

Figure S2

AAC	--MELVWQHRWAADAYPLDLSTMVIVLRVARCTPAQRVVRVHFVGDREPDFQ-----	49
LPL	-MQLAGIRHRPESED TAVLAAHQVRLQRTARD-DVAQVEILFADSYLWQEGTTA-----	53
KOX	MITISSLSHSAKSADSYAYNKETLHIRFRFTVKG-EVAKVSLWIGDPYQWEEGGLDGGNLG	59
TVU	-MLLEAIFHEAKGSYAYPISETQLRVRLRAKKG-DVVRCEVLYADRYASPEEE-----	51
AFL	-MLKEAVYHRPTDQFAYAYDDKTLHIRLRLTKKS-DVHAVSLLHGDPYCWEDRQWQ-----	53
GCA	-MFKEAIYHRPKDNFAYAYDEKTLHIRLRLTKKD-DVEAVHLLYADPYEWKDGWVQ-----	53
TSP	-MRKEAIHHRSTDNFAYAYDSETLHLRLQTKKN-DVDHVELLFGDPYEWHDGAWQ-----	53
BST	-MRKEAIYHRPADNFAYAYDSETLHLRLRLTKKD-DIDRVELLHGDPYDWQNGAWQ-----	53
BSP	-MFLEAVYHRPRKNWSYAYNGTTVHLRIRTKKD-DMTAVYALAGDKYMW DHT-----	50
LSP	MIMLEAVYHRMGQNSYAYNDSTLHIRIRTKRD-NVPRIDLHCGEKYDPEKY-----	51
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AAC	-----NSSEARRYSGDGTDFWFTCRVAAPTRRLKYAFVLSSTGRAVYLGEGGLA	99
LPL	-----LERRIMQPLATQGNQYQTTLLVPTNRVVAFLITDTIGATVGYGEGGFF	104
KOX	GSDAHGWSGGNEVHMEKEGQSESYDHWFAAFTPKRRSRYGFILYGNNGEKLLFGERRCV	119
TVU	-----LAHALAGKAGSDFDYFEALLECSTKRKVKYVFLLTGPOGEAVYFGETGFS	102
AFL	-----FTKTEMTKSGDALFDYWWVAISPPYRRLRYGFELVSGN-ERC VYTEKGGF	103
GCA	-----LNRAPMVKSGSDDLFDYWFIEVIPPYRRLRYGFELTSGN-EKIVYTEKGFY	103
TSP	-----FQTMPMRKTGSDGLFDYWLAEVKPPYRRLRYGFVLRAGG-EKLVYTEKGFY	103
BST	-----FQMMPMRKTGSDELFDYWFAEVKPPYRRLRYGFVLYSGE-EKLVYTEKGFY	103
BSP	-----MEYVPMTKLATDELFDYWECEVTPPYRRVKYGFLLQQGH-EKRWMT EYDFL	100
LSP	-----KETIPMERMASDGLFDYWQAAVQPRYRRLVYVFALHSDNGDAVYFMEKGGF	102
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AAC	DTL-----DAVQPFQYPIIHSRVLAVPDVWGHAVAYQIFPDRFAVGEQQLV-RP	148
LPL	DDV-----ATNWQTVGNVYFHMPLYMHVSDAELPPAVWKETVWYQIFPERFANGVRQNDPAN	159
KOX	DISKPPVAETELSNLSNFFCFPIINPGDVLSTPEWVKKTIWYQIFPERFCNGDPSISPEN	179
TVU	A-----ERS---KAGVFQYAYIHRSEVFTTPEWAKEAVIYQIFPERFANGDPSLNPPG	152
AFL	H-----EAPIDDIAYYFCFPFLNRIDVFHAPTWVKDTVWYQIFPERFANGDPSLNPKG	156
GCA	H-----EAPTDDTAYYFCFPFLNKIDVFHAPTEWVKDTVWYQIFPERFANGNPAIDPEG	156
TSP	H-----EAPSDDTAYYFCFPFLHRVDLFEAPDWVKDTVWYQIFPERFANGNPAISPKG	156
BST	F-----EVPTDDTAYYFCFPFLHRVDLFEAPDWVKDTVWYQIFPERFANGNPSISPEG	156
BSP	T-----EPPR-NPDRLEFYFPINPVDVFPQPAWVKDAIFYQIFPERFANGDTRNDPEG	152
LSP	D-----QPPKVMYEGLFDFPYLNRQDVHTPPAWVKEAIFYQIFPERFANGDPSNDPEG	155
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	CSR VI	
	β2	
AAC	TDPWDAR--PTPDSVFGGNLRGIVDKLPYLSDLGVNLMYLTPIFQAPSNEKMDTQDYFAV	206
LPL	VQPWGAG-RVHRDSFYGGDLPGITAHLLDAA LGVNGLYLCPIFTSPSNEKMDTIDHFEI	218
KOX	VQPWGTP--PDSKNFMGGDLQGIINKLDYLQDLGVNGLYLCPIFTANASNEKMDTVDYFNV	237
TVU	TEQWAKDARPRHDSFYGGDLKGVIDRLPYLEE LGVTALYFTPIFASPSNEKMDTADYLA I	212
AFL	TIPWGSA-DPTPTSFFGGDFAGIMERLDYLVELGINGIYFTPIFKASSNEKMDTIDYFEI	215
GCA	TLPWASE-DPTPTSFFGGDLEGI IQHLDYLVDLGVNGIYLTPIFHAPSNEKMDTTNYFEI	215
TSP	ARPWGSE-DPTPTSFFGGDLQGIIDHLDYLADLGITGIYLTPIFRAPSNEKMDTADYFEI	215
BST	SRPWGSE-DPTPTSFFGGDLQGIIDHLDYLVDLGITGIYLTPIFRSPSNEKMDTADYFEV	215
BSP	TLPWGSA-DPTPSCFFGGDLQGVIDHLDHLSK LGVNAVYFTPIFKATTNEKMDT EYFQI	211
LSP	VQEWGGT--PSAGNFFGGDLQGVIDHLDYLSDLGVNALYFNPLFAATTNEKMDTADYMKI	213
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	CSR I	
	β3	
AAC	DPAFGTLGDLQLLVREAHRLGIRVVLDVAVFNHSGFQFAPFQDVIARGTASPYWSWFFVQG	266
LPL	DPHFGTKADFQALVDGAHARGMRVMLDAVFNHFGEQSPQWQDVIKNGEQSRFADWFH IHG	278
KOX	DPHFGGNDRFKELVQKAHQRGMKVMLDAVFNHIGNQSPWLVDVVKNGDKSPYADWFWIKK	297
TVU	DPQFGDLPTFRRLVDEAHRRGIKIILDVAVFNHAGDQFFAFRDV LQKGEQSRYKDWFFIED	272
AFL	DPQFGDKPTFKRLVERCHQKIRVMLDAVFNHSGHFFAPFQDVLKHGEKSKYKDWFWH IHE	275
GCA	DPHFQDKQTFKKLVDLCEKGI R VMLDAVFNHCGYYFAPFQDVLKNGEASKYKDWFWH IHE	275
TSP	DPHFQDKETLKT LVKRCHEKGI R VMLDAVFNHCGYEFAPFQDVLKNGAASRYKDWFWH IRE	275
BST	DPHFQDKETLKTLDRCHEKGI R VMLDAVFNHCGYEFAPFQDVWKNGESSKYKDWFWH IHE	275

GCA	RRGKIITFMDASDES NHLIYTKTYLDETILVM--INNSSKEIDITVPLS-----LKG-	552
TSP	RRGDVAFLTADDEVNHLVYAKTDGNETVMI I--INRSNEAAEIPMPID-----ARG-	552
BST	RRGEISFLHADDEMNYLIYKKTGDGETVLVI--INRSDQKADIPIPLD-----ARG-	552
BSP	RTGTFKFLTAEKNSRQIAYLREDDQDTILVV--MNNDKAGHTLRCLSG-----M---	547
LSP	RGSGLRFLPVLEHPQLLVYERWDDNERFLIM--LNNEDAPVNVVIPAA-----QPG-	551
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AAC	RKILIWEC DSTEGCGHDS-----RADGFHNQ-----	578
LPL	-----GVSPILTAGITANEMAPGGFELTVA-----	574
KOX	-----RTLSLTPFGFYIIGLDHKDIIS	598
TVU	---KTWLDCLTGEEVHGKQGQLKLTLPYQGMILWNGR----	585
AFL	---KKLTDLWTKQFVSEENEWTVTLAPYGFMLFIVE-----	586
GCA	---KWLVLNLTNEQFAAEAETLRAAIPYS-----	579
TSP	---KWLVNLLTGERFAAEAETLCVSLPPYGFVLYAVESW----	588
BST	---TWLVNLLTGERFAAEAETLCTSLPPYGFVLYAIEHW----	588
BSP	-----HSGPICGTTMS-----	558
LSP	--ASWRVTNNGEPCAVVEESSIQAALPPYGYAILHAPIAGTAE	591

Fig. S2. Sequence alignment of the α -amylase family GH13_20 representatives from *Alicyclobacillus acidocaldarius* (AAC) (accession Q9WX32), *Thermoactinomyces vulgaris* (TVU) (Q08751), *Klebsiella oxytoca* (KOX) (Q48398), *Bacillus* sp. I-5 (BSP) (Q59226), *Lysinibacillus sphaericus* (LSP) (Q08341), *Anoxybacillus flavithermus* (AFL) (Q5BLZ6), *Geobacillus caldoxylosilyticus* (GCA) (CoLZ63), *Thermus* sp. IM6501 (O69007), *Bacillus stearothermophilus* (BST) (P38940), and *Lactobacillus plantarum* WCFS1 (LPL) (F9URM8). Alignment was done using the program ClustalOmega. Residues that are identical (*), conserved (:), or semiconserved (.) in all sequences are indicated. Dashes indicated gaps introduced to maximize similarities. The GH13 three domains are highlighted in blue (domain A), green (domain B), and yellow (domain C). The N-terminal domains are indicated in dark blue. The seven conserved sequence regions (CSR) found in GH13_20 subfamily α -amylases are also indicated. The residues of the GH13 catalytic triad (Asp-171, Glu-200, and Asp-227 in Lp_0179) are highlighted in pink colour. Conserved residues in amylolytic enzymes are highlighted in red (Lee et al., 2002). The conserved VAnE and MPKLn motifs, in the CSR II and V respectively, are indicated in grey colour.