

SUPPORTING INFORMATION

***Pantoea stewartii* WceF is a glycan biofilm modifying enzyme with a bacteriophage tailspike-like fold**

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Running title: Phage tailspike-like biofilm glycosidase from *P. stewartii*

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 WP_034887639.1/1-737 704 RALPASSVETSGHGFVSVVINGKQVALPYFAIKAS-- 737

Figure S1: Sequence alignment of conserved, WceF-like proteins within *Erwinia* and *Pantoea* species (see also Table S1). Sequences were aligned with Clustal with default settings ("Clustal W and Clustal X version 2.0", Bioinformatics, 23: 2947 (2007)).

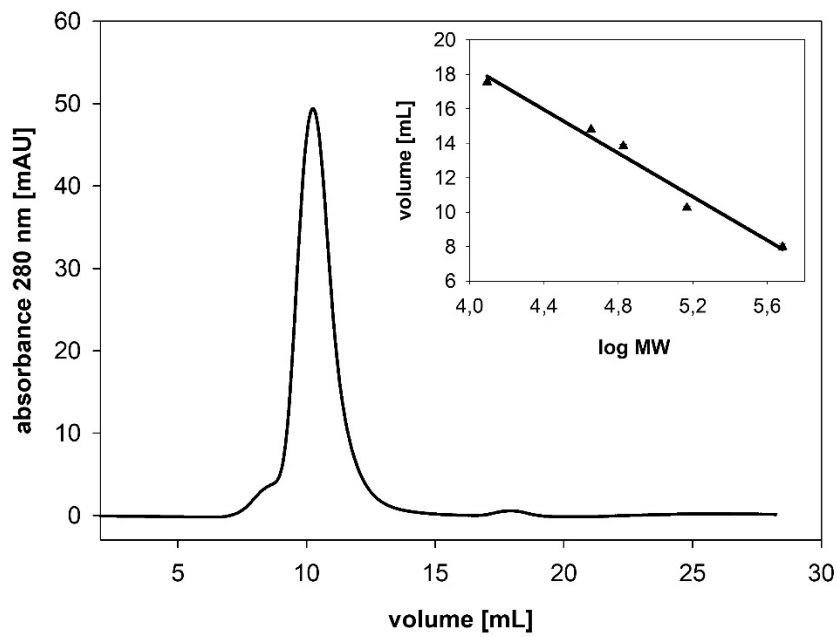


Figure S2: Size exclusion chromatography of native WceF. WceF was analyzed on a Superdex® 200 10/300. The inset shows a calibration curve using ferritin (equine, 480 kDa), aldolase (rabbit, 147 kDa), albumin (bovine, 67 kDa), albumin (chicken, 45 kDa) and cytochrome C (equine, 12.4 kDa). WceF eluted at 10.2 mL, resulting in an apparent molecular weight of 204.2 kDa for the native trimer (a single WceF chain has a calculated MW of 77.7 kDa).

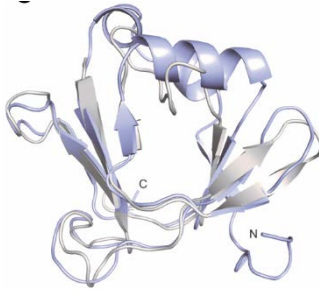


Figure S3: Comparison of WceF head domain with bacteriophage P22 head domain. Superposition of WceF head domain (aa 47-149) in light blue onto the P22 tailspike (PDB: 2XC1) head domain (aa 26-104) in gray, showing a similar overall fold (rmsd 1.27 Å).

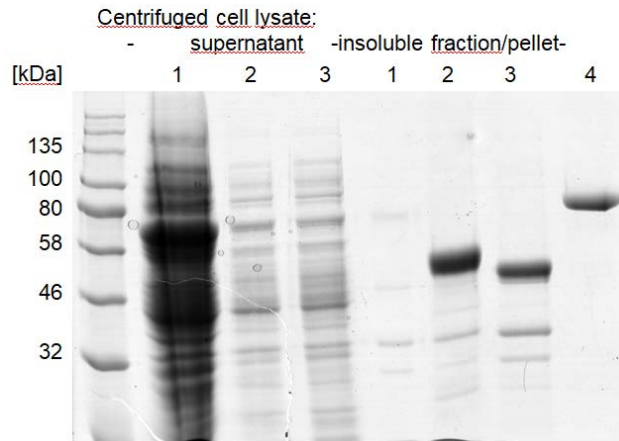


Figure S4: SDS PAGE of soluble and non-soluble fractions after cell lysis of *E. coli* BL21(DE3) expression of N-terminally truncated WceF constructs. (1) WceF lacking the head domain (residues 147-736, 67.3 kDa), (2) the neck domain (residues 230-736, 57.8 kDa) or (3) the β -helix capping domain (residues 266-736, 54.0 kDa). (4) A purified sample of purified, soluble full-length WceF (residues 29-736, 80.3 kDa) is shown for comparison.

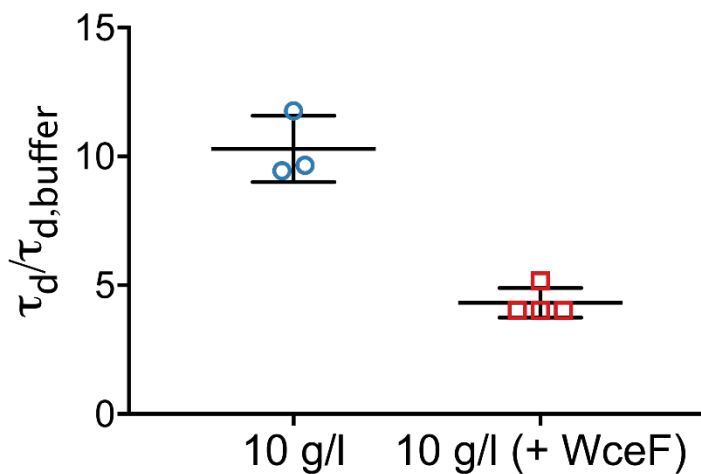


Figure S5: WceF reduces the viscosity of stewartan solutions after 75 h at room temperature. Fluorescence correlation spectroscopy was used to analyze hindrance of 60 nm latex beads ($\tau_d/\tau_{d,buffer}$) in the stewartan matrix (10 g/l) in absence (blue) or presence of WceF (red) (for a description of the method see Dusing, V.; Irmscher, T.; Barbirz, S.; Chiantia, S. Purely Polysaccharide-Based Biofilm Matrix Provides Size-Selective Diffusion Barriers for Nanoparticles and Bacteriophages. *Biomacromolecules* **2019**, *20*, 3842-3854, doi:10.1021/acs.biomac.9b00938.).

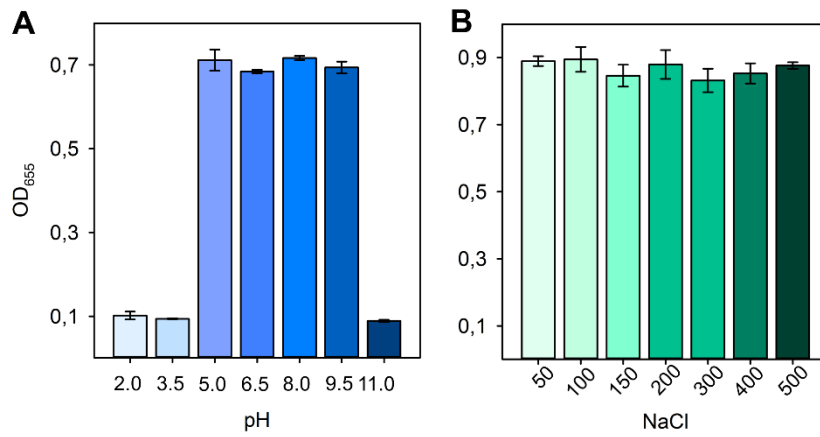


Figure S6: Activity of ϕ Ea1h TSP at varying pH and salt concentrations. ϕ Ea1h TSP (141 nM) was incubated with 1 mg ml⁻¹ stewartan for 72 h at varying pH (A) or salt concentration (B).

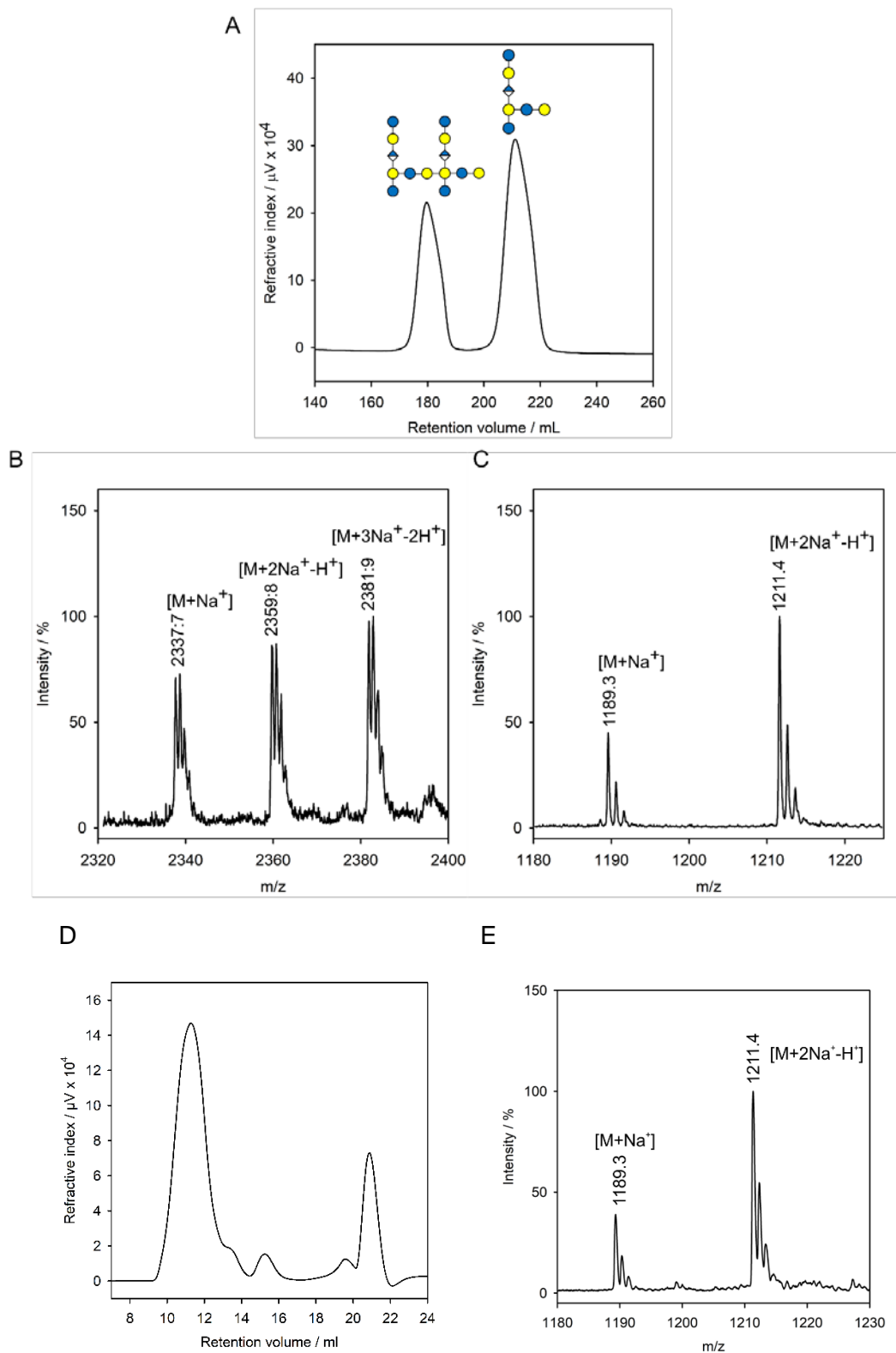


Figure S7: Size exclusion chromatography and MALDI-MS analysis of stewartan exopolysaccharide digests with ΦEa1h TSP or WceF. (A) Size-exclusion chromatography (SEC, Superdex® 30 26/60) after stewartan cleavage by ΦEa1h TSP. (B-C) MALDI-MS spectra of oligosaccharide fragments. Fractions pooled after SEC at ca. 180 ml (B) or ca. 215 ml (C) revealed masses corresponding to two RU (B) or one RU (C), respectively. (D) SEC (Superdex® 30 10/300) of stewartan after 197 h of incubation with WceF. (E) MALDI-MS of WceF-stewartan digest fraction pooled 15 ml corresponding to one RU. Eluent in all SEC runs was 100 mM acetic acid.

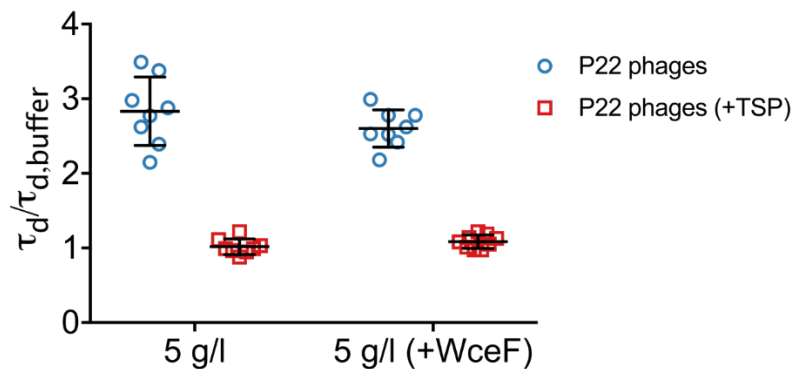
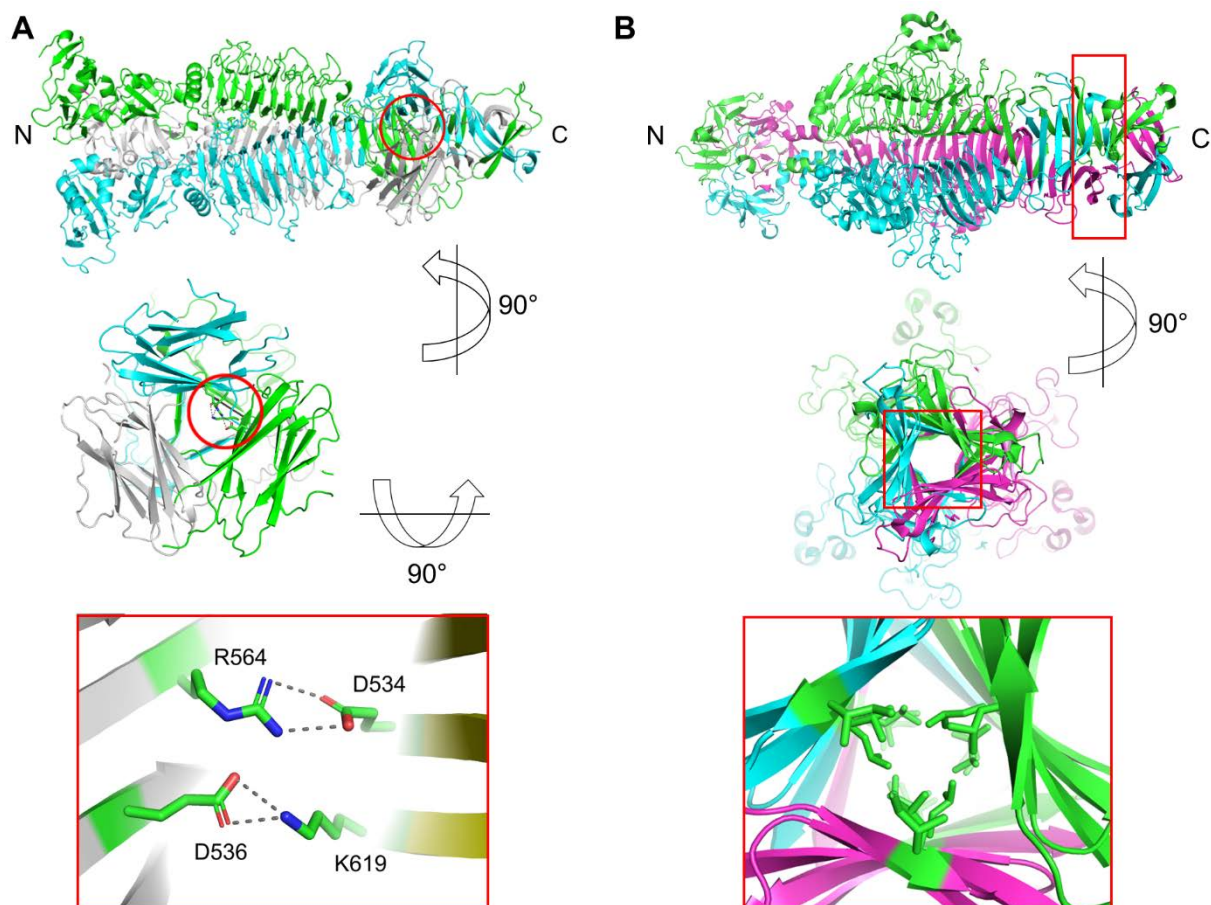


Figure S8: WceF does not hinder access of bacteriophage Φ Ea1h TSP depolymerase to cleavage sites in stewartan. Fluorescence correlation spectroscopy was used to analyze hindrance of particles ($\tau_d / \tau_{d,buffer}$) in the stewartan matrix (5 g/l) (for a description of the method see Dunsing, V.; Irmscher, T.; Barbirz, S.; Chiantia, S. Purely Polysaccharide-Based Biofilm Matrix Provides Size-Selective Diffusion Barriers for Nanoparticles and Bacteriophages. *Biomacromolecules* **2019**, *20*, 3842-3854, doi:10.1021/acs.biomac.9b00938.) Bacteriophage particles (0.2 nM) are hindered by the matrix (blue circles), but addition of the TSP (ϕ Ea1h depolymerase, 141 nM) cleaves stewartan and restores free diffusion of phage particles (red squares). When we did this experiment in the presence of WceF (1.5 μ M), the TSP still could restore free diffusion of the phages.



Protein / residues	Interface area / \AA^2	Calculated interface energy / kcal mol^{-1}
WceF / 34 - 736	5140	-40.5
WceF / 232 - 736	4844	-17.1
P22 / 113 - 666	4607	-35

Figure S9: Details of C-terminal trimer interfaces in WceF and P22TSP and interface area analysis. Stabilization of native trimers of WceF and P22TSP. (A) Salt bridges interconnecting two neighboring C-terminal sandwich domains in the WceF trimer. (B) Hydrophobic core of the β -prism domain in P22TSP.

Table S1: Conservation of WceF within *Erwinia* and *Pantoea* species

BLAST search against database of non-redundant protein sequences with WceF from *Pantoea stewartii* (NCBI accession number WP_006119865.1, 736 aa) as query sequence. For an alignment of the sequences in this table see Figure S1.

Protein/Species	Size / aa	Sequence identity / %	Origin of bacterial isolate	NCBI accession
<i>AmsF / Pantoea ananatis</i>	736	96	Sweet corn, ananas, rice, onion	WP_047714099.1
<i>AmsF / Pantoea allii</i>	736	95	Onion	WP_109717354.1
<i>AmsF / Pantoea agglomerans</i>	738	81	ubiquitous	WP_086906097.1
<i>AmsF / Pantoea vagans</i>	738	80	Eukalyptus	WP_050680143.1
<i>AmsF / Pantoea cypripedii</i>	736	78	Orchid	WP_084875998.1
<i>AmsF / Erwinia mediterraneensis</i>	738	75	Human skin	WP_130830746.1
<i>AmsF / Erwinia gerundensis</i>	740	71	Rosacea (e.g. apples, pears)	WP_067432085.1
<i>AmsF / Erwinia tasmaniensis</i>	740	67		WP_012441069.1
<i>AmsF / Erwinia amylovora</i>	743	61		WP_004162445.1
<i>AmsF / Erwinia typographi</i>	737	54	Gut contents of bark beetles (<i>Coleoptera</i>)	WP_034887639.1

Table S2: X-ray crystallographic data collection and refinement statistics

	WceF (SeMet - phasing)	WceF (native)
Data collection		
Beamline	BESSY 14.1	BESSY 14.2
Wavelength (Å)	0.9798	0.9184
Space group	P2 ₁	P2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	100.7, 97.6, 129.5	100.2, 97.2, 128.9
α , β , γ (°)	90.0, 106.3, 90.0	90.0, 106.4, 90.0
Resolution (Å)*	34.6 – 2.9 (2.98 – 2.90)	48.60 – 2.55 (2.70 – 2.55)
<i>R</i> _{meas} *	23.6 (60.5)	23.1 (128.1)
$\langle I / \sigma(I) \rangle$ *	9.72 (1.89)	6.77 (1.02)
CC1/2*	0.987 (0.668)	0.985 (0.360)
Completeness* (%)*	99.2 (96.7)	96.4 (81.4)
Redundancy	7.0	3.82
Refinement		
Resolution (Å)		2.55
No. unique reflections		74949
<i>R</i> _{work} / <i>R</i> _{free} (%)		22.42 / 26.93
No. atoms		
Protein		15839
Ligand		194
Water		617
Mean <i>B</i> factor (Å ²)		47.4
R.m.s deviations		
Bond lengths (Å)		0.015
Bond angles (°)		1.65
Mol/AU		3

Table S3: WceF structural homolog prediction with HHPred

The WceF protein sequence (UniProt H3REJ8) was subjected to HHPred structural homolog prediction.¹ Search was performed against PDB_mmCIF70_3_Aug on August 22nd, 2019. 25 best hits according to probability are shown.

No	pdb code	Homolog prediction	Probability	E-value	aa covered	Total length of homolog
1	5W6H_C	Bacteriophage CBA120 tailspike protein 4	100.0	2,00E-35	403	(697)
2	6NW9_B	Bacteriophage CBA120 tailspike protein 3	99.9	4.1E-28	371	(633)
3	2XC1_C	Bacteriophage P22 tailspike O-antigen recognition domain	99.9	1.1E-24	528	(666)
4	5W6S_A	Bacteriophage CBA120 tailspike protein 2	99.9	1.2E-24	324	(680)
5	1LKT_F	Bacteriophage P22 tailspike capsid binding domain	99.9	2.7E-25	104	(104)
6	6E1R_E	Acinetobacter phage vB_ApiP_P1, tailspike	99.9	1.7E-22	338	(554)
7	4OJ5_A	Bacteriophage CBA120 tailspike protein 1	99.8	1.1E-21	462	(776)
8	2VNL_A	Bacteriophage P22 tailspike capsid binding domain	99.8	4.8E-23	118	(151)
9	5W5P_A	Acinetobacter phage AM24 tailspike	99.8	7.1E-21	313	(623)
10	6EU4_C	Acinetobacter phage vB_AbaP_AS12 tailspike	99.8	1.5E-20	290	(716)
11	1RMG_A	<i>Aspergillus aculeatus</i> rhamnogalacturonase A	99.5	4.7E-15	262	(422)
12	4C2L_A	<i>Aspergillus tubingensis</i> endoxylogalacturonan hydrolase	99.5	2.5E-14	278	(388)
13	2VBK_A	Bacteriophage Sf6 tailspike	99.4	5.7E-14	325	(514)
14	1CZF_B	<i>Aspergillus niger</i> polygalacturonase II	99.4	8.3E-14	252	(362)
15	4XOT_A	Bacteriophage HK620 tailspike	99.4	3.8E-14	281	(597)
16	2IQ7_A	<i>Colletotrichum lupine</i> endopolygalacturonase	99.4	2.5E-13	239	(339)
17	3JUR_C	<i>Thermotoga maritima</i> Exo-poly-alpha-D-galacturonosidase	99.4	8.1E-13	262	(448)
18	4MXN_B	<i>Parabacteroides merdae</i> pectate lyase	99.3	2.7E-13	219	(247)
19	1H80_A	<i>Alteromonas</i> sp. ATCC43554 iota-carragenase	99.3	4.1E-13	233	(464)
20	2UVF_A	<i>Yersinia enterocolitica</i> exopolygalacturonase	99.3	2.6E-12	324	(608)
21	5OLP_A	<i>Bacteroides thetaiotaomicron</i> galacturonidase	99.3	3.2E-12	300	(452)
22	4RU5_A	Pseudomonas phage phi297 tailspike	99.3	6.2E-12	290	(605)
23	1IB4_B	<i>Aspergillus aculeatus</i> polygalacturonase	99.3	1.4E-12	253	(339)
24	4RU4_F	Pseudomonas phage LKA1 tailspike	99.3	1.3E-11	386	(602)
25	1BHE_A	<i>Chaetomium thermophilum</i> beta-1,3-glucanase	99.2	9.9E-12	295	(376)

1. Zimmermann, L., Stephens, A., Nam, S. Z., Rau, D., Kubler, J., Lozajic, M., Gabler, F., Soding, J., Lupas, A. N., and Alva, V. (2018) A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHPred Server at its Core, *J. Mol. Biol.* 430, 2237-2243.