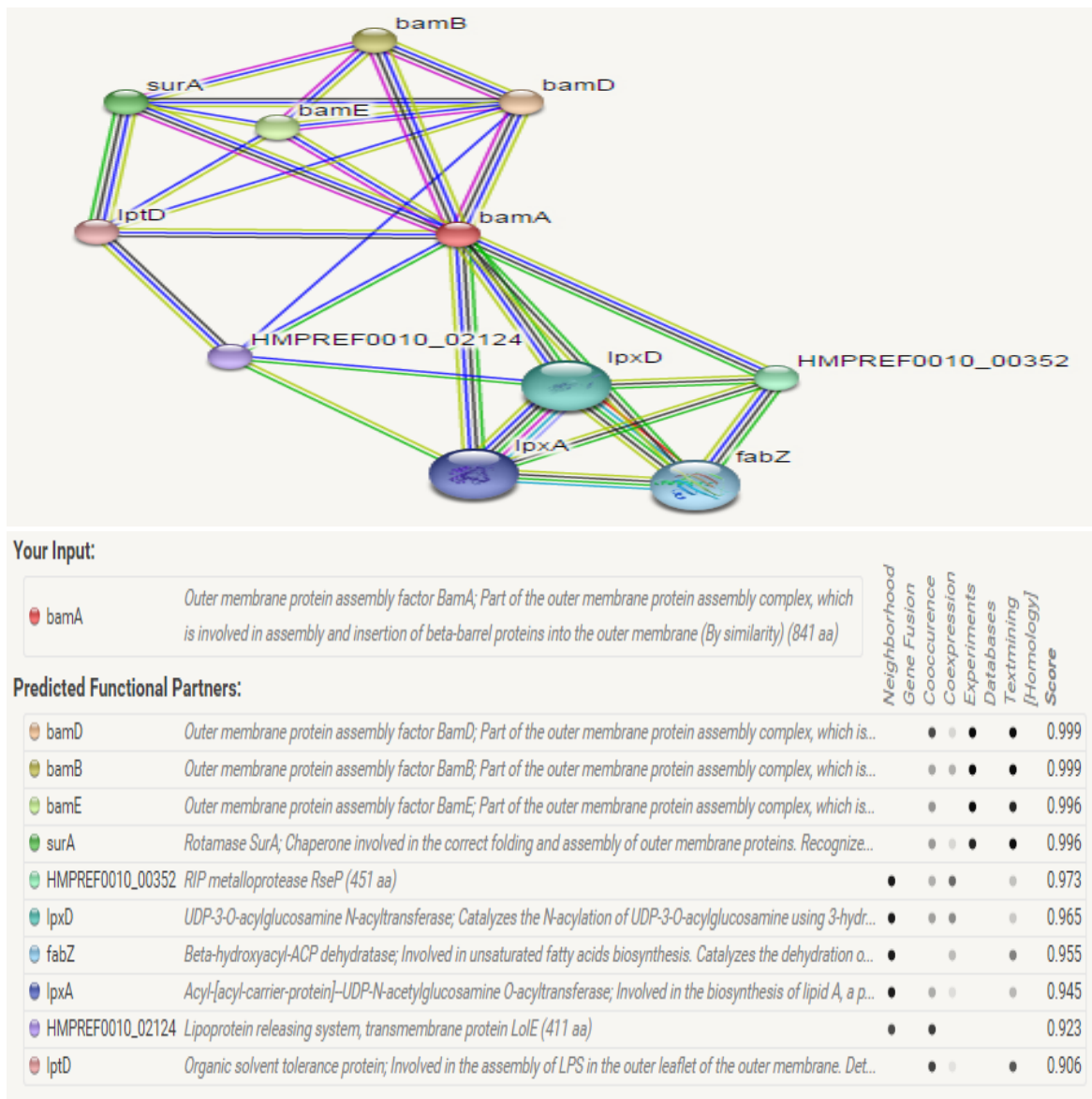


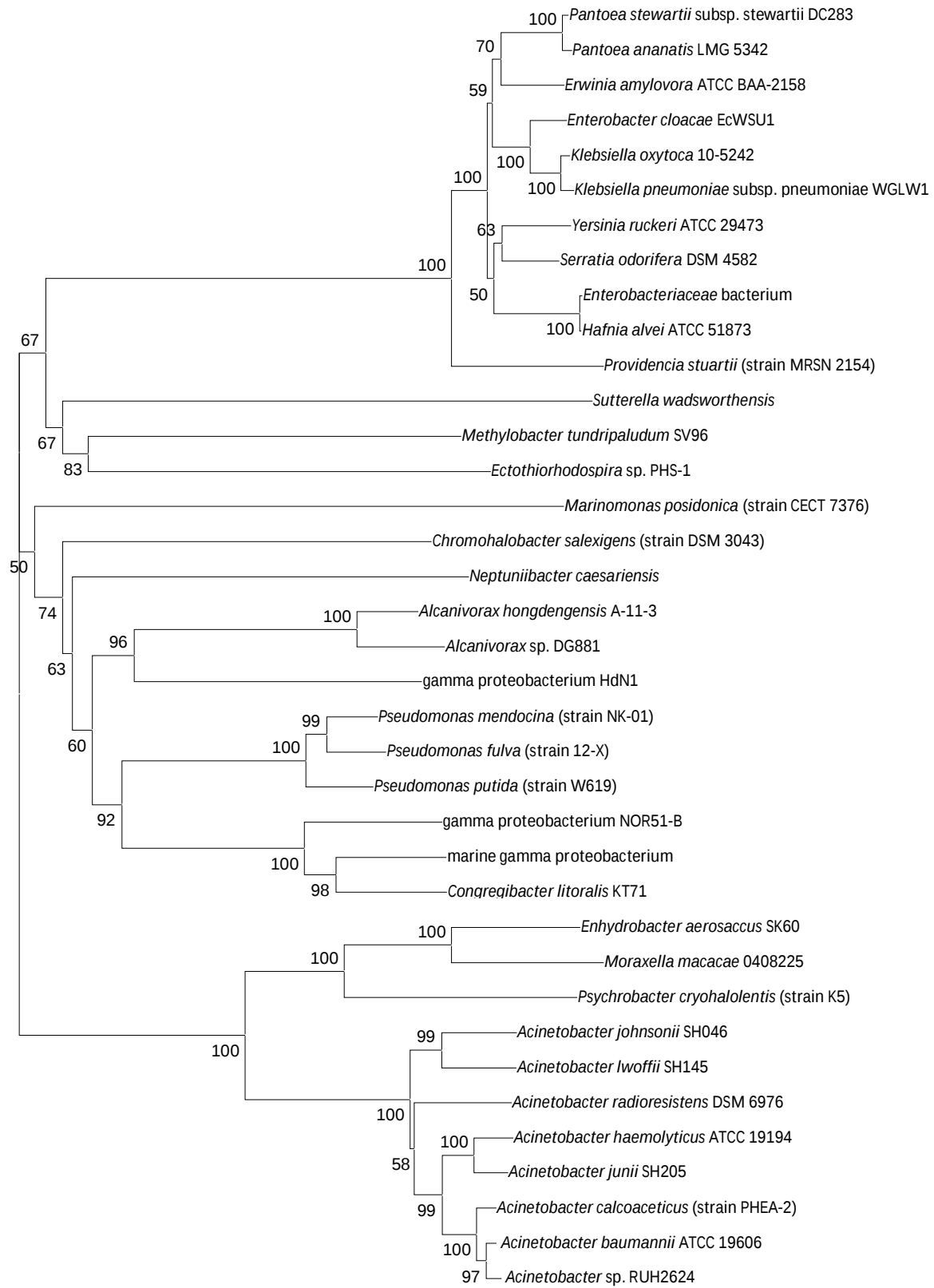
Supplementary data:

Immunoprotective potential of BamA, the outer membrane protein assembly factor, against MDR *Acinetobacter baumannii*

Ravinder Singh¹, Neena Capalash², Prince Sharma^{1*}

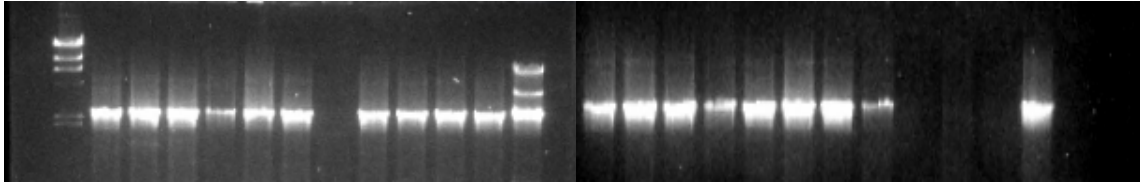


Suppl. Fig. 3: Graphic display of BamA interacting proteins by STRING analysis

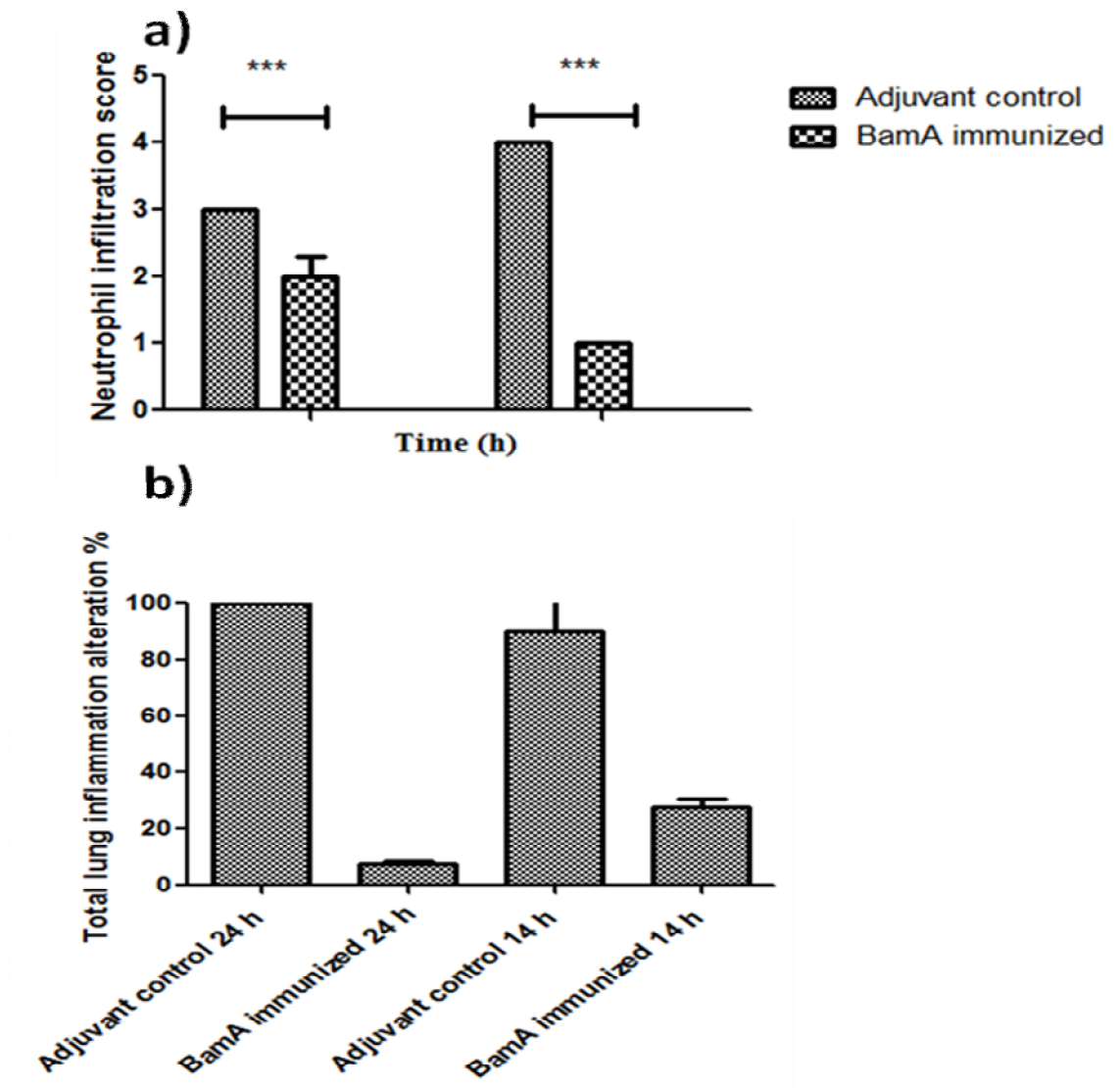


0.1

Suppl. Fig. 4: A phylogenetic tree based on BamA sequences present in various organisms was constructed using MEGA software, version 4.0, based on maximum likelihood.



Suppl. Fig. 5: *bam A* prevalence was checked in clinical isolates by colony PCR. *bamA* was present in 19/20 *A. baumannii* clinical isolates



Suppl. Fig. 6: a) Fig. 5: Lung histopathology (HE, 100X). Groups of female BALB/c mice (n=10) were immunized intraperitoneally with 20 µg BamA formulated with Al(OH)₃ adjuvant on day 1, 14 and 28. The mice were intranasally challenged with 10⁹ CFU of *A. baumannii* clinical isolate P562 at day 45. The mice were sacrificed 12 and 24 h post-challenge and lungs were collected for histopathology. a) Neutrophil infiltration score (B) Total inflammation alteration %age scored in tissue section of murine lungs stained with H&E. The data are pooled from at least two independent experiments (n = 6). Values represent the mean standard error of the mean (SEM) and compared with adjuvant control 24 h (100%). *p < 0.001.**

Suppl. Table 1: Physico-chemical parameters of BamA

S. No.	Parameter	BamA
1.	Gene ID	DOC6H3
2.	Number of aminoacids	841
3.	Molecular weight	93.78 kDa
4.	Theoretical pI	5.21
5.	Total number of negatively charged residues (Asp + Glu)	103
6.	Total number of positively charged residues (Arg + Lys)	88
7.	Sub-cellular localization	Outer membrane
8.	Signal peptide	1-24 aminoacids
9.	Adhesion probability	0.539
10.	Number of trans-membrane helices	0
11.	Similarity with human, mouse and pig proteome	No
12.	Grand average of hydropathicity (GRAVY)	-0.447
13.	Extinction coefficient	93770M ⁻¹ cm ⁻¹

Suppl.Table 2: B cell epitopes predicted by IEDB B cell prediction tool and T cell epitopes of BamA predicted by ProPred and ProPred I.

B cell epitopes with score more than 0.85 are mentioned. All the highest scorer CTL and HTL epitope sequences were selected at a threshold of 4% for ProPred 1 with proteasome filter at 5% & 1% for ProPred. Only epitopes binding to more than 10 MHC alleles are shown.

IEDB			Propred			Propred I		
Amino acid position	B cell epitopes sequence	score	Amino acid position	Predicted CTL epitopes sequence	Number of MHC class II binding alleles	Amino acid position	Predicted HTL epitopes sequence	Number of MHC class I binding alleles
261	EVAVDEGSQFKFGQTK	0.94	7	FLMPLALVS	23	15	AMAAVQQAY	15
435	GYSQSGGITFQAGLSQ	0.93	13	LVSAMAAVQ	30	32	DIRVNLVR	12
759	KCNIDNSVYGNGMKI	0.92	30	VRDIRVNL	17	49	MLPINSGDR	11
574	NGDSQYDTEKGECKVP	0.91	31	LVRLTPANV	49	67	IRTLYATGL	12
563	TSKGTYCPTDANGDSQ	0.91	46	VYTMLPINS	38	100	KLEFKGNKL	15
498	TKLNDDYNVNNYVTDS	0.91	67	IRTLYATGL	14	133	ALQTIETEL	12
486	GVSRGYNVYYRKTCLN	0.9	90	FNVIERP	15	222	KMAASLEAL	14
148	QGRYDADVTVDTVARP	0.9	108	LIPKEALEQ	19	226	LEALRAMYL	11
826	PGDETKEIQFEIGRTF	0.89	118	LKKMGIAEG	17	280	DALYKPEEL	14

747	VLFAEGGQVFDTKCNI	0.89	230	LRAMYLNKG	23	434	VGYSQSGGI	11
464	LSRSETQDYNNLSVTD	0.88	239	YINFNINNS	33	479	DPYFTIDGV	13
22	AYAADDFVVRDIRVNG	0.88	313	LRKYGNAGY	13	522	YPIDENQSL	19
94	ERPIISKLEFKGNKLI	0.87	373	MRQMEGALA	24	600	EFFTYNLNT	11
76	FDDIKASKENDTLVFN	0.87	404	IKPARIPNS	24	607	NLGWSYNL	11
683	GGYGSVRGYDNSTLGP	0.86	494	YYRKTCLND	16	652	FPIGSTGFV	14
313	LRKYGNAGYYFADVNI	0.86	546	YVSTYVRDY	23	697	GPKYPSVNL	13
270	FKFGQTKFLGDALYKP	0.86	550	YVRDYLLAN	18	722	ALVQFGTEL	16
692	DNSTLGPKYPSVNLQE	0.85	662	LRGYGKLG	37	754	QVFDTKCNI	11
543	TGPYVSTYVRDYLLAN	0.85	723	LVQFGTELV	19	814	LSLSYAFPL	16
294	YKDGDTYSQEKVNAVK	0.85	808	ITMIGPLSL	21	833	IQFEIGRTF	12

Supp. Table 3: *A. baumannii* clinical isolates and their antibiotic sensitivity

S. no.	Name	Source	Resistant to	Sensitive to
1.	P-562	Tracheal (ICU)	AMK, TOB, CRO, SAM, IPM	CST/PMX
2.	P-563	Tracheal (ICU)	AMK, CRO, SAM, IPM	TOB, CST/PMX
3.	P-565	Tracheal (ICU)	AMK, TOB, CRO, SAM, IPM	CST/PMX
4.	P-568	Tracheal (ICU)	AMK, CRO, SAM, IPM	TOB, CST/PMX
5.	P-569	Tracheal (ICU)	AMK, TOB, CRO, SAM, IPM	CST/PMX

AMK, Amikacin; TOB, Tobramycin; CRO, Ceftriaxone; SAM, Ampicillin/Sulbactam; IPM, Imipenem; CST, Colistin; PMX, Polymyxin B.

Suppl. Table 4: Vaccine candidate proteins predicted by Vaxign fulfilling the criteria of ideal vaccine candidate localized in outer membrane, with adhesion probability greater than 0.5 and less than 2 trans-membrane helices.

S. No.	UniProt ID	Protein Note	Outer membrane probability	Adhesin Probability	Trans-membrane helices	Protein Length
1	D0CBN6	Carbapenem-associated resistance protein	0.952	0.668	0	249
2	D0CDB7	TonB-dependent vitamin B12 receptor	0.993	0.649	0	616
3	D0CDV8	Outer membrane receptor protein	0.952	0.502	0	772
4	D0C9R5	Type VI secretion system OmpA/MotB	1	0.606	0	217
5	D0CDG4	Outer membrane receptor protein	1	0.519	0	723
6	D0CC45	TonB-dependent receptor	1	0.557	0	924
7	D0CDE3	Porin B	0.993	0.654	1	417
8	D0CE63	Porin	1	0.525	0	414
9	D0CF71	Outer membrane protein	0.952	0.554	0	299
10	D0CAH3	Outer membrane receptor for monomeric catechols	1	0.612	0	743
11	D0C7X6	Outer membrane usher protein mrkC	1	0.631	0	809
12	D0C682	Outer membrane receptor protein	1	0.547	0	745
13	D0C7J7	Ferric aerobactin receptor	1	0.597	0	772
14	D0CFN5	Predicted protein	0.949	0.752	0	209
15	D0CC21	Ferric anguibactin receptor	0.995	0.629	0	766
16	D0C9M4	Competence lipoprotein comL	0.992	0.509	0	385
17	D0CFK2	TonB-dependent receptor protein	1	0.593	0	736
18	D0C803	Putative uncharacterized protein	0.952	0.651	0	443
19	D0C6S0	Porin B	1	0.613	0	439
20	D0C7C2	Outer membrane receptor protein	1	0.628	0	735
21	D0C771	Putative uncharacterized protein	0.949	0.572	0	324
22	D0CB83	Putative uncharacterized protein	0.952	0.645	0	270
23	D0C6Y7	FhaB protein	0.995	0.671	0	1072

S. No.	UniProt ID	Protein Note	Outer membrane probability	Adhesin Probability	Trans-membrane helices	Protein Length
24	D0CDL7	Outer membrane transporter	0.952	0.635	0	476
25	D0CDY8	Outer membrane receptor protein	1	0.505	0	704
26	D0CF50	Porin	0.952	0.599	0	255
27	D0C998	Copper resistance protein B	0.993	0.549	1	272
28	D0C689	TonB-dependent siderophore receptor	1	0.591	0	718
29	D0C7P2	Outer membrane receptor protein	1	0	0	862
30	D0C9N1	Putative uncharacterized protein	0.952	0.571	0	394
31	D0C8G8	Putative uncharacterized protein	0.949	0.56	0	389
32	D0CBI7	Outer membrane efflux family protein	0.952	0.629	1	499
33	D0CEV8	Periplasmic protein	0.993	0.522	0	366
34	D0C6M9	Ferrichrome-iron receptor protein	1	0.556	0	698
35	D0CB85	Porin D	0.993	0.549	0	438
36	D0C912	Autotransporter adhesin	0.995	0.702	1	2258
37	D0CD61	Putative uncharacterized protein	0.949	0.635	0	397
38	D0C7H9	FhuE receptor	1	0.652	0	721
39	D0C9Y2	Putative uncharacterized protein	0.949	0.513	0	448
40	D0C6Y1	Long-chain fatty acid transporter	0.952	0.548	0	457
41	D0C9S5	Small protein A	0.992	0.536	0	132
42	D0CFI7	Nucleoside-binding outer membrane protein	0.993	0.574	0	241
43	D0C8Y7	Lipoprotein	0.993	0.513	0	159
44	D0C985	FilF	0.949	0.678	0	641
45	D0C6H3	β-barrel assembly machine protein BamA	1	0.539	0	841
46	D0C8R6	FhaB protein	0.948	0.684	2	1898
47	D0C982	Pilus assembly protein	0.949	0.673	0	398
48	D0CEI5	Nuclease	0.952	0.533	0	812
49	D0C856	Putative uncharacterized protein	0.952	0.626	0	394
50	D0C5V0	Outer membrane lipoprotein blc	0.977	0.546	0	177

S. No.	UniProt ID	Protein Note	Outer membrane probability	Adhesin Probability	Trans- membrane helices	Protein Length
51	D0CEW2	Peptidyl-prolyl cis-trans isomerase	0.929	0.59	0	188