ACCSCIENCE PUBLISHING

Microbes & Immunity

ORIGINAL RESEARCH ARTICLE

Identification and characterization of novel outer membrane proteins of *Brachyspira pilosicoli*

Supplementary File

Table S1. Nine strains of *Brachyspira pilosicoli* searched for predicted outer membrane protein variations compared to the reference genome (95/1000)

Organism name	Strain	Assembly	Level	Size (Mb)	GC %	Scaffolds	Coding sequences
Brachyspira pilosicoli	P43/6/78	GCA_000325665.1	Complete	2.556	28	1	2,208
	MEI7141	GCA_030168385.1	Complete	2.596	28	1	2,295
	MEI4046	GCA_030168405.1	Complete	2.595	28	1	2,290
	MEI4028	GCA_030168425.1	Complete	2.592	28	1	2,287
	ZH1243	GCA_030168345.1	Complete	2.578	28	1	2,261
	ZH1145	GCA_030168365.1	Complete	2.551	28	1	2,226
	ZH1268	GCA_030168305.1	Complete	2.539	28	1	2,209
	ZH1244	GCA_030168325.1	Complete	2.528	28	1	2,220
	B2904	GCA_000296575.1	Complete	2.765	28	1	2,658

Abbreviation: GC %: Percentage of guanine (G) and cytosine (C) nucleotides.

Table S2. Comprehensive results from various computational tools employed to predict outer membrane β-barrel proteins

Protein accession no.	Locus identifier	Protein name[a]	Pepstats		SignalP	Cleavage position	LipoP	DEG match	PSORTb		CELLO	OMPdb match	MCMBB	TMBETADISC. RBF_AADP	TMbed	
			Length	Molecular weight (kDa)					Localization	Score					Tmbed Count_B	Tmbed Count_b
Group A																
WP_013244106.1	BP951000_ RS05730	OMP assembly factor BamA	872	100.1	SP	CS pos: 24–25	SpI	DEG 10280066	OM	9.92	OM	500	0.005	OMP	73	77
WP_013244995.1	BP951000_ RS10215	Variable surface protein VspE	367	40.0	SP	CS pos: 21–22	SpI	-	Unknown	2	OM	163	0.046	OMP	70	82
WP_013243917.1	BP951000_ RS04760	Variable surface protein VspD	392	42.8	SP	CS pos: 21–22	SpI	-	Unknown	2	OM	205	0.039	OMP	56	69
WP_013243193.1	BP951000_ RS01125	CsgG/HfaB family protein	489	55.1	LIPO	CS pos: 14–15	CYT	-	Unknown	5.48	OM	13	0.018	OMP	40	40
WP_013243655.1	BP951000_ RS03440	OMBB protein	240	26.4	SP	CS pos: 21–22	SpI	-	Unknown	4.72	OM	5	0.058	OMP	39	42
WP_013244081.1	BP951000_ RS05600	TolC family protein	500	57.5	SP	CS pos: 26–27	SpI	-	Cytoplasmic	8.96	OM	52	0.002	OMP	31	28
WP_013244750.1	BP951000_ RS09000	TolC family protein	484	54.7	SP	CS pos: 20–21	SpI	-	Unknown	2.5	OM	54	0.035	OMP	30	28
WP_041747714.1	BP951000_ RS06235	TolC family protein	452	51.6	SP	CS pos: 21–22	SpI	-	OM	10	OM	58	0.048	OMP	20	20
WP_013243940.1	BP951000_ RS04880	Serpentine receptor domain-containing protein	214	23.4	SP	CS pos: 21–22	SpI	-	OM	9.52	OM	5	0.03	OMP	39	42
WP_013243377.1	BP951000_ RS02055	Serpentine receptor domain-containing protein	256	29.3	SP	CS pos: 20–21	SpI	-	OM	9.52	OM	5	0.045	OMP	42	44
WP_013243376.1	BP951000_ RS02050	Serpentine receptor domain-containing protein	254	28.6	SP	CS pos: 20–21	SpI	-	OM	9.49	OM	1	0.043	OMP	40	43
WP_013244459.1	BP951000_ RS07540	Serpentine receptor domain-containing protein	213	23.4	SP	CS pos: 21–22	SpI	-	OM	9.52	OM	5	0.031	OMP	40	37
WP_013242998.1	BP951000_ RS00180	Serpentine receptor domain-containing protein	224	25.0	SP	CS pos: 20–21	SpI	-	Unknown	2	OM	5	0.065	OMP	40	42
Group B																
WP_041747843.1	BP951000_ RS09575	Lipopolysaccharide-assembly protein (LptD)	970	111.9	SP	CS pos: 19–20	SpI	DEG 10200216	OM	9.52	OM	0	0.036	OMP	111	118

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Table S2. ***(Continued)***

Protein accession no.	Locus identifier	Protein name[a]	Pepstats		SignalP	Cleavage position	LipoP	DEG match	PSORTb		CELLO	OMPdb match	MCMBB	TMBETADISC. RBF_AADP	TMbed	
			Length	Molecular weight (kDa)					Localization	Score					Tmbed Count_B	Tmbed Count_b
WP_041747581.1	BP951000_ RS03215	TonB-dependent siderophore receptor	649	75.2	SP	CS pos: 18–19	Cyt	-	OM	9.49	OM	1	0.015	Non-OMP	91	102
WP_013243854.1	BP951000_ RS04405	Toxin A	354	42.3	SP	CS pos: 21–22	SpI	-	OM	9.52	OM	0	0.029	OMP	68	76
WP_013244879.1	BP951000_ RS09655	DUF5723 domain-containing protein	494	53.8	SP	CS pos: 20–21	SpI	-	Unknown	2.5	OM	0	0.038	OMP	73	73
WP_013243861.1	BP951000_ RS04440	Hypothetical protein	334	38.5	SP	CS pos: 17–18	SpI	-	Unknown	4.72	OM	0	0.018	OMP	60	63
WP_013244607.1	BP951000_ RS08285	Trep protein	926	108.1	Other	None	Cyt	-	Unknown	4.69	OM	0	0.021	OMP	70	75
WP_015274839.1	BP951000_ RS04505	Variable surface protein VspH	423	46.8	Other	None	TMH	-	Unknown	2	OM	205	0.024	Non-OMP	68	71
WP_013244641.1	BP951000_ RS08455	PorV/PorQ family protein	334	36.8	LIPO	CS pos: 21–22	TMH	-	Unknown	4.72	OM	0	0.049	OMP	49	56
WP_013243185.1	BP951000_ RS01090	Variable surface protein VspH	361	40.6	SP	CS pos: 18–19	SpI	-	OM	9.52	OM	0	0.023	OMP	64	68
WP_013244339.1	BP951000_ RS06935	Hypothetical protein	302	35.1	SP	CS pos: 21–22	SpI	-	Cytoplasmic membrane	9.82	OM	0	0.016	OMP	52	56
WP_041747935.1	BP951000_ RS11380	Toxin A	289	33.1	SP	CS pos: 19–20	TMH	-	Unknown	2	OM	0	0.025	OMP	46	59
WP_013243647.1	BP951000_ RS03405	Hypothetical protein	327	39.3	SP	CS pos: 19–20	SpI	-	Unknown	2	OM	0	0.006	OMP	51	54
WP_013242999.1	BP951000_ RS00185	Hypothetical protein	300	34.4	SP	CS pos: 20–21	SpI	-	OM	9.49	OM	0	0.031	OMP	57	58
WP_041747873.1	BP951000_ RS10320	Hypothetical protein	419	46.7	SP	CS pos: 20–21	SpI	-	Unknown	2	OM	0	0.023	OMP	48	45
WP_013244050.1	BP951000_ RS05445	DUF3575 domain-containing protein	201	22.5	SP	CS pos: 21–22	Cyt	-	Unknown	2	OM	0	0.025	OMP	34	36
WP_013244610.1	BP951000_ RS08300	Tia invasion determinant	205	23.5	SP	CS pos: 21–22	SpI	-	Unknown	2	OM	12	0.025	Non-OMP	33	37
WP_013244059.1	BP951000_ RS05490	Tia invasion determinant	202	22.9	SP	CS pos: 23–24	Cyt	-	Unknown	2	OM	0	0.023	OMP	33	38

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Table S2. ***(Continued)***

Protein accession no.	Locus identifier	Protein name[a]	Pepstats		SignalP	Cleavage position	LipoP	DEG match	PSORTb		CELLO	OMPdb match	MCMBB	TMBETADISC. RBF_AADP	TMbed	
			Length	Molecular weight (kDa)					Localization	Score					Tmbed Count_B	Tmbed Count_b
WP_187287137.1	BP951000_RS07500	Hypothetical protein	232	25.5	SP	CS pos: 19–20	SpI	-	Cytoplasmic membrane	9.82	OM	0	0.032	OMP	37	42
WP_181893515.1	BP951000_RS01590	Hypothetical protein	270	29.4	SP	None	TMH	-	Cytoplasmic membrane	9.82	OM	0	0.036	OMP	41	41
WP_228369485.1	BP951000_RS08295	Tia invasion determinant	198	22.9	SP	CS pos: 18–19	SpI	-	Unknown	4.69	Inner membrane	8	0.013	Non-OMP	34	37
WP_013244745.1	BP951000_RS08975	TonB-dependent receptor domain-containing protein	445	51.5	SP	CS pos: 16–17	Cyt	-	Unknown	5.48	OM	0	0.031	OMP	53	63
WP_013244338.1	BP951000_RS06930	Serpentine receptor domain-containing protein	211	24.3	Other	None	SpI	-	Cytoplasmic membrane	9.82	Inner membrane	0	0.03	OMP	36	40
WP_014936494.1	BP951000_RS03290	Serpentine receptor domain-containing protein	222	25.6	SP	CS pos: 23–24	SpI	-	Cytoplasmic membrane	9.86	OM	0	0.007	OMP	40	41
WP_014933009.1	BP951000_RS00765	Serpentine receptor domain-containing protein	232	26.4	SP	CS pos: 21–22	SpI	-	OM	9.49	OM	0	0.046	OMP	40	41
WP_013243225.1	BP951000_RS01280	Serpentine receptor domain-containing protein	256	28.4	SP	CS pos: 20–21	SpI	-	OM	9.52	OM	0	0.052	OMP	40	42
WP_013245039.1	BP951000_RS10445	Serpentine receptor domain-containing protein	230	25.6	SP	CS pos: 21–22	TMH	-	OM	9.49	OM	0	0.04	OMP	39	39
WP_013243037.1	BP951000_RS00365	Serpentine receptor domain-containing protein	226	25.5	SP	CS pos: 18–19	SpI	-	Unknown	2.5	OM	0	0.061	OMP	37	41
WP_013243896.1	BP951000_RS04620	Serpentine receptor domain-containing protein	234	25.7	SP	CS pos: 23–24	SpI	-	OM	9.52	OM	0	0.04	OMP	40	42
WP_013243815.1	BP951000_RS04220	Serpentine receptor domain-containing protein	254	28.6	SP	CS pos: 23–24	TMH	-	Cytoplasmic membrane	9.82	OM	0	0.043	OMP	39	39

Note: [a]Protein names follow annotations in the National Center for Biotechnology Information and UniProt databases, retrieved using protein accession numbers (accessed on March 28, 2024).
Abbreviations: CELLO: Subcellular localization predictive system; CS pos: Cleavage site position; Cyt: Cytoplasmic; DEG: Database of essential genes; DUF: Domain of unknown function; LIPO: Lipoprotein; OM: Outer membrane; OMBB: Outer membrane β-barrel; OMP: Outer membrane protein; OMpdb: Outer membrane protein database; SP: Signal peptide; SpI: Secretory signal peptide; TMH: Transmembrane helix; Trep: Transcriptional regulating protein.

Table S3. Structure- and sequence-based functional annotation

Locus identifier	Protein accession no.	Protein name[a]	Structure-based functional annotation		Sequence-based functional annotation		Consensus annotation
			DALI server	**Foldseek tool**	**PANNZER**	**eggNOG-mapper[e]**	
Group A							
BP951000_RS05730	WP_013244106.1	OMP assembly factor/BamA	OM assembly factor BamA (7NRE)	OMP assembly factor BamA from *Neisseria gonorrhoeae*	OM assembly factor BamA	OMP assembly complex, YaeT protein	OM assembly factor BamA
BP951000_RS10215	WP_013244995.1	VspE	Poly-β-1,6-N-acetyl-D-glucosamine export protein (PgaA) (4Y25)	Bacterial polysaccharide OM secretin from *Escherichia coli* K-12	VspB	-	Secretion with surface variability
BP951000_RS04760	WP_013243917.1	VspD	Cellulose synthase operon protein C (6TZK)	Bacterial polysaccharide OM secretin from *Escherichia coli* K-12	VspD	-	Secretion and adhesion
BP951000_RS01125	WP_013243193.1	CsgG/HfaB family protein	OM porin OmpF[b] (4RLC)	Uncharacterized protein from the marine metagenome	Curli production assembly protein CsgG	Curli production assembly transport component CsgG	Functions similar to CsgG and OmpF
BP951000_RS03440	WP_013243655.1	OMBB protein	OMP NspA (1P4T)	Uncharacterized protein from *Brachyspira murdochii* DSM 12563	OMBB domain-containing protein	-	OMBB acting as an adhesin protein
BP951000_RS05600	WP_013244081.1	TolC family protein	OMP TolC (6WXI)	Uncharacterized protein from a *Spirochaetes* bacterium	Integral OMP TolC, efflux pump component	Efflux transmembrane transporter activity	Efflux pump TolC
BP951000_RS09000	WP_013244750.1	TolC family protein	OMP TolC (6WXI)	Uncharacterized protein from a *Spirochaetes* bacterium	Integral OMP TolC, efflux pump component	Efflux transmembrane transporter activity	Efflux pump TolC
BP951000_RS06235	WP_041747714.1	TolC family protein	OMP TolC (6WXI)	OMP TolC from a *Spirochaetes* bacterium	OM efflux protein	OM efflux protein	Efflux pump TolC
BP951000_RS04880	WP_013243940.1	Serpentine receptor domain-containing protein	OMP NspA (1P4T)	Uncharacterized protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB acting as an adhesin protein
BP951000_RS02055	WP_013243377.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	OMBB protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS02050	WP_013243376.1	Serpentine receptor domain-containing protein	OMP A (9FZD)	OMBB protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins

(Cont'd...)

Table S3. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Structure-based functional annotation		Sequence-based functional annotation		Consensus annotation
			DALI server	Foldseek tool	PANNZER	eggNOG-mapper[e]	
BP951000_RS07540	WP_013244459.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS00180	WP_013242998.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hampsonii* 30446	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
Group B							
BP951000_RS09575	WP_041747843.1	Lipopolysaccharide-assembly protein LptD	Lipopolysaccharide-assembly protein LptD (5IXM)	Uncharacterized protein from *Actinomycetia* bacterium	Cell envelope biogenesis protein	lipopolysaccharide transport	Lipopolysaccharide-assembly protein (LptD)
BP951000_RS03215	WP_041747581.1	TonB-dependent siderophore receptor	Vitamin B_{12} transporter BtuB (3RGM)	TonB-dependent receptor from a *Bacteroidetes* bacterium	OM receptor	Cobalamin-transporting ATPase activity	TonB-dependent OM receptor
BP951000_RS04405	WP_013243854.1	Toxin A	Probable porin (4FRT)	Uncharacterized protein from *Treponema vincentii*	Toxin A	-	Porin-like protein
BP951000_RS09655	WP_013244879.1	DUF5723 domain-containing protein	CymA protein (4V3H)	Uncharacterized protein from *Euryarchaeota* archaeon	Cell surface protein	-	Inconclusive
BP951000_RS04440	WP_013243861.1	Hypothetical protein	Translocation and assembly module TamA (4C00)	Omp85 domain-containing protein from *Dracunculus medinensis*	Toxin A	-	Inconclusive
BP951000_RS08285	WP_013244607.1	Trep protein	TolB and TolB (2IVZ, 3IAX)	Uncharacterized protein from a *Spirochaetes* bacterium ADurb.Bin215	Trep	Involved in the TonB-independent uptake of proteins	TonB-independent uptake of proteins
BP951000_RS04505	WP_015274839.1	VspH	Poly-β-1,6-N-acetyl-D-glucosamine export protein (PgaA) (4Y25)	Bacterial polysaccharide OM secretin from *Escherichia coli* K-12	Cell surface protein	-	Polysaccharide secretion
BP951000_RS08455	WP_013244641.1	PorV/PorQ family protein	Long-chain fatty acid transport protein FadL (2R88)	PorV/PorQ family protein from a *Candidatus Margulisbacteria* bacterium	Uncharacterized protein	-	Hydrophobic substrates transporter

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Table S3. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Structure-based functional annotation		Sequence-based functional annotation		Consensus annotation
			DALI server	**Foldseek tool**	**PANNZER**	**eggNOG-mapper[e]**	
BP951000_RS01090	WP_013243185.1	VspH	CymA protein (4V3G)	Uncharacterized protein from a *Chitinophagaceae* bacterium	Variable surface protein VspH	-	Diffusion channel, VspH
BP951000_RS06935	WP_013244339.1	Hypothetical protein	Hemoglobin-binding protease hbp autotransporter (3AEH)	COG4313 outer membrane channel of *Pseudomonas putida* F1	Toxin A	-	Autotransporter, hydrophobic substrates transporter
BP951000_RS11380	WP_041747935.1	Toxin A	Serine protease EspP (2QOM)	Uncharacterized protein from a *Candidatus Margulisbacteria* bacterium	Toxin A	-	Inconclusive
BP951000_RS03405	WP_013243647.1	Hypothetical protein	OM phospholipase A (1ILD)	DUF1207 domain-containing protein from an *Ignavibacteria* bacterium	OMP	-	OMP having enzymatic activity
BP951000_RS00185	WP_013242999.1	Hypothetical protein	OM phospholipase (OMPLA) (1QD6)	DUF1207 domain-containing protein from an *Ignavibacteria* bacterium	Toxin A	-	Bacterial virulence factor
BP951000_RS10320	WP_041747873.1	Hypothetical protein	OMP OpcA[c] (2VDF)	Uncharacterized protein from *Brachyspira murdochii* DSM 12563	Cell surface protein	-	Adhesin protein
BP951000_RS05445	WP_013244050.1	DUF3575 domain-containing protein	OM porin OmpF and OMP NspA (4RLC, 1P4T)[d]	Uncharacterized protein from *Mangrovimonas* sp. DI 80	Tia invasion determinant	-	Adhesin involved in host–pathogen interaction
BP951000_RS08300	WP_013244610.1	Tia invasion determinant	OMP NspA (1P4T)	OmpA family protein from *Brachyspira hampsonii* 30446	Tia invasion determinant	Exhibits lipid A 3-O-deacylase activity, hydrolyzing the ester bond at the 3-position of lipid A—a bioactive component of lipopolysaccharide—and releasing the primary fatty acyl moiety	Multifunctional OMP is involved in host interaction and immune modulation
BP951000_RS05490	WP_013244059.1	Tia invasion determinant	OMP NspA (1P4T)	Uncharacterized protein from *Mangrovimonas* sp. DI 80	Tia invasion determinant	-	Adhesin protein

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Table S3. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Structure-based functional annotation		Sequence-based functional annotation		Consensus annotation
			DALI server	**Foldseek tool**	**PANNZER**	**eggNOG-mapper[c]**	
BP951000_RS07500	WP_187287137.1	Hypothetical protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hampsonii*	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS01590	WP_181893515.1	Hypothetical protein	OMP OprG (2X27)	Uncharacterized protein from *Brachyspira pilosicoli* P43/6/78	Cell surface protein	-	OmpW family OMP
BP951000_RS08295	WP_228369485.1	Tia invasion determinant	OMP NspA (1P4T)	OmpA family protein from *Brachyspira hampsonii* 30446	Tia invasion determinant	Exhibits lipid A 3-O-deacylase activity, hydrolyzing the ester bond at the 3-position of lipid A—a bioactive component of lipopolysaccharide—and releasing the primary fatty acyl moiety	Multifunctional OMP is involved in host interaction and immune modulation
BP951000_RS08975	WP_013244745.1	TonB-dependent receptor domain-containing protein	Vitamin B_{12} transporter BtuB (2GSK)	TonB-dependent receptor from a *Chitinophagaceae* bacterium	Serine/threonine protein kinase	Cobalamin-transporting ATPase activity	TonB-dependent OM receptor
BP951000_RS06930	WP_013244338.1	Serpentine receptor domain-containing protein	OMP NspA (1P4T)	Uncharacterized protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB acting as an adhesin protein
BP951000_RS03290	WP_014936494.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hyodysenteriae*	Uncharacterized protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS00765	WP_014933009.1	Serpentine receptor domain-containing protein	OMP A (9FZD)	Uncharacterized protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS01280	WP_013243225.1	Serpentine receptor domain-containing protein	OMP NspA (1P4T)	OMBB protein from *Brachyspira hyodysenteriae*	OMBB domain-containing protein	-	OMBB acting as an adhesin protein

(Cont'd...)

Table S3. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Structure-based functional annotation		Sequence-based functional annotation		Consensus annotation
			DALI server	Foldseek tool	PANNZER	eggNOG-mapper[e]	
BP951000_RS10445	WP_013245039.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira murdochii* DSM 12563	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins
BP951000_RS00365	WP_013243037.1	Serpentine receptor domain-containing protein	OMP NspA (1P4T)	Uncharacterized protein from *Brachyspira hampsonii* 30446	Uncharacterized protein	-	OMBB acting as an adhesin protein
BP951000_RS04620	WP_013243896.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hampsonii* 30446	OMBB domain-containing protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins.
BP951000_RS04220	WP_013243815.1	Serpentine receptor domain-containing protein	OMP A (9FZC)	Uncharacterized protein from *Brachyspira hyodysenteriae*	Uncharacterized protein	-	OMBB functions in the maintenance of OM integrity and virulence, and acts as a receptor for bacteriophages and colicins.

Notes: [a]Protein names follow annotations in the National Center for Biotechnology Information and UniProt databases, retrieved using protein accession numbers (accessed on March 28, 2024). [b]OmpF in *Pseudomonas aeruginosa* belongs to the OmpA–OmpF porin (OOP) family. [c]Given that the top four hits were synthetic constructs of a transmembrane *β*-barrel, we considered the fifth hit—outer membrane protein OpcA from *Neisseria meningitidis*—for functional annotation. [d]The DALI structural homologs were selected based on the highest Z-score. As both structural homologs had identical Z-scores for this protein, both were included. [e]Rows with blank outputs indicate that eggNOG-mapper did not assign orthology due to sequence identity falling below the 40% threshold required for reliable prediction.

Abbreviations: DUF: Domain of unknown function; OM: Outer membrane; OMBB: Outer membrane β-barrel; OMP: Outer membrane protein; Trep: Transcriptional regulating protein; Vsp: Variable surface protein.

Table S4. Sequence variations among nine strains of *Brachyspira pilosicoli* in the intracellular loops, extracellular loops, β-barrel transmembrane region, and other regions of predicted outer membrane β-barrel proteins

Locus identifier	Protein accession no.	Protein name[a]	Total variations	Variations in ICL region	Variations in ECL region	Variations in TM region	Variations in other regions
Group A							
BP951000_RS05730	WP_013244106.1	OMP assembly factor BamA	Five variations: D60, A184, V465, A467, and F512	None	None	V465, A467, and F512	D60 and V184
BP951000_RS10215[b]	WP_013244995.1	Variable surface protein (VspE)	208 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS04760[b]	WP_013243917.1	Variable surface protein (VspD)	260 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS01125	WP_013243193.1	CsgG/HfaB family protein	Five variations: S63, D79, T190, I210, and L380	None	None	L380	S63, D79, T190, and I210
BP951000_RS03440	WP_013243655.1	OMBB protein	Six variations: F24, V47, V64, N110, D169, and A197	None	V47	F24, V64, N110, and A197	D169
BP951000_RS05600	WP_013244081.1	TolC family protein	Four variations: T246, N499	None	None	None	T246 and N499
BP951000_RS09000	WP_013244750.1	TolC family protein	S90, and S131	None	S90	S131	None
BP951000_RS06235	WP_041747714.1	TolC family protein	18 variations: K2, N3, F5, V6, F7, I8, I10, L12, S16, S25, N33, I42, E43, L93, S105, E136, I137, and T210	None	L93	S105	K2, N3, F5, V6, F7, I8, I10, L12, S16, S25, N33, I42, E43, E136, I137, and L210
BP951000_RS04880	WP_013243940.1	Serpentine receptor domain-containing protein	N69	N69	None	None	None
BP951000_RS02055	WP_013243377.1	Serpentine receptor domain-containing protein	Three variations: H101, N163, and M235	None	N163 and M235	H101	None
BP951000_RS02050	WP_013243376.1	Serpentine receptor domain-containing protein	Five variations: A27, L28, T108, A228, and I247	None	T108 and A228	A27, L28, and I247	None
BP951000_RS07540	WP_013244459.1	Serpentine receptor domain-containing protein	Six variations: M1, K2, K3, I4, I5, and L6	None	None	None	M1, K2, K3, I4, I5, and L6
BP951000_RS00180	WP_013242998.1	Serpentine receptor domain-containing protein	Nine variations: I64, M115,	None	None	I64 and M115	None
Group B							
BP951000_RS09575	WP_041747843.1	Lipopolysaccharide-assembly protein (LptD)	N14, G137, I257, I382, E454, D600, and G944	G944	D600	I382 and E454	N14, G137, and I257

(Cont'd...)

Table S4. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations	Variations in ICL region	Variations in ECL region	Variations in TM region	Variations in other regions
BP951000_RS03215[b]	WP_041747581.1	TonB-dependent siderophore receptor	59 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS04405	WP_013243854.1	Toxin A	23 variations: M1, H2, R3, I4, I6, L8, T9, M18, V19, T24, N32, S34, N41, F84, K90, N98, I101, S102, N104, S175, Q183, I264, and T303	None	N41, F84, K90, N98, S175, and Q183	T24, N32, S34, I101, S102, N104, I264, and T303	M1, H2, R3, I4, I6, L8, T9, M18, and V19
BP951000_RS09655[b]	WP_013244879.1	DUF5723 domain-containing protein	315 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS04440	WP_013243861.1	Hypothetical protein	13 variations: K104, K113, S117, Y124, I132, T134, N151, G153, L243, V252, L254, S308, and N321	None	K104 and L243	K113, S117, Y124, I132, T134, N151, G153, V252, L254, N321, and S308	None
BP951000_RS08285[b]	WP_013244607.1	Trep protein	43 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS04505[b]	WP_015274839.1	Variable surface protein (VspH)	247 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS08455	WP_013244641.1	PorV/PorQ family protein	Six variations: L12, S20, N22, A117, R187, and S253	S253	N22 and A117	R187	L12 and S20
BP951000_RS01090	WP_013243185.1	Variable surface protein (VspH)	E258	None	E258	None	None
BP951000_RS06935	WP_013244339.1	Hypothetical protein	Four variations: S9, I10, V13, and R298	None	None	None	S9, I10, V13, and R298
BP951000_RS11380	WP_041747935.1	Toxin A	Three variations: M126, M154, and I278	None	M126	M154 and I278	None
BP951000_RS03405	WP_013243647.1	Hypothetical protein	24 variations: M1, R2, L3, K4, F5, F6, F7, L8, I9, F10, L11, F12, L13, S14, L15, S16, L17, Y18, T19, Q20, D21, N22, E23, and A24	None	None	None	M1, R2, L3, K4, F5, F6, F7, L8, I9, F10, L11, F12, L13, S14, L15, S16, L17, Y18, T19, Q20, D21, N22, E23, and A24
BP951000_RS00185[b]	WP_013242999.1	Hypothetical protein	58 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]

(Cont'd...)

Table S4. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations	Variations in ICL region	Variations in ECL region	Variations in TM region	Variations in other regions
BP951000_RS10320	WP_041747873.1	Hypothetical protein	25 variations: L18, D48, E55, F251, G285, E400, Y401, G402, I403, F404, T405, K406, Q407, L408, A409, I410, S411, F412, I413, P414, I415, N416, I417, R418, and F419	G285	None	E400, Y401, G402, I403, F404, T405, K406, Q407, L408, A409, I410, S411, F412, I413, P414, I415, N416, I417, R418, and F419	L18, D48, E55, and F251
BP951000_RS05445	WP_013244050.1	DUF3575 domain-containing protein	Six variations: K2, I7, A79, N87, H89, and K158	K158	N87 and H89	A79	K2 and I7
BP951000_RS08300	WP_013244610.1	Tia invasion determinant	Three variations: L143, N156, and S200	None	None	L143, N156, and S200	None
BP951000_RS05490[b]	WP_013244059.1	Tia invasion determinant	61 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS07500[b]	WP_187287137.1	Hypothetical protein	183 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS01590	WP_181893515.1	Hypothetical protein	Three variations: V205, I215, and V221	None	None	V205, I215, and V221	None
BP951000_RS08295	WP_228369485.1	Tia invasion determinant	Six variations: N34, I49, V123, S141, I144, and V167	None	N34	I49, V123, S141, I144, and V167	None
BP951000_RS08975	WP_013244745.1	TonB-dependent receptor domain-containing protein	Two variations: D32 and T371	None	D32	T371	None
BP951000_RS06930[b]	WP_013244338.1	Serpentine receptor domain-containing protein	135 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS03290	WP_014936494.1	Serpentine receptor domain-containing protein	None	None	None	None	None
BP951000_RS00765[b]	WP_014933009.1	Serpentine receptor domain-containing protein	70 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]
BP951000_RS01280	WP_013243225.1	Serpentine receptor domain-containing protein	Five variations: V32, A83, V124, E210, and T237	None	E210	V32, A83, V124, and T237	None
BP951000_RS10445	WP_013245039.1	Serpentine receptor domain-containing protein	G228	G228	None	None	None

(Cont'd...)

Table S4. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations	Variations in ICL region	Variations in ECL region	Variations in TM region	Variations in other regions
BP951000_RS00365	WP_013243037.1	Serpentine receptor domain-containing protein	11 variations: V72, Q77, I84, D156, D159, V168, N177, A200, T216, I222, and Y226	Q77, N177, I222, and Y226	D156, D159, and A200	V72, I84, V168, and T216	None
BP951000_RS04620	WP_013243896.1	Serpentine receptor domain-containing protein	Four variations: K2, E95, A140, and V194	None	None	E95, A140, and V194	K2
BP951000_RS04220[b]	WP_013243815.1	Serpentine receptor domain-containing protein	218 variations	Not determined[c]	Not determined[c]	Not determined[c]	Not determined[c]

Notes: [a]Protein names follow annotations in the National Center for Biotechnology Information and UniProt databases, retrieved using protein accession numbers (accessed on March 28, 2024). [b]Sequence comparison of these proteins across nine strains of *Brachyspira pilosicoli* revealed variations at more than 40 positions. Therefore, they have been listed in this table. [c]Given that sequence variations were present at more than 40 positions, we did not determine whether these variations were located in the transmembrane region or the loop region of the predicted proteins.
Abbreviations: DUF: Domain of unknown function; ECL: Extracellular loop; ICL: Intracellular loop; OMBB: Outer membrane β-barrel; TM: Transmembrane; Trep: Transcriptional regulating protein.

Table S5. Sequence comparison across nine strains of *Brachyspira pilosicoli* for the proteins having a high number of variations

Locus identifier	Protein accession no.	Protein name[a]	Total variations
Group A			
BP951000_RS10215	WP_013244995.1	Variable surface protein VspE	F4, V8, T9, V11, F12, V13, L14, A16, S17, D26, N27, T28, L30, F31, I34, N44, V46, T49, G53, T54, V55, F59, K62, A63, N64, T65, G66, L67, T68, Q70, F72, T73, K75, G76, N77, K78, L80, E81, A82, G86, G92, G94, V97, V99, N106, N107, A108, A109, G111, N112, V113, D114, A115, F120, V121, F122, V125, N127, N128, V131, V133, S136, S138, S139, A141, D142, L143, N144, N145, G146, D149, F151, L153, I155, P156, A157, D166, Y170, F173, E174, F175, N176, Q179, S181, K183, E184, G185, T186, V187, N188, Y189, S190, A191, K192, N193, L194, S195, Q197, L198, L200, H201, F202, L203, N204, T205, V206, I207, E208, N209, T211, V212, N213, R217, D219, F220, A221, S222, T230, A231, I232, V233, G234, T235, L236, G237, T241, S242, D243, I244, K245, A246, W247, T248, V249, A250, G251, A252, A253, E254, A255, T256, A257, G258, Q259, E260, R264, Y267, D268, R270, I271, S274, I275, S276, L277, T278, V279, T281, N285, F286, I287, F288, P290, I292, R295, V296, E297, Y299, K301, Q302, G303, G304, K305, L306, E307, S309, V310, Y311, A314, V318, V320, R321, I323, P324, A325, M332, D333, V334, N335, G337, V338, P339, K340, L341, Q342, D343, Q344, S345, T346, T347, P349, I350, A351, G353, N355, S356, S364, N366, and K367

(Cont'd...)

Table S5. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations
BP951000_RS04760	WP_013243917.1	Variable surface protein VspD	V4, A8, I9, L11, L12, T13, I14, S16, T17, Y22, N26, S27, D28, I30, D31, V34, D35, A36, F41, V43, R44, M46, V49, D53, M54, I55, R56, V58, V59, V61, R62, D63, E64, S65, E66, T67, S68, F69, A71, F73, D74, S75, T76, T77, Y78, A79, P80, N81, G82, T83, T84, Q86, F87, L88, F90, I91, P92, A96, F102, I104, S106, I107, V109, R114, H116, H117, T118, T119, F120, N121, G122, L123, T124, K125, N126, Y128, G129, L130, S131, E132, L137, T138, M139, T140, M142, D144, S145, I148, I150, V152, Q153, V154, A155, V156, N158, G159, D160, I161, A162, N163, S164, T165, D166, K167, V168, K169, M171, V173, M175, D176, T177, E186, E190, L193, Y194, V195, K196, I199, Q201, L202, E203, N204, T205, E206, V207, A208, N209, S210, K211, E212, K213, A214, E215, S216, F217, G218, D220, F221, A223, Y224, F225, G226, A227, T228, V229, G230, D231, A233, L234, K235, K239, T241, Y242, D243, T244, N247, I252, A253, Y254, S255, Y256, N257, T258, G259, A260, A261, A262, T263, Y264, G265, D266, T267, G269, V270, K271, S272, S273, S274, A275, L276, V277, F278, V279, D280, D281, G282, T283, G284, T285, G286, E288, H289, W292, A293, N295, I296, T299, L300, G301, M302, S303, A304, S306, S310, I311, Y312, V313, A315, L317, G320, A321, V322, T324, Y326, K327, F328, N329, A330, A331, N332, A333, A334, N335, T336, G337, I338, L339, S341, M342, R343, N345, A346, I350, I352, N353, V355, K356, D357, Q359, A364, E365, I366, G367, G369, G370, A371, F372, T373, K374, N375, T376, L377, Q378, N380, G381, A382, T383, and T391
Group B			
BP951000_RS09655	WP_013244879.1	DUF5723 domain-containing protein	K2, K3, L4, L6, L8, T9, A10, L11, C12, I13, T14, A16, Y17, S18, A20, I22, P23, T24, A25, N26, M27, N28, T29, F30, D32, N33, S36, A42, G45, E46, F47, T49, D50, N55, V59, N63, I66, A69, G70, V72, G74, N76, S77, S78, D79, S80, T81, V83, A85, F86, V89, L92, N93, A95, V100, A101, V103, Y104, M106, N107, E108, T109, R110, V111, D112, P115, Y116, S117, A118, T119, S121, Q122, G124, I125, T126, K129, V131, T132, T133, G134, S135, T136, V149, I155, R157, G158, N159, S160, K161, Q162, V163, E165, S169, F170, I171, D172, D173, K174, L176, S177, V178, I179, T182, T183, G184, V189, H190, V192, G195, L196, V197, G199, E200, K202, K204, I205, V207, R208, L209, T210, I211, D213, A214, G215, N216, S217, K220, Q221, G223, L224, T225, K226, T227, D228, V229, A230, N231, Q232, Y233, E235, E236, T237, Q238, T239, G240, Y241, T244, G245, N246, N247, T248, M249, N250, S252, I253, A254, S258, M266, T267, G268, N270, G272, T274, G276, I279, G281, M282, G283, K285, S286, A287, Y288, Y289, S290, T291, Y292, K293, N294, T295, V297, N298, G299, T300, T301, G302, E303, R304, K305, Q306, S307, V309, T310, E313, G314, V315, F316, N319, A320, N323, A324, T327, S331, L332, A333, N335, R336, V337, R338, I340, M341, K344, V345, A348, I349, N350, V351, A354, N356, V357, K358, V360, E361, K362, E364, I365, T366, D367, Q368, T369, L371, E372, T373, T374, T375, S377, G378, A379, K380, S381, S382, L383, V384, Q385, I390, L392, I394, A397, F398, V401, D402, F406, V410, S411, R413, L414, G415, F416, A417, M418, T419, T420, G421, S422, Y423, A425, L426, G428, G429, Y431, K432, T433, F334, N435, Y436, N437, N438, G439, E441, T443, M444, N445, L446, T448, M450, I453, V454, G455, E456, D457, F458, I460, Y463, A465, A466, R467, A468, G469, N470, T471, A472, T473, T474, T475, P476, S477, P478, S479, L480, L481, G482, I483, D484, S485, W486, A488, R493, and L494
BP951000_RS08285	WP_013244607.1	Trep protein	M1, K2, K3, Y4, I5, L6, F7, F8, L9, I10, F11, S12, I13, S14, V15, L16, R205, W207, H240, T305, T322, A349, Q376, R379, E424, R509, V535, K538, T552, T564, S574, A676, L728, V783, A811, S813, S866, I873, A893, R906, S908, I913, and S919

(Cont'd...)

Table S5. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations
BP951000_RS04505	WP_015274839.1	Variable surface protein VspH	R3, I4, L5, M6, S7, I8, L9, L10, M11, V13, L14, S15, I16, Q17, F19, A20, K23, S24, D25, M27, M30, M37, I39, L44, V46, A48, P50, R51, W53, F55, V56, A57, G61, I64, A65, G67, L68, H71, T73, G74, A75, P76, N77, T78, Q79, Q80, E81, N82, T83, E84, N85, N86, Y87, K88, K89, G90, V91, D92, K93, A98, V100, A101, F102, D105, S106, D107, L108, F109, I111, A112, A113, N118, W119, S121, P122, T123, I130, L131, H132, M133, L136, S139, I146, V150, Q152, K153, Y155, V156, N157, D158, K159, S160, K162, T164, M165, I167, A170, I171, G173, A178, E179, D180, I181, A183, L184, L187, F189, Y190, S196, T197, I198, K199, A200, L201, D202, F203, K204, D205, A206, S207, F208, T209, G215, E216, F217, M219, K222, I223, L224, T225, E226, N227, I228, K229, I230, F234, R237, F238, A240, A243, T244, T245, Y246, K247, N248, I249, D250, E251, A252, N253, R254, G255, S256, I257, L258, S260, Y261, A262, V263, S264, F268, I269, D271, D272, P273, G274, G275, T276, G277, A278, N279, I280, A281, A282, G284, A285, N286, A287, S288, G289, T290, L291, Q292, S301, G302, Y303, K306, E307, L311, L313, I315, V317, T320, A324, E326, S329, F332, L338, I340, V341, E345, T348, F349, G350, Q351, H352,A353, W354, E355, D356, V357, A358, S359, R360, H361, R362, R363, T364, N365, Y368, A369, F370, A376, V383, K384, N385, T390, L392, S397,T398, V399, A400, G401, D402, L403, S404, T405, A406, S407, S408, T410, I411, F413, A415, S416, I419, and W421
BP951000_RS00185	WP_013242999.1	Hypothetical protein	M1, K2, R3, I4, Y5, I6, I7, T8, I9, L10, F11, F12, T13, F14, S15, F16, L17, L18, Y19, S20, Q21, D22, S23, V24, S25, N26, D27, Y28, S29, F30, T31, N32, P33, F34, R35, I36, Y37, D38, V39, D40, K41, Y42, Y43, V44, G45, W46, Q47, D48, P49, R50, A51, F52, I53, G54, R55, L56, M147, and P252
BP951000_RS00765	WP_014933009.1	Serpentine receptor domain-containing protein	Q163, M164, Y165, V166, I167, P168, Y169, I170, K171, L172, T173, F174, D175, W176, F177, F178, S179, D180, I181, N182, Y183, K184, T185, R186, E187, F188, V189, D190, T191, R192, D193, L194, G195, I196, G197, F198, Y199, L200, G201, Y202, N203, F204, G205, P206, K207, S208, K209, N210, Y211, I212, G213, T214, D215, S216, F217, D218, I219, G220, L221, Q222, L223, S224, L225, R226, F227, K228, P229, A230, K231, and N232
BP951000_RS04220	WP_013243815.1	Serpentine receptor domain-containing protein	K2, F3, V4, K5, Y6, F7, L8, L9, I10, N11, F13, I14, I15, T16, I17, T18, Y19, S20, A21, A23, S24, L25, I27, N28, F29, Q30, G31, H32, Y33, A36, F37, P38, F39, N40, S41, I42, K43, V44, N45, D46, N47, Y48, K49, N50, T51, I52, Y53, D54, S55, V56, D57, G58, T59, L60, F62, E63, G64, T65, L66, F67, F68, Q69, I70, N72, Y73, F74, Q75, L76, F77, E78, D80, Y81, T82, R83, I84, I85, K86, G87, V88, L90, F91, G92, D93, I94, F96, S97, I98, N99, A100, L101, M102, S103, D104, Y105, K106, E107, N108, N109, N110, K111, Y112, T113, E114, V115, L116, G117, F118, Y119, S120, M121, L122, V123, A125, T126, V127, L129, F131, S132, K133, M134, I136, G137, G139, T140, I142, I143, A144, Y147, A148, M149, V150, A151, S152, K154, G156, G157, V158, M159, S160, A161, P162, D163, D164,L165, D166, N167, W168, V170, N171, D172, M173, R174, N175, L176, F177, K178, A179, I181, M182, L187, T188, I189, E190, G191, F192, L193, L195, V196, P197, N198, F199, A200, V201, T202, L203, G205, M207, M208, N210, G212, M213, Q214, Y215, K216, D218, V219, V220, N221, N222, N223, L224, G225, G226, N227, I228, Y229, N230, Q231, Y232, N233, F234, D236, F237, S238, I239, G240, L241, I242, L243, S246, F247, G248, R249, S250, D251, G252, Y253, and N254

(Cont'd...)

Table S5. ***(Continued)***

Locus identifier	Protein accession no.	Protein name[a]	Total variations
BP951000_RS06930	WP_013244338.1	Serpentine receptor domain-containing protein	M1, I2, L3, R4, K5, T6, F7, F10, V11, L12, L13, I14, L15, F18, N19, L21, A22, N23, K25, F26, I28, S29, P30, R31, L32, A34, D35, I36, N37, I38, A39, E40, T41, K42, N43, Y44, N45, S46, L47, L48, L49, D50, L53, I55, F57, S58, I59, G62, K64, L65, N67, I68, N69, L70, L71, S72, G73, I74, D75, F80, N83, E84, H85, M86, L87, I89, V90, D91, K92, T94, S95, L96, R99, V100, T101, F103, G104, V105, Y106, A107, V109, D114, L115, S116, K117, N118, N119, Q120, N121, L123, I124, I126, Q131, N133, P134, L135, N139, Y140, K141, S142, R150, S152, R155, K156, F157, F158, E159, T160, K162, T163, D164, L166, A167, V169, H170, N175, N179, Y180, N181, M183, N188, Y189, H190, D191, V192, K193, Y195, L200, F202, F204, S205, F206, F207, E209, and N210
BP951000_RS05490	WP_013244059.1	Tia invasion determinant	M1, K2, V3, Y4, A8, V9, T14, F17, A21, T23, E24, E26, K28, K29, N30, K31, V32, L33, G35, I36, Y46, T51, F65, E71, N72, V79, N87, D89, F95, I99, T100, S103, K104, V106, T108, E110, A114, A117, A121, G129, T132, D133, H134, E140, L142, W144, K158, V163, D172, I173, Y174, A177, K178, R183, L185, M187, V188, M195, V197, I201, and W202
BP951000_RS03215	WP_041747581.1	TonB-dependent siderophore receptor	M1, K2, F4, I5, Y7, L9, F10, A11, S12, I13, M15, T20, K35, V36, N41, T42, Q43, T44, N45, N46, E47, Y48, S49, N50, V51, S52, K53, I55, N58, V59, N61, K62, T63, E70, N75, K78, I80, E82, A85, N92, G104, S105, A106, K141, S147, Q258, E285, V291, F306, K307, E308, L332, E342, D377, S483, A506, I569, Y591, and I593
BP951000_RS07500	WP_187287137.1	Hypothetical protein	M1, K2, K3, I4, I5, V6, L7, G8, L9, M10, L11, I12, S13, S14, S15, L16, V17, Y18, S19, H20, S21, L22, G23, I24, G25, M26, Y27, I28, P29, L30, G31, G32, S33, L34, P35, S36, F37, Y38, S39, D40, N41, A42, E43, A44, T45, S46, F47, F48, S49, P50, K51, S52, A53, F54, E55, V56, G57, V58, I59, F60, N61, P62, R63, V64, N65, F66, N67, I68, G69, D70, G71, T72, H73, T74, V75, S76, L77, G78, V79, D80, V81, G82, W83, Y84, R85, D86, A87, F88, K89, F90, A91, S92, S93, T94, D95, V96, T97, H98, E99, F100, D101, T102, V103, M104, T105, G106, L107, N108, L109, E110, W111, R112, P113, L114, L115, F116, Q117, L118, G119, V120, G121, G122, G123, V124, K125, F126, P127, F128, L129, G130, K131, Y132, I133, E134, G135, N136, N137, K138, M139, A140, L141, S142, G143, G144, A145, F146, A147, S148, R149, Y150, N151, N152, V153, F154, I155, P156, Y157, I158, R159, L160, Y161, T162, G163, I164, N165, I166, I167, F168, I169, S170, L171, S172, L173, Y174, V175, N176, F177, D178, I179, P180, Y181, L182, and Q183

Note: [a]Protein names follow annotations in the National Center for Biotechnology Information and UniProt databases, retrieved using protein accession numbers (accessed on March 28, 2024).

Abbreviations: DUF: Domain of unknown function; Trep: Transcriptional regulating protein.

Table S6. Basic local alignment search tool–protein (BLASTp) output of all identified outer membrane β-barrels from *Brachyspira pilosicoli* against other species of the genus *Brachyspira*

Identified Outer membrane β-barrel proteins (*Brachyspira pilosicoli*)	Pathogenic species				Non-pathogenic species	
	Brachyspira aalborgi	*Brachyspira hyodysenteriae*	*Brachyspira intermedia*	*Brachyspira alvinipulli*	*Brachyspira murdochii*	*Brachyspira innocens*
Group A						
WP_013244106.1	+	+	+	+	+	+
WP_013244995.1	−	+	+	−	+	+
WP_013243917.1	−	+	+	−	+	+
WP_013243377.1	+	+	+	+	+	+
WP_013243376.1	+	+	+	+	+	+

(Cont'd...)

Table S6. (*Conrtinued)*

Identified Outer membrane β-barrel proteins (*Brachyspira pilosicoli*)	**Pathogenic species**				**Non-pathogenic species**	
	Brachyspira aalborgi	***Brachyspira hyodysenteriae***	***Brachyspira intermedia***	***Brachyspira alvinipulli***	***Brachyspira murdochii***	***Brachyspira innocens***
WP_013244459.1	+	+	+	+	+	+
WP_013242998.1	−	+	+	+	+	+
WP_013243940.1	−	+	+	+	+	+
WP_013243193.1	+	+	+	+	+	+
WP_013243655.1	+	+	+	+	+	+
WP_013244081.1	+	+	+	+	+	+
WP_013244750.1	+	+	+	+	+	+
WP_041747714.1	+	+	+	+	+	+
Group B						
WP_041747843.1	+	+	+	+	+	+
WP_013244879.1	+	−	+	−	−	+
WP_013244607.1	+	+	−	+	+	+
WP_015274839.1	+	−	+	−	+	−
WP_013243854.1	−	+	+	+	+	+
WP_013243861.1	−	+	+	+	+	+
WP_013242999.1	+	−	+	+	+	+
WP_013244339.1	+	+	+	+	+	+
WP_013243647.1	+	−	+	+	+	+
WP_013244641.1	+	+	+	+	+	+
WP_041747935.1	+	+	+	+	+	+
WP_013243225.1	+	+	+	+	+	+
WP_013243896.1	−	+	+	−	+	+
WP_014933009.1	−	+	+	−	+	+
WP_014936494.1	+	+	+	+	+	+
WP_013243815.1	+	+	+	+	+	+
WP_013245039.1	+	+	+	+	+	+
WP_013243037.1	+	−	+	+	+	+
WP_013244338.1	+	+	+	+	+	+
WP_013244050.1	+	+	+	+	+	+
WP_013244610.1	+	+	+	+	+	+
WP_228369485.1	+	+	+	+	+	+
WP_013244059.1	+	+	+	+	+	+
WP_041747581.1	−	+	+	+	+	+
WP_041747873.1	+	+	+	+	+	+
WP_187287137.1	−	+	+	+	+	+
WP_181893515.1	+	+	+	+	+	+
WP_013244745.1	−	+	+	+	+	+
WP_013243185.1	+	+	+	+	+	+
WP_013243063.1	+	−	+	+	+	−

Notes: + Indicates the presence of homologues of the given protein across the six *Brachyspira* species taken for comparison; – Indicates the absence of homologues of the given protein across the six *Brachyspira* species taken for comparison.

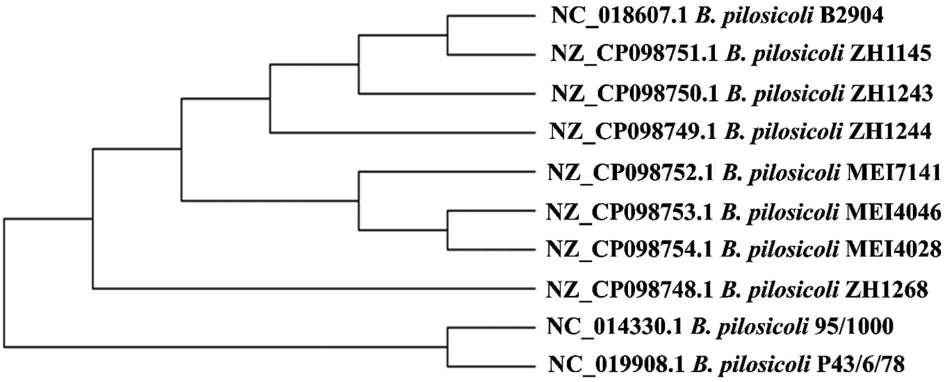

Figure S1. Phylogenetic analysis of *Brachyspira pilosicoli* strains based on 16S ribosomal RNA gene sequence data

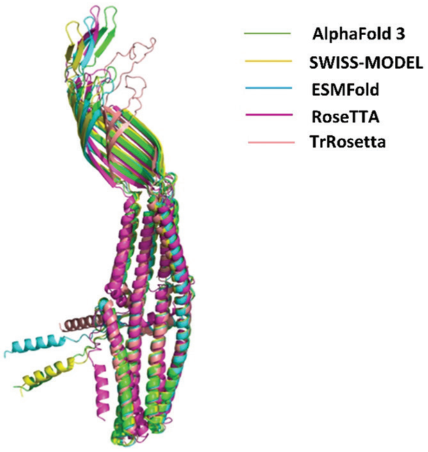

Figure S2. Structural alignment of monomeric BP951000_RS05600 models generated by different prediction tools (AlphaFold 3, ESMFold, RoseTTAFold, SWISS-MODEL, and TrRosetta) using the US-Align server, yielding a root mean square deviation of 3.96Å

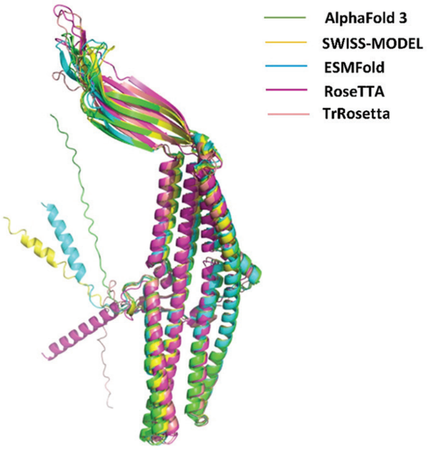

Figure S4. Structural alignment of monomeric BP951000_RS06235 models generated by different prediction tools (AlphaFold 3, ESMFold, RoseTTAFold, SWISS-MODEL, and TrRosetta) using the US-Align server, yielding a root mean square deviation of 3.26Å

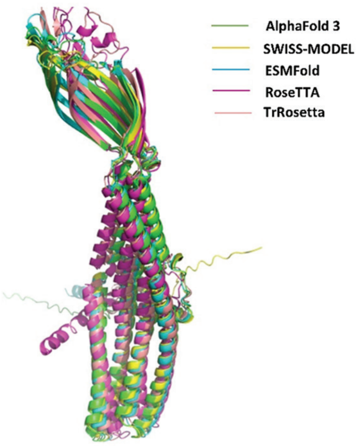

Figure S3. Structural alignment of monomeric BP951000_RS09000 models generated by different prediction tools (AlphaFold 3, ESMFold, RoseTTAFold, SWISS-MODEL, and TrRosetta) using the US-Align server, yielding a root mean square deviation of 3.85Å

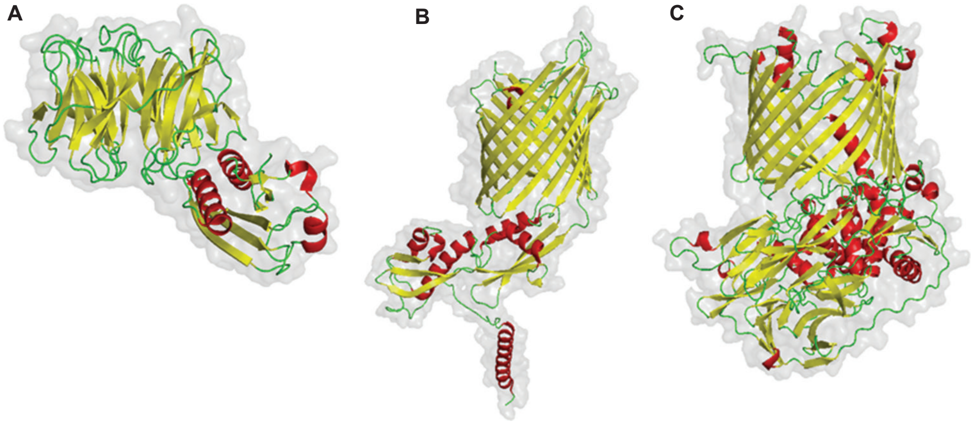

Figure S5. Structural models of (A) TolB protein from *Escherichia coli* K-12, (B) filamentous hemagglutinin transporter protein FhaC from *Bordetella pertussis*, and (C) Trep protein from *Brachyspira pilosicoli*

Figure S6. Structural alignment of BP951000_RS04760, BP951000_RS10215, BP951000_RS04505, and BP951000_RS01090 models using the US-align server, yielding a root mean square deviation of 4 Å. Cyan, green, magenta, and yellow represent BP951000_RS04760 (VspD), BP951000_RS10215 (VspE), BP951000_RS04505 (VspH), and BP951000_RS01090 (VspH, 14-stranded β-barrel protein), respectively.