Supplementary Material for:

Analysis of a keystone enzyme for agar degradation provides insight into unique features of glycoside hydrolysis

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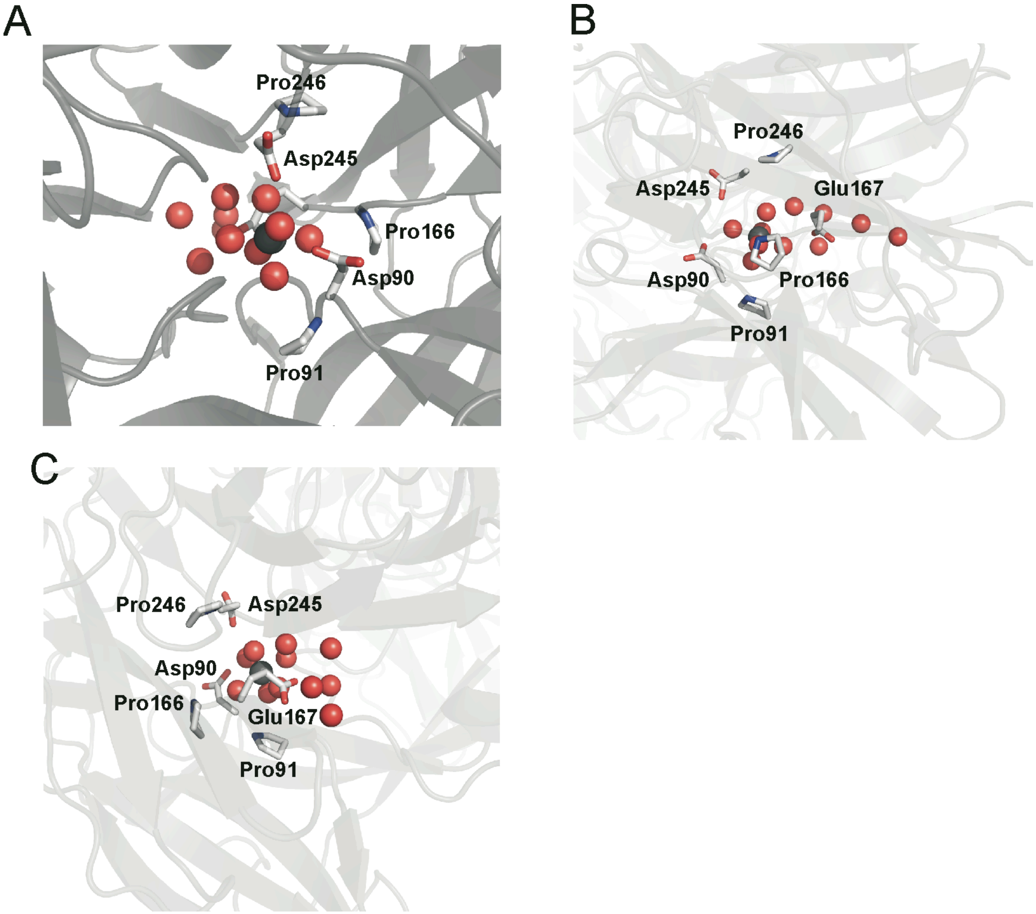
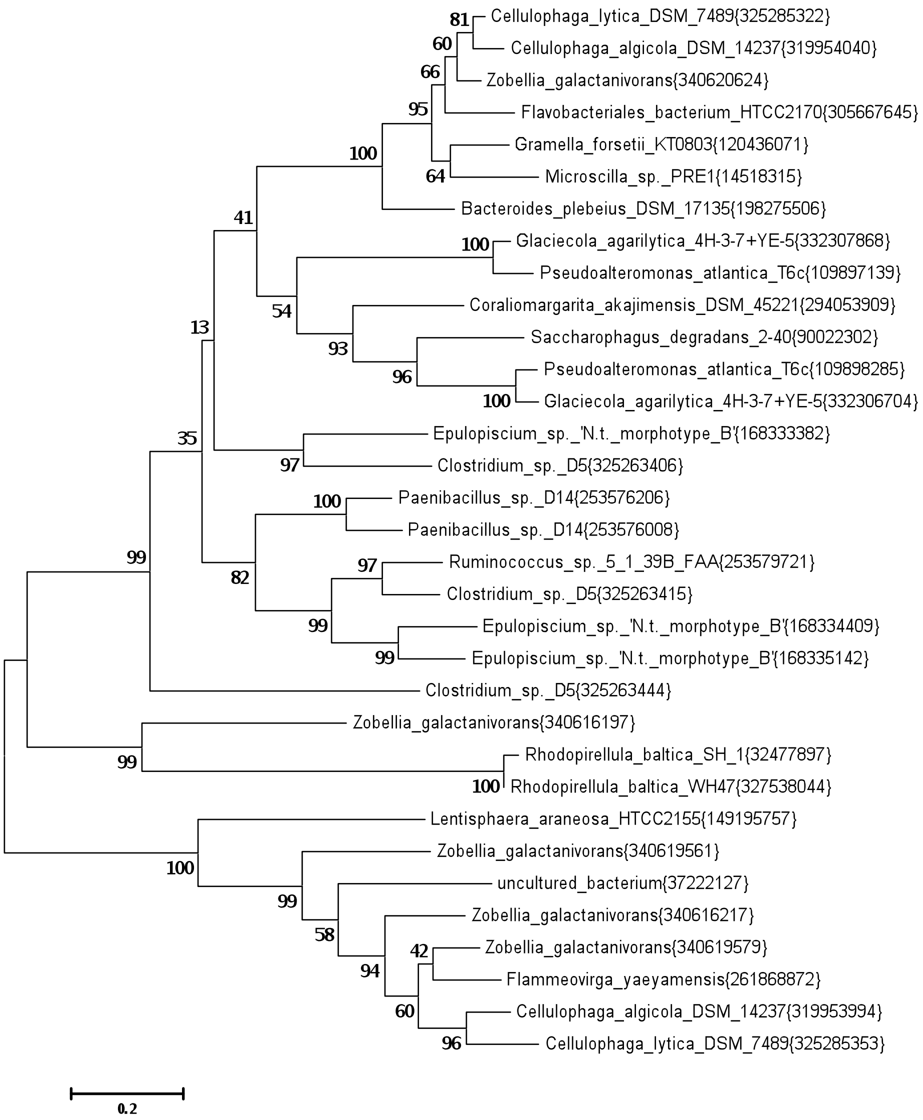
Supplementary Table 1. The occurrence of bacterial GH families that contain α-agarases and β-agarases.

Environment	**Phylum**	**Species**	**α-agarase**		**β-agarase**			**Reported agarolytic activity**
			GH117	**GH96**	**GH86**	**GH50**	**GH16**	
Intestinal	Bacteroidetes	*Bacteriodes xylanisolvens* XB1A	1	-	-	3	1	
Marine	Bacteroidetes	*Cellulophaga algicola* DSM 14237	2	-	1	-	8	John P. Bowman *et al.*(1)
Marine	Bacteroidetes	*Cellulophaga lytica* DSM 7489	2	-	1	-	8	Amrita Pati *et al.*(2)
Marine	Verrucomicrobia	*Coraliomargarita akajimensis* DSM 45221	4	-	16	1	18	
Marine	Bacteroidetes	*Flavobacteriales bacterium* HTCC2170	3	-	-	-	4	
Marine	Proteobacteria	*Glaciecola agarilytica* 4H-3-7+YE-5	2	-	1	4	6	Jeong-Joong Yong *et al.* (2007)(3)
Marine	Bacteroidetes	*Gramella forsetii* KT0803	1	-	-	-	7	Tristan Barbeyron *et al.* (2008)(4)
Freshwater	Bacteroidetes	*Pedobacter saltans* DSM 12145	2	-	-	-	2	
Marine	Planctomycetes	*Planctomyces brasiliensis* DSM 5305	1	-	-	-	-	
Marine	Proteobacteria	*Pseudoalteromonas atlantica* T6c	2	-	3	3	4	Masayo Akagawa-Matsushita *et al.* (1992)(5)
Marine	Planctomycetes	*Rhodopirellula baltica* SH 1	1	-	1	-	2	
Marshland	Proteobacteria	*Saccharophagus degradans* 2-40	1	-	2	2	9	NA Ekbork *et al.* (2005)(6)
-	Bacteroidetes	*Sphingobacterium* sp. 21	1	-	-	-	2	
Soil	Actinobacteria	*Streptomyces coelicolor* A3(2)	1	-	-	1	5	Mark J. Buttner *et al.* (1987)(7)
Marine (seaweed associated)	Bacteroidetes	*Zobellia galactanivorans* DsiJT	6	-	-	-	15	
Intestinal	Bacteroidetes	*Bacteroides plebeius*	1	-	2	n.d.	2	this study



Supplementary Figure 1. *Bp*GH117 contains a metal/water cluster which is located at the base of the active site. The metal and its hydration sphere are kept in place by hydrogen bonds to Asp245 and Asp90 on the active site and by Glu167 at the back. The three prolines, Pro246, Pro166, Pro91, disrupt the secondary element of beta-strands which leads to an extended cavity to hold the metal and its hydration sphere. The hydration sphere of the metal ion is connected with a continuous water channel that leads to the back of the enzyme. A) View from the active site along the axis of the beta propeller. B) View from the side perpendicular to the beta propeller. C) View from the back of the enzyme along the axis of the beta propeller.



Supplementary Figure 2. Phylogenetic anaylsis of GH117 α-agarases. The phylogenetic tree was calculated in MEGA5 (8) with the Maximum Likelihood method (9) using default parameters. The reliability of the tree was tested by bootstrap analysis using 100 resamplings of the dataset (10). The percentage of trees, in which the associated GH117 homologues clustered together, is shown next to the branches. The analysis involved 33 amino acid sequences extracted from genbank. All positions containing gaps and missing data were eliminated resulting in a total of 275 positions in the final dataset.

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