

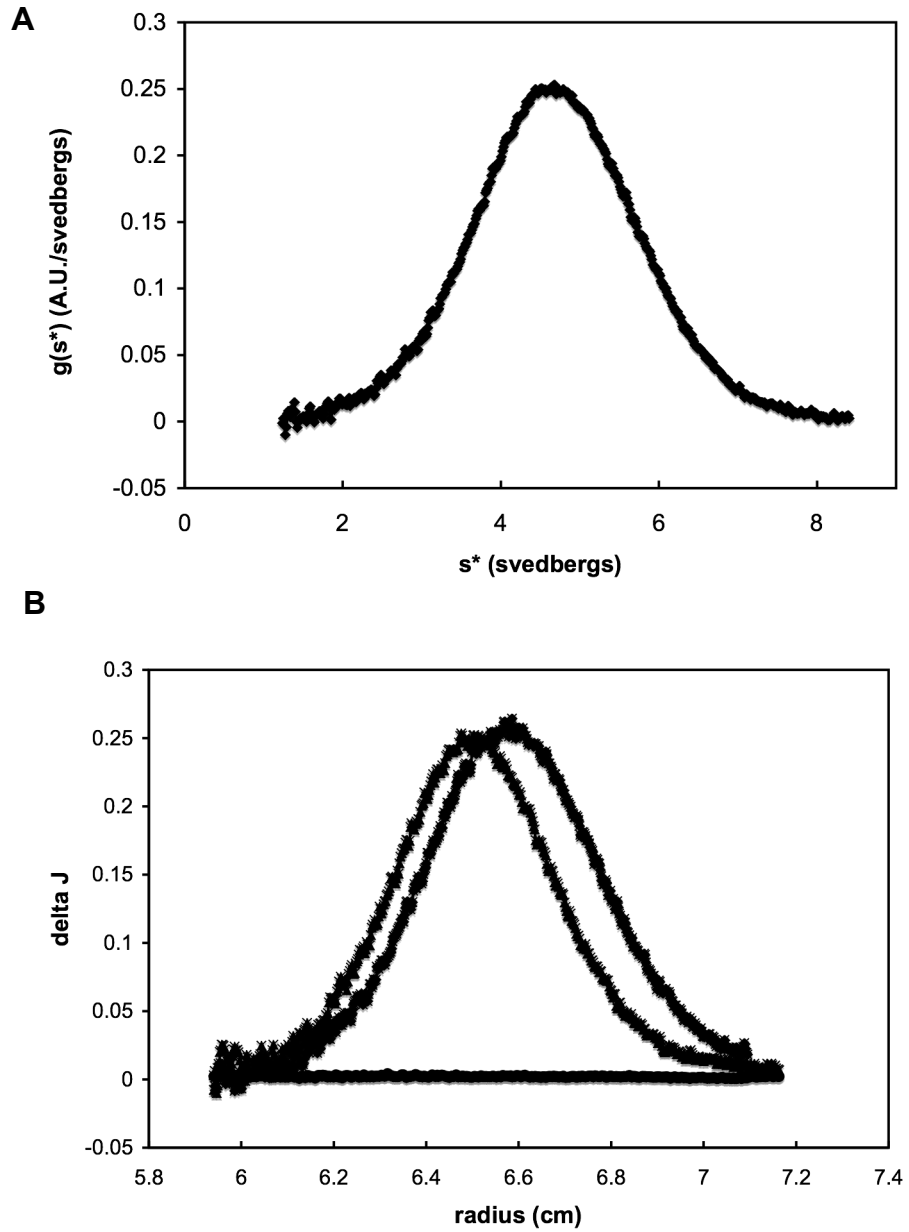
## Supporting Information

### **Structural Analysis of WbpE from *Pseudomonas aeruginosa* PAO1: A Nucleotide Sugar Aminotransferase Involved in O-antigen Assembly**

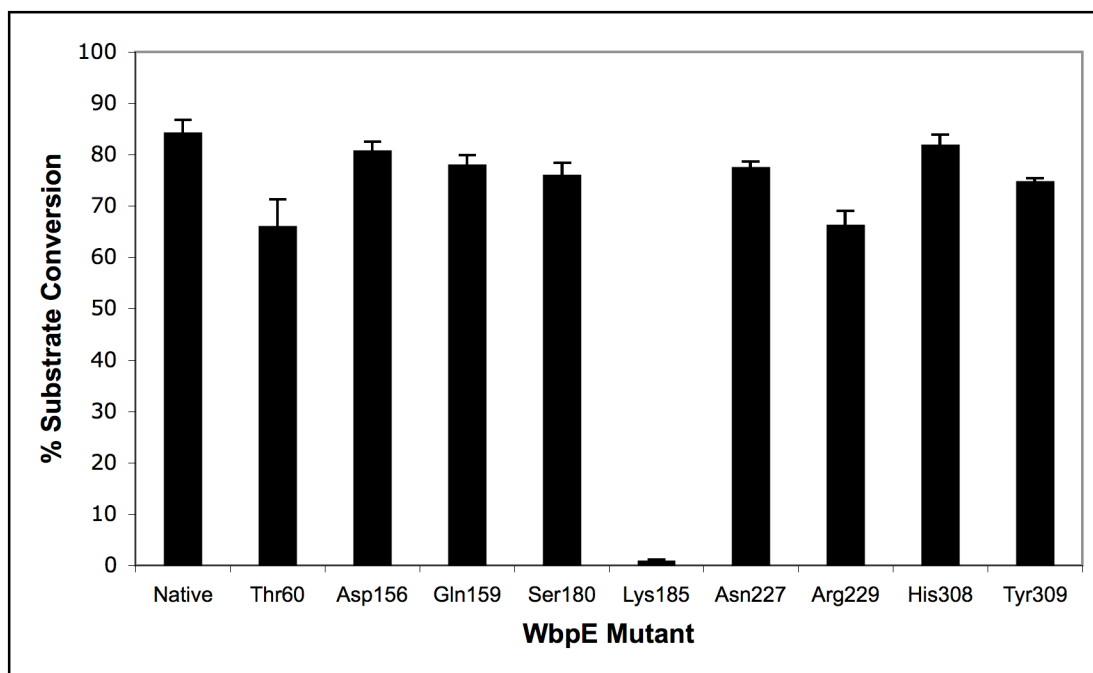
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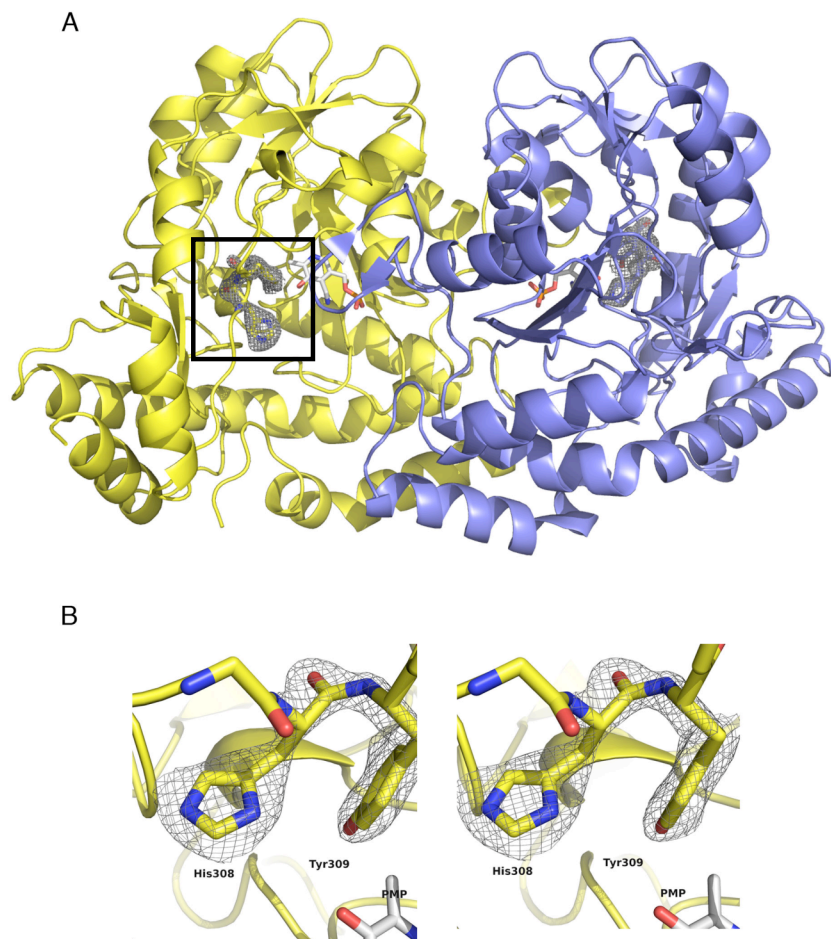
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**Figure S1:** Sedimentation velocity analytical ultracentrifugation data. A) Time derivative ( $g(s^*)$ ) plot of WbpE depicting a sedimentation coefficient of 4.72 svedbergs. B) Time difference residual for WbpE. The raw data (triangles), corresponding model (hatches) and residuals of the fit (boxes) for two pairs of scans taken over the course of the experiment.






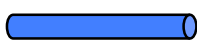



**Figure S2:** WbpE Alanine Mutant Assay. In order to test for function of the alanine mutants, the coupled WbpB and WbpE enzyme assay was performed. Reactions contained 2.5  $\mu\text{g}$  each of WbpB and WbpE mutant as described in the text and were incubated at 30 °C for 2 h. After filtration to remove protein, the crude reaction mixtures were analyzed by capillary electrophoresis to determine total percent of product turnover. Data represent the average of two experiments.



**Figure S3:** The non-prolyl *cis* amide bond between His308 and Tyr 309 in the external aldimine-bound structure of WbpE. A) The location of the amide bond within the homodimer, where the boxed area indicates the region shown in the stereo figure. B) A close-up view of the *cis* amide bond. The electron density represents a  $2(F_o-F_c)$  map contoured at  $2.5\sigma$  in which the His and Tyr residues were omitted from the calculation.


Legend:   $\alpha$ -Helix   $\beta$ -Sheet

				
P. aeruginosa	(1)	-----MIEFIDLKNOQARIKDKIDAGIQRVLRHGQYILGPEVTELEDRLADDFVGAK		
A. baumannii	(1)	-----MIDFIDLKAQQNRIDKIDAGIQNVLTHGQYILGPEVIELEEKLASYVGAK		
V. cholerae	(1)	-----MQFIDLGAQQARIKEKIDVGIQKVLAHGQYILGPEVAELEEOKLIAYTGAK		
B. pertussis	(1)	-----MQFIDLKTOYQARLDTINPRIQAVLDHGQFIMGPEVKELEAALCAYTGAK		
C. burnetti	(1)	-----MKFIDLNEOYLKIKQAVDGRMQAVLDHGQFIMGPEVKELEARLAEWVGR		
L. pneumophila	(1)	-----MQFIDLKTOYSLIENDILNSIKRVLNHGQYIMGPEIAELEKELATFVGVK		
P. acanthamoebae	(1)	-----MEFIDIKKOYLYKKEIDLRIQAVLDHGQFIMGPEVKELEAALAQYTNTK		
C. botulinum	(1)	-----MNIPLIDLKAQYKSISEDLDRVTKVLSANYIMGKNVSDFEKDFAQYTNTK		
A. butzleri	(1)	-----MKIDFANLQYQHLYKDEIENAILKVTRNCNFIMGNEVQELEKSLEDYIGVK		
B. neotomae	(1)	MRARYIGANMQFIDLGAQRARIENRLNAAISKVVAEGRYILGPEVAEFEKKLGEYLGVE		
C. jejuni	(1)	-----MNFINLQAQYLAYKDEINAEIESVLSSSSFIGGAKLNEFEQNLAHFLGVK		
H. cinaedi	(1)	-----MEFINLKAQYQAYKSSIDKAMQDVLDSQFIMGSAVGELESALAKYSGAK		


			<b>PLP Binding Site</b>				
							
P. aeruginosa	(52)	YCISCANGTDALQIVQMALGVGPGDEVITPGFTYVATAETVALLGAKPVYVDIDPRTYN					
A. baumannii	(52)	HCITCANGTDALQIAQMAFGIGPDEVITPGFTYIATAETVALLGAKPVYVDVNPKTYN					
V. cholerae	(51)	HCISCANGTDALQIVQMALGVGPGDEVITPGFTYVATAETVALLGAKPVYVDVCPKTYN					
B. pertussis	(51)	HCITVASGTEALLISLMALGVKAGDEVITTSFTFVATAEVIALLGAKPVFVDVEPDTCN					
C. burnetti	(51)	HCITVSSGTMALLIALMALGVGPGDEIITSSFSFFATAETIVFLGATPVFVDIDPKTYN					
L. pneumophila	(51)	HCITVASGTEALLISLMALGVKAGDEVITTSFTFVATAEVIALLGAKPVFVDIDPLTYN					
P. acanthamoebae	(51)	HCITVSSGTDLSLQIALMALGVGPGDEVITVPFTWISSTEVI GLVGATPVFVDIEDRTYN					
C. botulinum	(53)	HAI SVNGTDALVIALKSLGIGAGDEVITSTFTYFASAECISAVGAKPVFVDAEKDTFN					
A. butzleri	(53)	YAVSCNGTDALLLMMALDIKPGDEVITTPFTFIATAEMIAFLGAIPVFVDIDEKTYN					
B. neotomae	(60)	HVIACANGTDALQMLMTRGIGPGHAVFVPSFTFAATAEVVALVGAEPVFVDVDPDSYN					
C. jejuni	(51)	HAIGCSSGTSALYLALRALDIGKDEVIVPSFTFIATAEVVALVGAKPVFVDINLSNYN					
H. cinaedi	(51)	HAIACSSGTDALILALMALDVKSGDEIITSPFSFIASVEAIMLIGAKPVFVDIDEKTYN					

							
P. aeruginosa	(111)	LDPQLLEAAITP-----RTKAIIPVSLYGQCADFDAINAIASKYGIPIVEDAAQSFG					
A. baumannii	(111)	LDSEKLEAAITP-----RTKAIIPVSLYGQCADFVDINAIACKYSIPVIEDAAQSFG					
V. cholerae	(110)	LDPAKLEAAITP-----RTKAIIPVSLYGQCADFDAINTIAAKYGIPIVEDSAQSFG					
B. pertussis	(110)	IKVSEIEAKITP-----RTKAIIPVSLYGQCGDMDEVNAVAARHGLPVIEDAAQSFG					
C. burnetti	(110)	IDVSRIEAAITN-----RTKAIIPVSLYGQCADLVAINAIAERHGLPVIEDGAQSLG					
L. pneumophila	(110)	LDPEQLERAITK-----KTKAIMPVDLYGQCADYDVINAIATQYGIPIVEDAAQSFG					
P. acanthamoebae	(110)	IDVEQLEKAITP-----KTKAILPVSLFGQMPDYTAINEIANKYGLPVIEDGAQSFG					
C. botulinum	(112)	IDPSKIEEKITK-----KTKAIIPVHIFGQSADMDEINKIAKKYNLKVIEDACQAVG					
A. butzleri	(112)	INPDLIEEKITS-----KTKAIIPVSLYGQPADMDKVNQIAKMYNLKVIIDGAQSFG					
B. neotomae	(119)	MNVEQLEAAIAATIKEGRLEPKAIIPVDLFLGLAASYNRITAIAREGLFIIEDAAQSIG					
C. jejuni	(110)	LDFAVQKAITP-----KTKAVIAVSMFGQMSDLRALEEILKNKNITLIEDGAQSFG					
H. cinaedi	(110)	LSSSKLESSITD-----KTKAIIPVAIFGQMDMESINAIASKYKIPVIEDAAQSFG					


### Catalytic Domain




<i>P. aeruginosa</i>	(163)	AS-YK GK---RSCNLSTVACTSFFFP SKPLG CYGDGG AIFTN DDELATAIRQ IARHGQ--
<i>A. baumannii</i>	(163)	AT-YK SR---KSCNLSTVACTSFFFP SKPLG CYGDGG AIFTN DDELAKVIRQ IARHGQ--
<i>V. cholerae</i>	(162)	AC-YK GK---KSCNLSTIACTSFFFP TKPLG CYGDGG AIFTN DDELALVMRQ IARHGQ--
<i>B. pertussis</i>	(162)	AT-YK GR---KSCNLSTIGCT SFFFP SKPLG CYGDGG ALFTN DDEL AQAMREIRVHGQ--
<i>C. burnetti</i>	(162)	AT-HH GR---QSCGF TTI GCT SFFFP SKPLG CYGDGG ACFTN DDEL AQTMRLIRNHGQ--
<i>L. pneumophila</i>	(162)	AT-YK GK---YSCSLATIGCT SFFFP SKPLG GYGDSGACFTN DDVLAQK LIEIRIHGQ--
<i>P. acanthamoebae</i>	(162)	AT-QH GR---KSCSVTTIGCT SFFFP AKPLG CYGDGG ALFTN DDTLAAKMRAIRTHGG--
<i>C. botulinum</i>	(164)	AK-YK GK---MIGALS DMACFSFFFP TKNLSCAGD GGMIVTSD DNVATIARALRTHGSGE
<i>A. butzleri</i>	(164)	ST-YD GI---TDSALADISTTSFFFP AKPLG CYGDGG AVFTN DEEIANKMKSRLRHGQ--
<i>B. neotomae</i>	(178)	GK-RD NV---MCGAFGHV GATSFYPAKPLG CYGDGG AMFTN DAELADTLRSVLFHGKG-
<i>C. jejuni</i>	(162)	AS-FK GE---KSCSIAKISCT SFFFP SKPLG AYGDGG AIFCHDDEIAKKIRILLNHGQ--
<i>H. cinaedi</i>	(162)	ATQIQGKNQIKSCNASLLATTSFFFP SKPLG CYGDGG AVFTS DDALAEKLR YLLNHGQ--



<i>P. aeruginosa</i>	(215)	-----DRRYHHIRVGVNSRLDTLQAAIILLPKLEIFE
<i>A. baumannii</i>	(215)	-----DKRYHHIRVGVNSRLDTLQAAIILLPKLEILD
<i>V. cholerae</i>	(214)	-----DRRYHHIRVGVNSRLDTLQAAIILLPKLEVHE
<i>B. pertussis</i>	(214)	-----SGRYYHARIGVGG RMDTLQCAVVLGKLERFD
<i>C. burnetti</i>	(214)	-----EKRYHHVVRVGLNARFNTLQAAVLLAKLELFA
<i>L. pneumophila</i>	(214)	-----NARYCHHRVGINGRMDTIQAAIILLQKLIKIFS
<i>P. acanthamoebae</i>	(214)	-----ERRHHHTCLGMNGRLDTMQAAIILLAKFP HFD
<i>C. botulinum</i>	(219)	TGQKAYNLLNNINEDIDKSNTGDDTVYNPLKYNYLIGYNSRLDAIQAAIILKVKLPHLD
<i>A. butzleri</i>	(217)	-----SKRYHHKYIGMGGRLDTIQAAVNLVKKLYYP
<i>B. neotomae</i>	(232)	-----ETQYDNVRI GINSRLDTIQAAVLLLEKLAILE
<i>C. jejuni</i>	(214)	-----TORYKHEFIGINGRLDTLQAAIILNVKLYLE
<i>H. cinaedi</i>	(219)	-----TRRYEHSFIGLNARLDSIQAAVLLAKLPHLD



<i>P. aeruginosa</i>	(246)	EEIALRQKVA AEYDLSLK-QVGIG-TPFIEVN NISVYAQYTVRMDNRESVQASLKAAGV
<i>A. baumannii</i>	(246)	DEMQRQRVAEVYNRLFN-EVGIHTTPYIEAHNTSAWAQYTIQVDNRAEVQEKLKAQGI
<i>V. cholerae</i>	(245)	EEIELRNQVANTYTRLLN-KAGILSTPFVEAHNISAWAQYTI R VKNRAEIQHKLQEAGI
<i>B. pertussis</i>	(245)	WEIAQR IKIGARYQOLLADLPGGACTVTVRPDRDSVWAQFTVMVPNREAVIAQLKEAGI
<i>C. burnetti</i>	(245)	DELEQRQRVAEWYSEILG---ADFVTPYIAPNNMSAFAQYTLRVEQRERVOVALTEAGV
<i>L. pneumophila</i>	(245)	NEILLRQKVAKRYDQMLS---ELVKT PYVHECNASVYAQYTI EVENRDVFAKSMQASGI
<i>P. acanthamoebae</i>	(245)	QEAQARASIGHFYSQELA---GYCVIPEIQAGNTHVYAQYTI R TPREDLAKYLQAKGV
<i>C. botulinum</i>	(278)	KWNSKRKEIAKIYDKNFKD--SNVVT P SVREENESVYHQYVLQTEDREKMLNKLKDKGV
<i>A. butzleri</i>	(248)	KDLAKRAEVASKYTKALENK-SGLVLPFVDKKATSAWAQYSIRVKNRDEVQNLKEAGI
<i>B. neotomae</i>	(263)	DEMEARDRIARRYNEALK---DVKVPEL PVGNRSAWAQYSIESEN RDGLKAQLQAEGI
<i>C. jejuni</i>	(245)	KELDKRQKLAQTYNANLKN----CQIPQIDPNAFSAYAQYSVLVEDRASVLQKFEKANI
<i>H. cinaedi</i>	(250)	KELDKRQKLAQTYNANLKN----CQIPQIDPNAFSAYAQYSVLVEDRASVLQKFEKANI

*cis* amide 

P. aeruginosa	(303)	PTAVHYPIPLNKQPAVAD----EKAKLPVGDKAATQVMSLPMHPYLDTASIKIICAALT
A. baumannii	(304)	PTAVHYPIPLNKQPAVAD----SDIHLPIGDAIAEKVMSLPMHPYLAIDDQLKIVKAFG
V. cholerae	(303)	PTAVHYPIPLNKQPAVAD----SSIQLPIGDEIAEEVMSLPMHPYLTEQDIQRVVESLA
B. pertussis	(301)	PTAVHYPRPIHAQPAYEQYA-EGAGATPVSDDLAARVMSLPMHPDLDEATQDKIVAALR
C. burnetti	(301)	PTAVHYPKSLHEQPAIQAYL-KTDDHYPOQAASQQVLALPFHPYLTKETVRNVCDSELL
L. pneumophila	(301)	PTAIHYPIAMHQQALGYLN-YKLGDFPNSEKASQHVISLPMHPYLQEQDEQLKIVA AVR
P. acanthamoebae	(301)	PTGIYYPKCVHEQPVFKPLG-YARGSMVAEKMADEVISLPMHPWLTEEDQHIIINMIK
C. botulinum	(336)	AAGVYYPVPLHLQKVYKDFG-YKEGDMVAEYLSHRTFAIPVYPELTEEQIRYIIDS IK
A. butzleri	(306)	PTAVHYPMPLHLQECFKYLG-YKKGDFPISEIVSEEIMSLPMNPHYVTDEEINYISEQL-
B. neotomae	(320)	PSVIYYVKPLHLQATAYKHYS-VAPGGLPVSESLPSRILSLPMHPYLSEADQDKIIGVIR
C. jejuni	(300)	PYAIHYPTPLHKQPCFSEFS---NLELKNSEYASEHILSLPFSPFLSEEEQEQVICIFK
H. cinaedi	(305)	PYAVHYPIPLHLQEVVTKLYPYKKGDFPISEMISEEILSLPFSPFLTKEEQLSVIKAVN

P. aeruginosa	(358)	N-----
A. baumannii	(358)	-----
V. cholerae	(358)	Q-----
B. pertussis	(359)	QALN-----
C. burnetti	(359)	SIVSCKSVNG--
L. pneumophila	(359)	NALLVMNEEAMV
P. acanthamoebae	(359)	EFCCAPAAC---
C. botulinum	(394)	E-----
A. butzleri	(363)	-----
B. neotomae	(278)	GFHGKKA-----
C. jejuni	(356)	D-----
H. cinaedi	(364)	G-----

**Figure S4:** Sequence alignment of WbpE with closely related homologs from pathogenic bacteria. Homologs of WbpE were obtained using BLAST, and the alignment was conducted using ClustalW. Conserved residues are highlighted in black, while similar residues are indicated in gray. Secondary structure assignments were determined from analysis of the PMP-bound WbpE structure by PROCHECK. The following GenBank accession numbers of homologs from each indicated bacterial species are listed, allowing with sequence identity to WbpE:

- Acinetobacter baumannii* (ABO10550.2, 78%)
- Vibrio cholerae* (ZP\_04414211, 75%)
- Bordetella pertussis* (NP\_878993, 60%)
- Coxiella burnetti* (YP\_002303655, 57%)
- Legionella pneumophila* (YP\_095453, 51%)
- Parachlamydia acanthamoebae* (ZP\_06298915, 51%)
- Clostridium botulinum* (ZP\_04823710, 41%)
- Arcobacter butzleri* (YP\_001489598, 49%)
- Brucella neotomae* (ZP\_05964854, 46%)
- Campylobacter jejuni* (ZP\_02270922, 48%)
- Helicobacter cinaedi* (ZP\_03659225, 52%)