSIN -- PEHMGVAKEKVYHKMILEG -- EEVNLNTQSFDNLYIESYKQILAQGGEGL	
<i>B. pertussis</i>	240	TMLTYPQNLLEGTSITILCEKGTVRVGGVAVNRIDEWKFAEPPHDDDKIREANYETTSVYCF	
Consensus	*	
<i>P. gingivalis</i>	292	DEARNSTIOIVHDIRNASPVGLKGEYHPPFARLPLASHPFGWR R-----	323
<i>P. aeruginosa</i>	292	DDAARRHCVETVNTIRSAVIVPASDNEGHPPFVAAR -----	316
<i>H. pylori</i>	290	DDAMMASVKLAYELRNLSVSEPNEDSHVLCCKNKTDDQ -----	315
<i>B. pertussis</i>	300	GHPLLYYDNVINCLRGDCEPETDGREGLQSLALITAIYRSARDGVRIPPLD	350
Consensus	*	

Supplemental Figure 2

BP1629	1	-----	
BP3150	1	-----	
PGN_0613	1	-----	
PA WbpA	1	-----	
PGN_1243	1	MGQACIGSRPISFLWEVRTCRRFIGSYTLIRRIVSHVLSGSRHYIPATGRGNSRLSLDK	
BP1629	1	----- MPKKFSNPADVHLAVVGLGYVGLPLAVEFGKK-RPVIGFDINERRIAALKAG	
BP3150	1	----- MRIIDDVKLAVVGLGYVGLPLAVEFGKK-RPVIGFDINERRIAALKAG	
PGN_0613	1	- MKPYKALTINTKEMNNIRIATIIGLGYVGLPLARLFSTK-FPTIGFDINPNRVEELISG	
PA WbpA	1	- MIDVNTVVEKFKSRSQALIGIVGLGYVGLPLMLRYNAIGFDVLCIDIDDVVKVDKLNA G	
PGN_1243	61	ASPFYLTVPKNSISYYNMDIAVVGIGVGLVSATCFAELGANVRCIDTDRNKIEQLNSG	
Consensus		*****	
BP1629	52	HDHTLEVEDDELARAQQLS-YTAER-EELGKANVFIVTVVPTPIDEKQPDILTPLVKA	
BP3150	47	HDHTLEVEDDELARAQQLS-YTAER-EELGKANVFIVTVVPTPIDEKQPDILTPLVKA	
PGN_0613	59	YDSTGEIGNLLOEALOKG-LLCTTKIEEIRDNFYIVTVVPTPIDHNHTPDLRPLLAA	
PA WbpA	58	QCYIEHIPQAKIAKARASG-FEATTDFSRVSECDALILCVPTPLNKYREPDMSFVINT	
PGN_1243	121	TIPIYEPGLEKMIARNVKAGRRLRGTEIEQAVPEADIIIFIAVGTPAGEDGSADM SYVLDA	
Consensus		*****	
BP1629	107	SETIGAVLKRGDIVIYEESTVYPGATEEDCVPVLERVSGLKFNQDFYAGYSPERINPGDKA	
BP3150	102	SETIGAVLKRGDIVIYEESTVYPGATEEDCVPVLERVSGLKFNQDFYAGYSPERINPGDKA	
PGN_0613	116	SETIGKVIDQGDVVVYESTVYPGVTEDECIPPIIEKVGSLKYNVDFFAGYSPERINPGDKV	
PA WbpA	115	TDALKPYLRVGQVVSLESTTYPGTTEEELLPRVQEG-GLVVGRDIYLVYSPEREDPGNPNT	
PGN_1243	181	ARSICRAMSRYIILIVTKSTVPVGSYRLIRKAIQEELDKREVLIDFDIASNPEFLKEGN AI	
Consensus		*****	
BP1629	167	HRVNTIKKVTSGS-TPEVAELVDOLYN-EIITAGTHKASSIRVAEEAKVIENTQRDVNIA	
BP3150	162	HRVNTIKKVTSGS-TPEVAELVDOLYN-EIITAGTHKASSIRVAEEAKVIENTQRDVNIA	
PGN_0613	176	YTVENIKKVTSGS-TPEIADYVDRMYN-TVLVNGTHKASSIKVAEASKIIENAQRDVNIA	
PA WbpA	174	FETRTIPKVIGGH-TPQCLEVGIALYE-QAIDR-VVPVSSSTKAAEMTKLLENIRAVNIG	
PGN_1243	241	DDFMKPDVVGVDSDRARELITSLYKPMLLNNFRVLFMDIASAEMTKYANAMLATRIS	
Consensus		*****	
BP1629	225	LINELALIFNKGIDTEAVLQAAGTKWN--FLPFRPC-LVGGHCIGVDPYYLT HKAQAI G	
BP3150	220	LINELALIFNKGIDTEAVLQAAGTKWN--FLPFRPC-LVGGHCIGVDPYYLT HKAQAI G	
PGN_0613	224	FMNEIAKIFNAMEIDTREVLEAAATKWN--FLPFQPC-LVGGHCIGVDPYYL IQRAQVY G	
PA WbpA	231	LVNEMKIVADRKGIDIFEVVDAAATKPGF-FTPYYPCPGILGGHCIPIDPFYLTWKAREYG	
PGN_1243	301	FMNDVANLCERVGADVSMVRLGIGSDSRIGSKFLYPCCGYGGSCFPKDVKALIRTAEDNG	
Consensus		*****	
BP1629	282	YHPEIILAGRRLNDSMGGYVVSQLVKAMAKRRITHVEGARVLLMGLTFKENCPDLRNTRVV	
BP3150	277	YHPEIILAGRRLNDSMGGYVVSQLVKAMAKRRITHVEGARVLLMGLTFKENCPDLRNTRVV	
PGN_0613	291	VYPRVLMMSARRLNDSMGSYTASQTIKQMNKAGIMVKDARILILCFTFKENCPDLRNTKVI	
PA WbpA	290	LHTRFIELSGEVNQAMPEYVLGKLMMDGLNEAGRALKCSRVLVLCIAYKKNVDDMRESPSV	
PGN_1243	361	YRMEVLEAVEVNEKQKSILFDKFSTYYKGN--VQRCVAIWGLSFKPGTDDMREAPSL	
Consensus		*****	
BP1629	342	DIVRELGEYHVDVDVYDPWDPAAEAEHEYGI TPVAKPAAGA----- YDAIIILAVSHHQFVE	
BP3150	337	DIVRELGEYHVDVDVYDPWDPAAEAEHEYGI TPVAKPAAGA----- YDAIIILAVSHHQFVE	
PGN_0613	351	DTYSTLQEYTKNIIVHDPWANPSIAEQVYGI KIHNEFPKCK----- FDAIIIAVAHRDFSN	
PA WbpA	350	EIMELIEAKGGMVAYSDFHV PVFPKMREHHFELSSEPLTAENLARFDAVVLATDHDKF DY	
PGN_1243	418	VLIKEKILEVGCRVRVYDPVAMKEAQKRLGDKVEYTTDMYDAVRG-AEALFHVT EWKE FRM	
Consensus		*****	
BP1629	398	MGAFAIRKFGKAEHILYDLKYVLAPEQADLRL-----	429
BP3150	393	MGAFAIRKFGKAEHILYDLKYVLAPEQADLRL-----	424
PGN_0613	407	IDLEKS---STANTIIYDVKGILPQGKKYHKL-----	435
PA WbpA	410	ELIKAEAKLVVDSRGKYLRSPPAAHIKA-----	436
PGN_1243	477	PDWSALSQAMAASLVIDGRNVYELPADSDFTILNIGNSAIESASSK	522
Consensus		*****	

Supplemental Figure 3

WbpE	1	MIEFIDLENQQARIKKIDAGIQRVLRHGQVILGPEVTELEDRLADEVGAKYC
WlbC	1	MQFIDLEKTOYQALERTINPFHQAVLDHGQFIMGPEVKELAALCAYTGAKHC
PorR	1	MPTSIISQPIVMVVDLKKOYLQMKKQIDEAIHTVIDSTAPINGKEVHAFAEDLAAYLGKVHV
Consensus	MIEFIDLENQQARIKKIDAGIQRVLRHGQVILGPEVTELEDRLADEVGAKYCMQFIDLEKTOYQALERTINPFHQAVLDHGQFIMGPEVKELAALCAYTGAKHCMPTSIISQPIVMVVDLKKOYLQMKKQIDEAIHTVIDSTAPINGKEVHAFAEDLAAYLGKVHV
WbpE	54	ISCANGTDALQIVQMALGVGE[GDEVITEGFTYVATAETVALLGAKPVVVDIDPRTYNLDP
WlbC	53	ITVASGTEALLISLMALGVKA[GDEVITTSFTEVATAEVIALLGAKPVVVDVEPDTCNLIKV
PorR	61	IPCANGTDALQISLMALGV[KVGDEIIIVP[DFTYAAASAAIGLLGLTPVFADVDPITENITS
Consensus	ISCANGTDALQIVQMALGVGE[GDEVITEGFTYVATAETVALLGAKPVVVDIDPRTYNLDPITVASGTEALLISLMALGVKA[GDEVITTSFTEVATAEVIALLGAKPVVVDVEPDTCNLIKVIPCANGTDALQISLMALGV[KVGDEIIIVP[DFTYAAASAAIGLLGLTPVFADVDPITENITS
WbpE	114	QLLEAAITPRTKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----K
WlbC	113	SEIEAKITPRTKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----R
PorR	121	KGC[EKVLSDETKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----R
Consensus	QLLEAAITPRTKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----KSEIEAKITPRTKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----RKGC[EKVLSDETKAIIPVSLYGGQCADFDAINATAASKY[GIPVIEDAAQSFGA[SYKG-----R
WbpE	169	RSCNLSTVACTSFFPSKPLGCYGDGGAI[FTNDDELATAIRQIARHGGDRRYH[HIRVGVNS
WlbC	168	KSCNLSTIGCTSFPPSKPLGCYGDGGAI[FTNDDELAQAMREIRVHGQSGRYYHARIGVGG
PorR	191	KTGTMGHIGCA[SFPPSKNLGCYGDGGAVITNDDELAKRVRMIANHGQKIKYHDIIGONS
Consensus	RSCNLSTVACTSFFPSKPLGCYGDGGAI[FTNDDELATAIRQIARHGGDRRYH[HIRVGVNSKSCNLSTIGCTSFPPSKPLGCYGDGGAI[FTNDDELAQAMREIRVHGQSGRYYHARIGVGGKTGTMGHIGCA[SFPPSKNLGCYGDGGAVITNDDELAKRVRMIANHGQKIKYHDIIGONS
WbpE	229	RLDTLQAAILLPKLEIFEEEIALROKVAAEYDLSLKQVGI[TPPIEVN--NI SVYAQFTV
WlbC	228	RMDTLQCAVWLCKLERFDWEIAQRIKIGARYQQLIADLPGGACTVTVRPDRDSVWAQFTV
PorR	241	RLDTLQAAILRVLQYLDRFNALRNEVASHYTSI[EGIEWLQTPTSQQSSHVYHQYTLK
Consensus	RLDTLQAAILLPKLEIFEEEIALROKVAAEYDLSLKQVGI[TPPIEVN--NI SVYAQFTVRMDTLQCAVWLCKLERFDWEIAQRIKIGARYQQLIADLPGGACTVTVRPDRDSVWAQFTVRLDTLQAAILRVLQYLDRFNALRNEVASHYTSI[EGIEWLQTPTSQQSSHVYHQYTLK
WbpE	297	RMDN--RESVQASLEAAGVPTAVHYPIPLNEQ---PAVADEKAKLPVGDKAATQVMSLPM
WlbC	298	MVPN--REAVIAQOLKEAGIPTAVHYPRPIHAQPAYEQYAEGAGATPVSDDLAARVMSLPM
PorR	301	LIDQNTRDGLREHITNHHIASMIYYPPIPLRQPAFVGIAHWGESLDVSDSISRTVLSIPI
Consensus	RMDN--RESVQASLEAAGVPTAVHYPIPLNEQ---PAVADEKAKLPVGDKAATQVMSLPMMVPN--REAVIAQOLKEAGIPTAVHYPRPIHAQPAYEQYAEGAGATPVSDDLAARVMSLPMLIDQNTRDGLREHITNHHIASMIYYPPIPLRQPAFVGIAHWGESLDVSDSISRTVLSIPI
WbpE	342	HPIYLDTASIKIICAAALTN-----
WlbC	346	HPIYLDTASIKIICAAALTN-----
PorR	361	YPEMEIEQIHAVVSAIKTFEPSLQ-----
Consensus	HPIYLDTASIKIICAAALTN-----HPIYLDTASIKIICAAALTN-----YPEMEIEQIHAVVSAIKTFEPSLQ-----

Supplemental Figure 4

P. aeruginosa 1 MSYYQHPSAIVDDGAQIGSDSRVWHFVHICAGARIGAGVSLGQNPFVGNKVVIGDRCKIQ
B. pertussis 1 -MTTIHPTAIVDDEGARIGANSRISHWVWHICGGAEIGAGCAGSLGQNPFVGNRVRIGDRVKIQ
P. gingivalis 1 ---MIHPTAIVEDGCVLGGQGTRVWHFSHLMCGAEVGENCNIGQNVVIMPEVRLGRGCKVQ
Consensus .***.***...*. . *.***....*....*.....*****.....*....*.*

P. aeruginosa 61 NNVSVYDNVTLLEEGLVFCGPSMVFTNVNVNPRLIERKDQYRNTLVLKKGATLGANCTIVCGV
B. pertussis 60 NNVSVYDNVFLLEDDVFCGPSMVFTNVNVNPRAAIERKNEYRDTLVRQGATLGANCTIVCGA
P. gingivalis 58 NNVSIYSGVVCEDYVFLGPSCVFTNVINPRAFIERKSEYRPTHLHEGVSIGANATILCGI
Consensus *****,*,*,*,*,*,*,****,****,****,****,****,*,*,*,*,*,*,*,*,****,****,****.

P. aeruginosa 121 TIGAYAFVGAGAVINKNVPSYALMVGVPARQIGWMSEFGEQLQLNEQG--EAVCSHSGAR
B. pertussis 120 TVGRYAFVGAGAVVNKDVPDFALVVGVPARQIGWMSSRHGEQLDLPLAGNGQARCPTHGDL
P. gingivalis 118 TIGAYAMVGACTVVIRDVPPYALVVGNPARRIGWVSRACHRLSFDDKG--MAVCPETER
Consensus *.*.****,*....**.****,****,****,*.****.****,*.****.****,****,****,****,****,****.

P. aeruginosa 179 YVENGKILSKVDV-- 191
B. pertussis 180 YILENGVCRLE--- 191
P. gingivalis 176 YREVEETGTIEPVSD 190
Consensus *...

Supplemental Tabe 1. Bacterial strains used in this study.

<i>P. gingivalis</i> strain	Description	Source or reference
33277	Wild type	ATCC
HG66	Naturally non-pigmented strain	Gift from J. Potempa ^a
HG66/pWbpB+	HG66/pTCB-wbpB	This study
KDP220	<i>wbpB</i> ::Em ^r	This study
KDP221	<i>porR</i> :Tc ^r	This study
KDP222	PGN_0002::Em ^r	This study
KDP223	KDP222/pTCB-PGN_0002	This study
KDP224	KDP222/pTCB-P. a. wbpD	This study
Pugd1	<i>ugdA</i> ::Em ^r	Sato et al. 2009
W83	Wild type	Gift from M. J. Duncan ^b

<i>E. coli</i> strain	Description	Source or reference
S17-1	RP-4-2-Tc::Mu <i>aph</i> ::Tn7 <i>recA</i> , Sm ^r	Simon et al. 1983
XL-1 Blue	General purpose host strain for cloning	Stratagene

<i>P. aeruginosa</i> strain	Description	Source or reference
PAO1	Wild-type	Gift from J. S. Lam ^c

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b Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland.

c Department of Molecular and Cellular Biology, University of Guelph, Ontario, Canada.

Supplemental Table 2. Plasmids used in this study.

Plasmid	Description	Source or reference
pBluescript II SK(-)	Ap ^r , cloning vector	Stratagene
pBSSK-cat pro-rgpBter	Ap ^r , pBSSK-cat pro-rgpBter	This study
pBSSK-cat pro-wbpB-rgpBter	Ap ^r , pBSSK-cat pro-wbpB-rgpBter	This study
pBSSK-cat pro-PGN_0002-rgpBter	Ap ^r , pBSSK-cat pro-PGN_0002-rgpBter	This study
pBSSK-cat pro-P. a. wbpD-rgpBter	Ap ^r , pBSSK-cat pro-P. a. wbpD-rgpBter	This study
pCR4	Ap ^r Km ^r , PCR Topo cloning vector	Invitrogen
pCR4-cat pro	Ap ^r Km ^r , pCR4-cat pro	This study
pCR4-porRup	Ap ^r Km ^r , pCR4-porRup	This study
pCR4-porRdw1	Ap ^r Km ^r , pCR4-porRdw1	This study
pCR4-porRdw2	Ap ^r Km ^r , pCR4-porRdw2 (NotI self-ligated of pCR4-porRdw1)	This study
pCR4-porRupdw2	Ap ^r Km ^r , pCR4-porRup-porRdw2	This study
pGEM-T Easy	Ap ^r , PCR cloning vector	Promega
pKD375	Ap ^r , Tc ^r , pUC19- <i>tetQ</i>	Shi et al. 1999
pKD740	Ap ^r Em ^r , pGEM-T Easy-hbp35up- <i>ermF</i> -hbp35dw	Shoji et al. 2010
pKD854	Ap ^r , pBSSK-sKGp-rgpBter	Sato et al. 2005
pKD892	Ap ^r Em ^r , pGEM-T Easy-PGN_0168up- <i>ermF</i> -PGN_0168dw	This study
pKD893	Ap ^r Em ^r , pGEM-T Easy-PGN_0002up- <i>ermF</i> -PGN_0002dw	This study
pKD894	Ap ^r Km ^r Tc ^r , pCR4-PGN_1236up- <i>tetQ</i> -PGN_1236dw	This study
pKD955	Ap ^r , pBSSK-p-porK+-T	Sato et al. 2010
pTCB	Ap ^r Tc ^r , shuttle vector plasmid	Nagano et al. 2007

pTCB-wbpB	Ap ^r Tc ^r , pTCB-PGN_0168	This study
pTCB-PGN_0002	Apr Tc ^r , pTCB-PGN_0002	This study
pTCB- <i>P. a.</i> wbpD	Ap ^r Tc ^r , pTCB-wbpD from <i>P. aeruginosa</i> PAO1	This study
pUC118	Ap ^r , PCR cloning vector	Takara
pUC118-wbpB	Ap ^r , pUC118-PGN_0168	This study
pUC118-PGN_0002	Ap ^r , pUC118-PGN_0002	This study
pUC118- <i>P.a.</i> wbpD	Ap ^r , pUC118-wbpD from <i>P. aeruginosa</i> PAO1	This study

Supplemental Table 3. Primers used in this study.

Primer name	Nucleotide sequence (5'-3')	underline	double underline
p6-34-F-KpnI	<u>GGTACCTTCGTCGTCAATCAGCATCCCAG</u>	KpnI	
p6-34-R-SalI	<u>GTCGACTGTTTGTCTCTTATTAAAGTTA</u>	SalI	
PGN_0002upFw	<u>GCATGCTCCAGCAGCGTACGCTTCAAAGC</u>	SphI	
PGN_0002upRev	<u>GGATCCAAGACCTGCTATCAATTGGCGA</u>	BamHI	
PGN_0002dwFw	<u>CTGCAGTCTACACCAAATAGCGGCATATC</u>	PstI	
PGN_0002dwRev	<u>GAGCTCCGATTACATACTCGGTTACAACC</u>	SacI	
PGN_0168upFw	<u>GCATGCATGCACAACTTGCCATCATCGG</u>	SphI	
PGN_0168upRev	<u>GGATCCCTCGCATATCACATCCGCTCCAA</u>	BamHI	
PGN_0168dwFw	<u>CTGCAGAAAGGAATTCTATAATCTTATC</u>	PstI	
PGN_0168dwRev	<u>GAGCTCCGCTGTGCACCGATGTCGGACAC</u>	SacI	
PGN_1236upFw	<u>GGTACCGTGAAACGATAGCGAAGGGAAAGCA</u>	KpnI	
PGN_1236upRev	<u>GGATCCAGGCGTGTGTAGGGATTCTGGT</u>	BamHI	
PGN_1236dwFw	<u>GGATCCAAAAGAGGAGGGAGGCTCGTCTA</u>	BamHI	
PGN_1236dwRev	<u>GCGGCCGCCAGATTATTGCCAGACCTTGA</u>	NotI	
PGN_0168compFw	<u>GTCGACATGCACAACTTGCCATCATCGT</u>	SalI	Start codon
PGN_0168compRev	<u>TCTAGATTAGCGTCTCCATCCGAAAGGATG</u>	XbaI	Stop codon
PGN_0002compFw	<u>GTCGACATGATCCATCCCACAGCGATAGTA</u>	SalI	Start codon
PGN_0002compRev	<u>TCTAGATTAGTCGATACCGGTTCTATTGT</u>	XbaI	Stop codon
<i>P.a. wbpD</i> compFw	<u>GTCGACATGAGTTATTATCAGCACCCCCAGCGCGATCGTGGAC</u>	SalI	Start codon
<i>P.a. wbpD</i> compRev	<u>TCTAGATTACACGTCCACCTTGCTCAGGAT</u>	XbaI	Stop codon