Supplementary material

Crystal structure of the catalytic domain of a GH16 β -agarase from a deep-sea bacterium, *Microbulbifer thermotolerans* JAMB-A94

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Table S1. Comparison of residues involved in the subsites of *MtAgaA*, *ZgAgaA*, and *ZgAgaB*

Subsite	<i>MtAgaA</i>	<i>ZgAgaA</i>	<i>ZgAgaB</i>
-4 and -3	Asn70	Asn71	Asn107
	Trp72	Trp73	Trp109
	Ser144	Glu144	Asp181
	Phe68	Tyr69	Tyr105
-2	Arg177	Arg176	Arg219
	Phe180	Phe179	Phe217
	Trp138	Trp138	Trp175
-1	Asp136	Ala136	Asp173
	His173	His172	His215
	Glu257	Glu254	Glu308
	Glu147	Glu147	Glu184
+1	Glu152	Glu152	Glu189
	Tyr154	Tyr154	Tyr191
	Gln259	Gln256	Gln310
+2	His169	His168	His211
	Gln184	Gln183	Gln226
	Trp261	Trp259	Trp312
+3	Trp191	Trp190	Trp233
+4	Trp163	Trp162	Tyr205

Table S2. Features of arginine and proline residues of *MtAgaA*

Residue	Exposed surface area (%) ^a	Exposed/Buried ^b	Corresponding residue in ZgAgaA	Corresponding residue in ZgAgaB	β-turn type	Rank
Arginine						
Arg59	43	Half-exposed	Ala60	Glu96		Variable
Arg63	22	Half-exposed	Lys64	Lys100		Variable
Arg98	25	Half-exposed	Gln98	Arg135		
Arg125	31	Half-exposed	Lys125	Arg162		
Arg158	23	Half-exposed	Asp157	Tyr195		Variable
Arg167	25	Half-exposed	Arg166	Lys209		
Arg177	47	Half-exposed	Arg176	Arg219		Conserved
Arg201	14	Half-buried	Arg199	Asn241		
Arg206	13	Half-buried	Arg204	Arg246		Conserved
Arg212	33	Half-exposed	Arg210	Arg252		Conserved
Arg227	11	Half-buried	Arg225	Arg267		Conserved
Arg262	3	Buried	Arg260	Arg313		Conserved
Arg279	24	Half-exposed	Lys273	Asn337		Variable
Arg288	1	Buried	Arg282	Arg346		Conserved
Proline						
Pro27	63	Exposed	Pro27	Pro64		Conserved
Pro29	28	Half-exposed	Pro29	Pro66		Conserved
Pro32	11	Half-buried	Pro32	Ala69		
Pro42	64	Exposed	Asp42	Glu79	Type I <i>i</i> +1	Variable
Pro52	58	Exposed	Ser53	Pro89		
Pro71	86	Exposed	Gly72	Ala108		Variable
Pro75	13	Half-buried	Pro76	Pro112		Conserved
Pro82	37	Half-exposed	Ala83	Arg119		Variable
Pro100	73	Exposed	Ala100	Pro137	Type II <i>i</i> +1	
Pro115	35	Half-exposed	Lys115	Arg152		Variable
Pro119	20	Half-exposed	Pro119	Pro156		Conserved
Pro159	84	Exposed	Arg158	Ser196	Type II <i>i</i> +1	Variable
Pro179	73	Exposed	Pro178	Pro221		Conserved
Pro185	3	Buried	Pro184	Pro227		Conserved
Pro214	11	Half-buried	Pro212	Pro254	Type I <i>i</i> +1	Conserved
Pro237	44	Half-exposed	Pro234	Pro277	Type I <i>i</i> +1	Conserved
Pro249	42	Half-exposed	Ala246	Glu300		Variable
Pro269	3	Buried	Ala263	Pro327		
Pro277	60	Exposed	Asp271	Ile335	Type II <i>i</i> +1	Variable
Pro292	11	Half-buried	Pro286	Pro350		Conserved
Pro294	65	Exposed	Ala288	Glu352		Variable

^a Calculated using Areaimol from CCP4^b Exposed surface area: more than 50%, exposed; 49-20%, half-exposed; 19-10%, half-buried; less than 10%, buried.

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MtAgaA CBM6      314 LGNFHNTGKDGANVTGDTVLGFNKNG--NNINYN TKGDWADYTVNLPAAGE
SdAgaB CBM6-1    321 MANFSATGKEGSAVAGDTFTGFNPSGANNINYN TLGDWADYTVNFPAAGN
SdAgaB CBM6-2    469 AENFNAVGGTFSDGQAQPVSVYTVNGNTAINYN VNQGDYADYTIAVAQAGN
      **  .*  :  .:  .  :.  .*  .  ***  .  **:****:  .  **:

MtAgaA CBM6      363 YRVDLVIASPMSSGLGAELTFA--GNAAKTVTLSENTGGWESYQTFTLPQT
SdAgaB CBM6-1    371 YTVNLIAASPVTSGLGADILVD--SSYAGTIPVSSTGAW EYNTFSLPSS
SdAgaB CBM6-2    519 YTISYQAGSGVTGGSIEFLVNENGSWASKTVTAVPNQG WDNFQPLNG--G
      *  :.  .*  :.*  :  .  :  *:  .  .*:  ::  :.

MtAgaA_CB M6      411 ISVSSPGNYNFRLKSTGSSNWQWNGDEIRFVK 432
SdAgaB_CB M6-1    419 IYIASAGNHTIRVQSSGGS AWQWNGDELRF TQ 450
SdAgaB_CB M6-2    567 SVYLSAGTHQVRLHGAGSNNWQWNL DKFTLSN 598
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Fig. S1. Sequence alignment of CBM6 domain of *MtAgaA* and two CBM6 domains, CBM6-1 and CBM6-2, of *SdAgaB*.

The amino acid residues in *SdAgaB* CBM6-2 that interact with the ligand are shown with a black background. The alignments were performed using the Clustal Omega server (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).