

Three Acidic Residues Are at the Active Site of a β -Propeller Architecture in Glycoside Hydrolase Families 32, 43, 62, and 68

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ABSTRACT Multiple-sequence alignment of glycoside hydrolase (GH) families 32, 43, 62, and 68 revealed three conserved blocks, each containing an acidic residue at an equivalent position in all the enzymes. A detailed analysis of the site-directed mutations so far performed on invertases (GH32), arabinanases (GH43), and bacterial fructosyltransferases (GH68) indicated a direct implication of the conserved residues Asp/Glu (block I), Asp (block II), and Glu (block III) in substrate binding and hydrolysis. These residues are close in space in the 5-bladed β -propeller fold determined for *Cellvibrio japonicus* α -L-arabinanase Arb43A [Nurizzo et al., Nat Struct Biol 2002;9:665–668] and *Bacillus subtilis* endo-1,5- α -L-arabinanase. A sequence–structure compatibility search using 3D-PSSM, mGenTHREADER, INBGU, and SAM-T02 programs predicted indistinctly the 5-bladed β -propeller fold of Arb43A and the 6-bladed β -propeller fold of sialidase/neuraminidase (GH33, GH34, and GH83) as the most reliable topologies for GH families 32, 62, and 68. We conclude that the identified acidic residues are located at the active site of a β -propeller architecture in GH32, GH43, GH62, and GH68, operating with a canonical reaction mechanism of either inversion (GH43 and likely GH62) or retention (GH32 and GH68) of the anomeric configuration. Also, we propose that the β -propeller architecture accommodates distinct binding sites for the acceptor saccharide in glycosyl transfer reaction. Proteins 2004;54:424–432.

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INTRODUCTION

Glycoside hydrolases (GHs) have been classified into 87 families according to the similarity of their amino acid sequences, which imply both structural and mechanistic relationships.^{1,2} Some GH families are grouped at a higher hierarchical level described as clans.² Family GH32 comprises sucrose-6-phosphate hydrolases, invertases, inulinases, levanases, eukaryotic fructosyltransferases, and bacterial fructanotransferases; family GH43 includes β -xylosidases, β -xylanases, α -L-arabinases, and α -L-arabino-

furanosidases; family GH62 groups some α -L-arabinofuranosidases; and family GH68 includes the majority of bacterial fructosyltransferases and two invertases. Thus, each of these families includes enzymes with a glycofuranosidase activity. Families GH32 and GH68 are combined into the clan GH-J, whereas GH43 and GH62 compose the clan GH-F^{3–5} (for updates see the CAZy server at <http://afmb.cnrs-mrs.fr/cazy/families.html>). On the basis of sequence similarity, these four families compose the furanosidase superfamily,⁵ which also includes a family of enzymatically uncharacterized proteins, known as GHLP⁵ or COG2152 (<http://www.ncbi.nlm.nih.gov/cog/>). For the discussion that follows, we refer to the GH families 32, 43, 62, and 68. It should be stressed that not all furanosidases belong to these families. For example, inulin fructotransferase III [IFTaseIII, Enzyme Commission (EC) 2.4.1.93] of *Arthrobacter globiformis* is included into GH91, and its crystal structure revealed a right-handed β -helix fold.⁶ Furthermore, α -L-arabinofuranosidase from *Geobacillus stearothermophilus* (AbfA T-6, EC 3.2.1.55) belongs to GH51 and has a catalytic domain with a $(\beta/\alpha)_8$ -barrel fold.⁷

Enzymatic hydrolysis of the glycosidic bond occurs via a general acid catalysis that requires at least two critical residues, a proton donor and a nucleophilic base, and involves two major mechanisms giving rise to either an overall retention or inversion of anomeric configuration.^{8–10} The proton donor is positioned within hydrogen-bond distance of the glycosidic oxygen in both the retaining (e.g., GH32 and GH68) and the inverting (GH43) enzymes, but the nucleophilic base is more distant than the anomeric carbon in the inverting enzymes that need to accommodate a water molecule between the nucleophilic residue and the sugar. This difference results in an average distance between the two catalytic residues of ~ 4.5 to 5.5 Å in retaining enzymes as opposed to ~ 9.0 to 9.5 Å in inverting enzymes.^{9,10}

Site-directed mutations on yeast and plant invertases, two bacterial α -L-arabinanases, and various bacterial fruc-

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tosyltransferases have revealed a direct implication of Asp and Glu residues in substrate hydrolysis.^{11–20} The representative GH43 crystal structures of *Cellvibrio japonicus* α -L-arabinanase Arb43A and *Bacillus subtilis* endo-1,5- α -L-arabinanase show a 5-bladed β -propeller fold with 3 invariant carboxylates close in space at the catalytic center.^{14,15} The 6-bladed β -propeller fold of sialidases and neuraminidases is the other example of a β -propeller architecture representative of GH families 33, 34, and 83 (clan GH-E).²¹ Crystal structures at high resolution have not been elucidated for members of the GH families 32, 62, and 68. Previous work on the development of methods for the detection of catalytic residues using homology were proposed by Casari et al.,²² Fetrow and Skolnick,²³ and Aloy et al.²⁴

In this article, we have aligned 160 protein sequences of GH families 32, 43, 62, and 68 to identify conserved blocks containing acidic residues. The integrative analysis of our sequence alignments, the sequence–structure compatibility search, and the previous site-directed mutagenesis studies allow us to propose the existence of three key acidic residues located at the active site in a β -propeller architecture for GH families 32, 43, 62, and 68. The three strictly conserved acidic residues operate with the canonical reaction mechanism of inversion (GH43) or retention (GH32 and GH68) of anomeric configurations.

MATERIALS AND METHODS

Protein sequences were retrieved from the SwissProt/TREMBL database. The MACAW program version 2.0.5 Win32i²⁵ and CLUSTALW service (<http://www.ebi.ac.uk/clustalw>) were used for multiple-sequence alignments. Sequences of the GHLP family were not analyzed, since they are homologous only to the C-terminal part of the other sequences of the furanosidase superfamily.⁵ The MACAW program with a maximum of 30 proteins selected from GH32 (8 sequences), GH43 (8 sequences), GH62 (4 sequences), and GH68 (10 sequences) was used to identify the conserved regions. Block I was revealed by aligning the first 150 amino acids of the selected proteins; blocks II and III were identified using the complete amino acid sequences. The probability of obtaining the observed level of similarity by chance—*P* value—was 10^{-19} or lower (search space $N = 5.457e + 055$ for block I, and $N = 1.539e + 080$ for blocks II and III). Blocks I, II, and III of the total 160 sequences were extracted from CLUSTALW alignments for each GH sequence. The fold-recognition methods 3D-PSSM (<http://www.sbg.bio.ic.ac.uk/servers/3dpssm/>), INBGU (<http://www.cs.bgu.ac.il/~bioinbgui/query.html>), mGenTHREADER (<http://bioinf.cs.ucl.ac.uk/psiform.html>), and SAM-T02 (<http://www.soe.ucsc.edu/research/compbio/hmm-apps/t02-query.html>) were used to study structural compatibility between β -propeller architectures and the amino acid sequences of GH families 32, 62, and 68. The structural comparison method CE (<http://cl.sdsc.edu>) was used for superposition of β -propeller crystal structures. The calculation of the distance between the catalytic residues in the crystal structure of Arb43A was done using the WHAT IF program.²⁶ The complete list of fold-

recognition results, structural comparisons, and other additional information is available at <http://bio.cigb.edu.cu/~pons/>.

RESULTS AND DISCUSSION

Three Key Acidic Residues Are Conserved in GH Families 32, 43, 62, and 68

The comparison of 160 protein sequences of GH families 32, 43, 62, and 68 using MACAW and CLUSTALW revealed three equivalent blocks containing acidic residues in all the enzymes (Fig. 1). Previous site-directed mutagenesis studies have revealed a functional role for these residues in members of the families GH32, GH43, and GH68.^{11–20} To our knowledge, the catalytic residues of GH62 enzymes have not been identified.

The conserved residues Asp42 (block I) and Glu223 (block III) (Asp23 and Glu204 in the mature protein) were recognized as the nucleophilic base and the proton donor, respectively, in *Saccharomyces cerevisiae* invertase.^{11,12} The same functions were attributed to the corresponding Asp54 (block I) and Glu236 (block III) of invertase from the plant *Chenopodium rubrum*.¹³ For this family (GH32), there are no mutagenesis data for the conserved aspartate residue in block II. Concerning family GH43, the separate substitution of the three conserved carboxylates—Asp38 (block I), Asp158 (block II), and Glu221 (block III)—of *C. japonicus* α -L-arabinanase Arb43A inactivated the enzyme or considerably reduced its activity. Asp38 lies 6 Å from the anomeric carbon of the substrate and may function as the nucleophilic base. Glu221 is adjacent to the glycosidic oxygen atom in the site of bond cleavage, and it must be the proton donor. Asp158 is important in catalysis presumably by modulating pKa and orienting the proton donor.¹⁴ Similar results were obtained for *B. subtilis* endo-1,5- α -L-arabinanase. In this case, mutation of Asp44 (block I), Asp163 (block II), and Glu215 (block III) (Asp14, Asp133, and Glu185 in the mature protein) to alanine abolished the enzyme activity, showing that they form the basis of the catalytic center.¹⁵

In bacterial fructosyltransferases (GH68), the three conserved carboxylates have been independently mutated.^{16–19} Site-directed mutagenesis of Asp135 (block I) in *Gluconacetobacter diazotrophicus* levansucrase resulted in an inactive enzyme (Hernández, unpublished data). Similarly, the substitution Asp → Asn in block I of the *Lactobacillus reuteri* fructosyltransferases (Asp272 in inulosucrase and Asp249 in levansucrase) nearly abolished the enzyme activity.¹⁹ The mutation of the equivalent Asp residues in block II of *G. diazotrophicus* levansucrase,¹⁶ *Streptococcus salivarius* fructosyltransferase,¹⁷ *Zymomonas mobilis* levansucrase,¹⁸ and *L. reuteri* inulosucrase and levansucrase¹⁹ either abolished or reduced dramatically sucrose hydrolysis. The substitution of Glu278 (block III) by His in *Z. mobilis* levansucrase reduced 210-fold the k_{cat} for sucrose hydrolysis.¹⁸ *L. reuteri* inulosucrase and levansucrase became almost inactive when Glu in block III was changed to Gln.¹⁹ Visual inspection of the active site in the crystal structure of Arb43A,¹⁴ using the WHAT IF program, shows that blocks I, II, and III are

| Identifier | block I | block II | block III | EC# | GH | organism |
|-------------|---|----------|-----------|-----|-----------|---|
| Q8UGU1 | 92 HFFPPFGWMDPNSGFR-----FEGRPHLPHYQHS<103>TPDFRDPVYFVRGD-----GLWKKLGSQS<30>RYKTTAIECPCLPLDGP | | | 245 | n.d. | 32a <i>Agrobacterium tumefaciens</i> |
| Q8K871 | 32 HLAAPVGLLNDPNSGID-----WDGTYHLFYQWHP<094>TAHFDRDPKVMKRD-----GIWYLVIAQT<32>GDFGYMMECPDLFHLDOE | | | 257 | n.d. | 32a <i>Bacillus halodurans</i> |
| Q8QM36 | 29 HMAFANWINDPNGLVQ-----YKGEYHVFYQHP<099>SHHFRDPKVMKHE-----GFWYMLNGST<28>GDLGYMMECPDFELDOE | | | 260 | n.d. | 32a <i>Bacillus megaterium</i> |
| Q9L864 | 30 HLMKPVGFMDPNGLIE-----INGEYHVFYQHP<097>TRHFRDPKVMKGN-----DKWYMLNGAQ<32>DPLGYMMECPDLFELDOE | | | 259 | 3.2.1.26 | 32a <i>Clostridium acetobutylicum</i> |
| Q92HJ7 | 30 HIEMFGLINDPNGLSY-----YDNKFHIFYQWNP<095>TAHFDRDPVFIED-----DTYNYMLGVS<28>KDFGYMMECPDLFELDOE | | | 260 | 3.2.1.26 | 32a <i>Clostridium beijerinckii</i> |
| Q8XK71 | 28 HFEPPFGLINDPNGLSY-----YKGEYHVFYQWNP<095>TAHFDRDPVFIED-----GYNYMLGVS<28>EDFGYMMECPDLFELDOE | | | 264 | n.d. | 32a <i>Clostridium perfringens</i> |
| RAFD_ECOLI | 27 HLAAPVGLLNDPNSGLI-----FNGRYHAFQHPH<097>IMHFRDPKVMHED-----GSWNVIAQAR<28>AGSYMMECPDFPKGNF | | | 252 | 3.2.1.26 | 32a <i>Escherichia coli</i> K12 |
| Q94466 | 33 HLAAPVGLLNDPNSGLI-----WNGYHVFYQWNP<094>TAHFDRDPKVMKRD-----GIWYLVIAQT<32>GDFGYMMECPDLFELDOE | | | 214 | 3.2.1.26 | 32a <i>Oecobacillus stearothermophilus</i> |
| SCRB_KLEFN | 30 HLAAPVGLLNDPNSGFC-----VAGRYHVFYQWNP<094>THHFRDPKVMKRD-----GRWYMLNGAQ<32>ANAGYMMECPDLFELDOE | | | 238 | 3.2.1.26 | 32a <i>Klebsiella pneumoniae</i> |
| SCRB_LACLA | 36 HIEFETGLLNDPNSGFSY-----FNEKWHLFYQHP<096>TDHFRDPKVMHED-----GQYICLIGAS<30>EKMGYMIIECPDLFELDOE | | | 239 | 3.2.1.26 | 32a <i>Lactococcus lactis</i> |
| Q9U079 | 45 HLAAPVGLLNDPNSGFSY-----FRDHYHVFYQHP<099>YVHFRDPKVMHED-----GRWNVIAQAR<31>DNVPMMECPDYFTIGSR | | | 266 | n.d. | 32a <i>Leishmania major</i> |
| Q9C214 | 232 HYTFYQWINDPNGLI-----WNGYHVFYQWNP<104>SAGFRDPKVMHED-----TANNWVVG- SG<33>GSRGYMMECPDFELDOE | | | 252 | n.d. | 32a <i>Neurospora crassa</i> |
| Q9CJ20 | 56 HLAPEFTGLLNDPNGLVF-----DGEKYHIFYQWNP<094>TEHVRDPKVFPE-----EGKIRILGAOR<31>NOQVPMMECPDLKLOE | | | 245 | n.d. | 32a <i>Pasteurella multocida</i> |
| SCRB_PEDPE | 36 HIQPTSGLLNDPNSGFSY-----FDGQWHLFYQHP<096>TSSFRDPKVMHED-----HGVALIGAT<28>NARGYMIIECPDLFELDOE | | | 269 | 3.2.1.26 | 32a <i>Pediococcus pentosaceus</i> |
| Q8XG3 | 18 HLCPPQGLLNDPNSGLIF-----WKGATYHVFYQWNP<095>TGHFDRDPKVMKRD-----DHVYLVIAQT<28>L-FCYMIIECPDLFELDOE | | | 261 | n.d. | 32a <i>Ralstonia solanacearum</i> |
| SCRB_SALTY | 30 HIAFVGLLNDPNSGFSY-----FACRYHVFYQWNP<094>TGHFDRDPKVMHED-----DLYVYLVIAQT<32>DDVYMIIECPDLFELDOE | | | 238 | 3.2.1.26 | 32a <i>Salmonella typhimurium</i> |
| SCRB_STAXY | 37 HIQPTSGLLNDPNSGLIF-----FKGNYVYSHQWNP<096>TQHFRDPKVMHED-----GVYAMIAAQ<28>DDFGYMMECPDYENLNGY | | | 261 | 3.2.1.26 | 32a <i>Staphylococcus xylosum</i> |
| SCRB_STRMU | 36 HIEPPTGLLNDPNSGFSY-----FMGKFWLFYQWNP<095>TEHFRDPKVMHED-----GQYVIAQAS<30>SKSEYMIIECPDLFELDOE | | | 246 | 3.2.1.26 | 32a <i>Streptococcus mutans</i> |
| Q97P86 | 36 HIEPPTGLLNDPNSGFSY-----FMGKFWLFYQWNP<095>TEHFRDPKVMHED-----GQYVIAQAS<30>SKSEYMIIECPDLFELDOE | | | 251 | n.d. | 32a <i>Streptococcus pneumoniae</i> |
| Q97P49 | 30 HFSAPYGLLNDPNSGFSY-----FRGEYHVFYQHP<099>AADFRDPKVMHED-----GRWNVIAQAR<28>EHQGYMMECPDYFELDOE | | | 210 | n.d. | 32a <i>Streptococcus pneumoniae</i> |
| Q99Y90 | 36 HIEPPTGLLNDPNSGFSY-----FNGRYHVFYQWNP<095>TEHFRDPKVMHED-----GQYVIAQAS<30>SGTEYMIIECPDLFELDOE | | | 246 | n.d. | 32a <i>Streptococcus pyogenes</i> |
| BFR_A_THERM | 6 HFFPTTGMNDPNGLIF-----WKGATYHVFYQWNP<096>THAFDRDPKVMHED-----GQYVIAQAS<30>SKSEYMIIECPDLFELDOE | | | 232 | 3.2.1.26 | 32a <i>Thermotoga maritima</i> |
| SCRB_VIBAL | 40 HIAFVGLLNDPNSGLI-----FNGEHHIFYQWNP<093>TEHFRDPKVMHED-----DDVYLVIAQT<28>SDSGYMIIECPDLFELDOE | | | 251 | 3.2.1.26 | 32a <i>Vibrio alginolyticus</i> |
| Q56660 | 97 HIEPPTGLLNDPNSGFSY-----HGEYHVFYQHPH<105>TLDFRDPKVMHED-----GQYVIAQAS<28>GNVGYMMECPDYFELDOE | | | 253 | 3.2.1.26 | 32a <i>Vibrio cholerae</i> |
| INV_A_ZYMMO | 32 HVTPLTGMNDPNGLIF-----FKGEYHVFYQHP<096>VAHFRDPKVMHED-----GRWNVIAQAR<37>GKAPYMIIECPDLFELDOE | | | 273 | 3.2.1.26 | 32a <i>Zymomonas mobilis</i> |
| O50585 | 41 HMTFPGSLWCDPQRPVH-----TNGAYQLVYHSG<105>ABWFRDPKVMHED-----NEWCVICIAR<22>NHAGGIECPDLFELDOE | | | 275 | 2.4.1.- | 32b <i>Arthrobacter nicotianovorans</i> |
| Q9KJ00 | 43 HMTFPGSLWCDPQRPVH-----THGAYQLVYHSG<107>ABWFRDPKVMHED-----NEWCVICIAR<22>NHAGGIECPDLFELDOE | | | 275 | 2.4.1.- | 32b <i>Arthrobacter ureafaciens</i> |
| Q94200 | 32 HFTPDQWMDPNGLIK-----IGSTWHLFYQHPH<108>GLESFRDPKVMHED-----GNWIMVLAAG<29>SSDITGMVECPDYFELDOE | | | 273 | 3.2.1.7 | 32b <i>Aspergillus ficum</i> |
| Q42801 | 32 HFTPDQWMDPNGLIK-----HNGTYHLFYQHPH<123>YQFRDPKVMHED-----GNWIMVLAAG<24>NAQGVMECPDYFELDOE | | | 286 | 2.4.1.99 | 32b <i>Aspergillus foetidus</i> |
| Q74641 | 32 HFTPDQWMDPNGLIK-----IGSTWHLFYQHPH<108>GLESFRDPKVMHED-----GNWIMVLAAG<29>SSDITGMVECPDYFELDOE | | | 273 | 3.2.1.7 | 32b <i>Aspergillus niger</i> |
| SACC_BACSU | 38 HFTPEANWMDPNGLIY-----YAGEYHVFYQHPH<098>KDFDRDPKVMHED-----KKWNVIAQAR<23>GSHGYMMECPDLFELDOE | | | 444 | 3.2.1.65 | 32b <i>Bacillus subtilis</i> |
| Q91003 | 47 HFTPEANWMDPNGLIY-----FDGQWHLFYQHPH<099>TDHFRDPKVMHED-----KKWNVIAQAR<23>GSHGYMMECPDLFELDOE | | | 276 | n.d. | 32b <i>Bacillus subtilis</i> |
| Q45155 | 136 HFTPLTGMNDPNGLIF-----FKGEYHVFYQHPH<097>LKFDRDPKVMHED-----GRWNVIAQAR<23>GKAPYMIIECPDLFELDOE | | | 292 | 3.2.1.65 | 32b <i>Bacteroides fragilis</i> |
| Q94224 | 33 HLTTPGVGMNDPNGLIY-----SESTYHVFYQHPH<102>STQFRDPKVMHED-----NQWNVIAQAR<24>GYGVYMIIECPDLFELDOE | | | 298 | 3.2.1.26 | 32b <i>Candida utilis</i> |
| Q97180 | 48 HFTPEANWMDPNGLIY-----FDGEYHVFYQHPH<099>TKDFRDPKVMHED-----NKWNVIAQAR<20>NNIGIIECPDLFELDOE | | | 273 | n.d. | 32b <i>Clostridium acetobutylicum</i> |
| Q9RBJ1 | 46 HFSAPYGLLNDPNSGLI-----LDGVYHVFYQHPH<106>SRQFRDPKVMHED-----GCWINTVIAQ<26>RPGMLWMTPLVPLKLD | | | 282 | 3.2.1.65 | 32b <i>Glucanacetobacter diazotrophicus</i> |
| INV1_KLUMA | 42 HFTSPQGMNDPNGLIY-----KEEDWHLFYQHPH<102>SSNFRDPKVMHED-----NQWNVIAQAR<24>GYGVYMIIECPDLFELDOE | | | 307 | 3.2.1.7 | 32b <i>Kluyveromyces marxianus</i> |
| Q93R69 | 75 HFTPEANWMDPNGLIY-----LDGVYHVFYQHPH<111>RDFDRDPKVMHED-----GRWNVIAQAR<20>NNIGIIECPDLFELDOE | | | 340 | n.d. | 32b <i>Microbacterium laevaniforme</i> |
| Q9BQV9 | 52 HMTFPGSLWCDPQRPVH-----TNGAYQLVYHSG<107>ABWFRDPKVMHED-----NEWCVICIAR<22>NHAGGIECPDLFELDOE | | | 275 | 2.4.1.- | 32b <i>Microbacterium</i> sp. AL-210 |
| Q45372 | 115 HFEPPFGWMDPNSGFR-----FEGRPHLPHYQHS<103>TPDFRDPVYFVRGD-----GLWKKLGSQS<30>RYKTTAIECPCLPLDGP | | | 245 | 3.2.1.65 | 32b <i>Penicillium polymyxa</i> |
| O00056 | 32 HFCFANWMDPNGLIK-----IDSTWHLFYQHPH<108>GLESFRDPKVMHED-----GNWIMVLAAG<29>SSDITGMVECPDYFELDOE | | | 272 | 3.2.1.65 | 32b <i>Penicillium purporeum</i> |
| INV1_HANAN | 31 HLTDPQGMNDPNGLIY-----KKLWYHVFYQHPH<102>SSQFRDPKVMHED-----NQWNVIAQAR<24>GYGVYMIIECPDLFELDOE | | | 317 | 3.2.1.26 | 32b <i>Pichia anomala</i> |
| Q91AL1 | 14 HFTPETNMDPNGLIY-----YGEYHVFYQHPH<103>MTDFRDPKVMHED-----NKWNVIAQAR<23>GSHGYMMECPDLFELDOE | | | 287 | 2.4.1.- | 32b <i>Pseudomonas mucidolens</i> |
| INV2_YEAST | 31 HFTPETNMDPNGLIY-----KDAKWHLFYQHPH<102>STQFRDPKVMHED-----GNWIMVLAAG<24>GYGVYMIIECPDLFELDOE | | | 299 | 3.2.1.26 | 32b <i>Saccharomyces cerevisiae</i> |
| INV1_SCHPO | 86 HFTPETNMDPNGLIY-----TGCVYHVFYQHPH<106>SLQFRDPKVMHED-----NQWNVIAQAR<24>GYGVYMIIECPDLFELDOE | | | 291 | 3.2.1.26 | 32b <i>Schizosaccharomyces pombe</i> |
| FRUA_STRMU | 447 HYSVTKGWMDPNGLIY-----YNGVYHVFYQHPH<099>NQDFRDPKVMHED-----NQWNVIAQAR<16>TYPLDHTCEPDMYPIVAN | | | 789 | 3.2.1.80 | 32b <i>Streptococcus mutans</i> |
| Q8G179 | 49 HFTPEANWMDPNGLIY-----LDGVYHVFYQHPH<101>VADFRDPKVMHED-----GRWNVIAQAR<20>NNIGIIECPDLFELDOE | | | 275 | 3.2.1.64 | 32b <i>Streptomyces exfoliatus</i> |
| Q02490 | 110 HFTPEANWMDPNGLIY-----LDGVYHVFYQHPH<097>CRDFRDPKVMHED-----DQWNVIAQAR<23>GSHGYMMECPDLFELDOE | | | 248 | 3.2.1.26 | 32b <i>Trichomonas foetus</i> |
| Q9RLU2 | 115 HFEPPFGWMDPNSGFR-----FEGRPHLPHYQHS<103>TPDFRDPVYFVRGD-----GLWKKLGSQS<30>RYKTTAIECPCLPLDGP | | | 245 | n.d. | 32b <i>Xanthomonas oryzae</i> |
| Q96VC5 | 49 HILFAEGQIDGCAHYTD-----STGLFHVGLHNG<104>VTAFRDPKVMHED-----GRWNVIAQAR<26>RPGMLWMTPLVPLKLD | | | 352 | n.d. | 32c <i>Aspergillus niger</i> |
| Q9P853 | 46 HVLPPGQIDGCAHYND-----ATGLFHVGLHNG<104>VTAFRDPKVMHED-----GRWNVIAQAR<41>KRWGYMIIECPDLFELDOE | | | 406 | 2.4.1.- | 32c <i>Aspergillus sydowii</i> |
| F92916 | 74 HFTPETNMDPNGLIY-----YKGVYHVFYQHPH<099>RDFDRDPKVMHED-----STHIVVLSGS<29>VDRGVMECPDYFELDOE | | | 335 | 2.4.1.- | 32d <i>Allium cepa</i> |
| O81083 | 153 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<099>KDFDRDPKVMHED-----DWRIVVLSGS<27>VERGVMECPDYFELDOE | | | 336 | 3.2.1.26 | 32d <i>Allium cepa</i> |
| O81082 | 86 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<100>PHDFRDPKVMHED-----STWMLVLSGS<28>KDSVGMCEPDLFELDOE | | | 334 | 2.4.1.99 | 32d <i>Allium cepa</i> |
| Q39041 | 124 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<099>PKDFRDPKVMHED-----GKWRIVVLSGS<26>VPMTCMECPDYFELDOE | | | 341 | 3.2.1.26 | 32d <i>Arabidopsis thaliana</i> |
| Q38801 | 51 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<099>GSAFRDPKVMHED-----GKWRIVVLSGS<26>VPMTCMECPDYFELDOE | | | 339 | 3.2.1.26 | 32d <i>Arabidopsis thaliana</i> |
| Q43866 | 55 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<101>ASSFRDPKVMHED-----KRWIVVLSGS<26>DDSGYMIIECPDLFELDOE | | | 328 | 3.2.1.26 | 32d <i>Arabidopsis thaliana</i> |
| Q9W413 | 53 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<099>GSAFRDPKVMHED-----GKWRIVVLSGS<26>DDSGYMIIECPDLFELDOE | | | 339 | 3.2.1.26 | 32d <i>Arabidopsis thaliana</i> |
| Q9RA55 | 112 HPPANWMDPNGLIY-----HDCSYHVFYQHPH<112>VADFRDPKVMHED-----NQWNVIAQAR<33>DGSAGYMIIECPDLFELDOE | | | 283 | 3.2.1.7 | 32d <i>Arthrobacter</i> sp. S37 |
| O43732 | 121 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<100>ATDFRDPKVMHED-----STWMLVLSGS<27>VERGVMECPDYFELDOE | | | 339 | 3.2.1.26 | 32d <i>Asparagus officinalis</i> |
| O52973 | 823 HAITPPGMNDPNGLIY-----YKGVYHVFYQHPH<101>HNEFRDPKVMHED-----DKWYLVVLSGS<33>PELGTWELFVLLPLD | | | 472 | 2.4.1.- | 32d <i>Bacillus circulans</i> |
| INV1_CAPAN | 115 HFPQPNWMDPNGLIY-----HKGWYHVFYQHPH<099>VKDFRDPKVMHED-----GQWLVVLSGS<23>VFGTGMMECPDYFELDOE | | | 328 | 3.2.1.26 | 32d <i>Capsicum annuum</i> |
| Q42691 | 93 HFPQPNWMDPNGLIY-----FKGIVYHVFYQHPH<102>ATSYRDPKVMHED-----GNWIVVLSGS<27>YERGVMECPDYFELDOE | | | 327 | 3.2.1.26 | 32d <i>Chenopodium rubrum</i> |
| Q9Z896 | 93 HFPQPNWMDPNGLIY-----HKGWYHVFYQHPH<099>LKDYRDPKVMHED-----GKWRIVVLSGS<26>VPMTCMECPDYFELDOE | | | 325 | 2.4.1.100 | 32d <i>Cichorium intybus</i> |
| Q9Z854 | 49 HFPQPNWMDPNGLIY-----YKGVYHVFYQHPH<100>DDCFRDPKVMHED-----GKWRIVVLSGS<26>ADATGMMECPDYFELDOE | | | 332 | 3.2.1.26 | 32d <i>Cichorium intybus</i> |

Fig. 1. Multiple-sequence alignment of proteins from GH32, GH43, GH62, and GH68 families. The distance from the protein termini and length of variable spacers are indicated. The sequence identifiers are according to the nomenclature in the SwissProt/TREMBL database. At the top, three conserved blocks (I, II, and III) are indicated by asterisks. EC#, Enzyme Commission number; n.d., not determined. Fructosyltransferases, fructan:fructan 6G-fructosyltransferase, or sucrose fructan 6-fructosyltransferase (2.4.1.-); levansucrase (2.4.1.10); sucrose:sucrose 1-fructosyltransferase (2.4.1.99); fructan:fructan 1-fructosyltransferase (2.4.1.100); inulinase (3.2.1.7); xylanase (3.2.1.8); invertase or sucrose 6-phosphate hydrolase (3.2.1.26); β -xylosidase (3.2.1.37); α -L-arabinofuranosidase (3.2.1.55); levnanase (3.2.1.65); fructan β -fructosidase (3.2.1.80); endo-arabinase (3.2.1.99). *These enzymes have the α -L-arabinofuranosidase (EC 3.2.1.55) activity as well. GH: family (GH32, GH43, GH62, and GH68 according to <http://afmb.cnrs-mrs.fr/CAZY/families.html>) and subfamily (a/b/c/d/e/f/g, according to Naumoff⁵ and Naumoff and Livshits³⁴) of the sequences.

close in space. Also, the nucleophilic base residues Tyr406+Glu277 in neuraminidase of subtype N9 from an avian influenza virus are in close vicinity to Asp38 (block I) [Fig. 2(A and B)].

Five- and Six-Bladed β -Propeller Topologies Predicted for GH32, GH62, and GH68

We previously predicted a 6-bladed β -propeller catalytic domain for fructofuranosidases included in GH32 and GH68.^{28,29} In this work, sequence-structure compatibility searches using 3D-PSSM, INBGU, mGenTHREADER, and SAM-T02 programs showed the 5-bladed β -propeller fold of Arb43A and the 6-bladed β -propeller fold of sialidase/neuraminidase (clan GH-E) as the most reliable topologies for GH families 32, 62, and 68 (Table I). The choice of

divergent amino acid sequences [*S. cerevisiae* invertase (GH32), *G. diazotrophicus* levansucrase (GH32), *C. japonicus* α -L-arabinofuranosidase C (GH62), and *G. diazotrophicus* levansucrase (GH68)] and four prediction methods using different approaches provide confidence in our results. 3D-PSSM, mGenTHREADER, INBGU, and SAM-T02 identified several β -propeller structures with 4-, 5-, 6-, 7-, and 8-bladed topologies among the first hits for each method (Table I). The 5- and 6-bladed topologies of Arb43A and sialidase/neuraminidase are identified with highest frequency and their superposition revealed their high structural similarity (Fig. 2).

A general conclusion can be drawn from inspection of the recently determined three-dimensional (3D) structures, and the sequence-structure compatibility searches

THREE KEY ACIDIC RESIDUES IN GH FAMILIES

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| Identifier | block I | block II | block III | EC# | GH | organism |
|------------|--|------------------------------------|-----------|-----------|-----|---|
| Q92855 | 24 HFQPPQNNMNDPNCMC-----YKGVYHLFYQYNP<100>PDDFRDPTTAMLEED---- | GTWRLLVGSQK<26>VSGTGMWECVDFPVPVWD | 326 | 3.2.1.26 | 32d | <i>Cichorium intybus</i> |
| Q24459 | 109 HFQPPQNNMNDPNCMPY-----HGMWYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GEYRMWGSKH<27>VPHGTGMWECVDFPVPVST | 331 | 2.4.1.99 | 32d | <i>Cichorium intybus</i> |
| Q65778 | 93 HFQPPQNNMNDPNCGLP-----HGMWYHLFYQYNP<099>LTDYRDFSTVMTGPD----- | QKGRMIITGKR<26>VPHGTGMWECVDFPVPVST | 325 | 2.4.1.100 | 32d | <i>Cynara scolymus</i> |
| Q23786 | 106 HFQPPQNNMNDPNCMPY-----HGMWYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GEYRMWGSKH<27>VPHGTGMWECVDFPVPVST | 331 | 2.4.1.99 | 32d | <i>Cynara scolymus</i> |
| INV1_DAUCA | 63 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<102>ATAFRDPTTAMLDKS----- | GHWMLVGSKR<26>QANTGMWECDFPVPVSLK | 327 | 3.2.1.26 | 32d | <i>Daucus carota</i> |
| INV2_DAUCA | 64 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>SSSFRDPTTAMFDG----- | VHMKLVGSRR<26>KDRGTGMWECDFPVPVAPK | 328 | 3.2.1.26 | 32d | <i>Daucus carota</i> |
| INV3_DAUCA | 57 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>PSAFRDPPTTAMFDG----- | GHWMLVGSRR<26>KASTGMWECDFPVPVSPR | 326 | 3.2.1.26 | 32d | <i>Daucus carota</i> |
| INV6_DAUCA | 134 HFQPPQNNMNDPNCGLP-----HGMWYHLFYQYNP<099>STDFRDPSTTAMTGRD----- | GKWRITIGSVR<26>VPGTGMWECVDFPVPVST | 328 | 3.2.1.26 | 32d | <i>Daucus carota</i> |
| Q95812 | 104 HFQPPQNNMNDPNCGLP-----HGMWYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRIITIGSVR<26>VPGTGMWECVDFPVPVSLA | 267 | 3.2.1.26 | 32d | <i>Daucus carota</i> |
| O81985 | 91 HFQPPQNNMNDPNCGLP-----HGMWYHLFYQYNP<099>LTDYRDFSTVMTGPD----- | QKGRMIITGKR<26>VPHGTGMWECVDFPVPVST | 325 | 2.4.1.100 | 32d | <i>Helianthus tuberosus</i> |
| O81986 | 97 HFQPPQNNMNDPNCMPY-----HGMWYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GEYRMWGSKH<27>VPHGTGMWECVDFPVPVST | 333 | 2.4.1.99 | 32d | <i>Helianthus tuberosus</i> |
| Q96466 | 85 HFQPPQNNMNDPNCGLP-----YKGVYHLFYQYNP<101>TKDFRDPMTANTYDSSD----- | ETWRLTIGSKD<30>VVRGTGMWECVDFPVPGR | 324 | 2.4.1.- | 32d | <i>Hordeum vulgare</i> |
| Q927X2 | 134 HFQPPQNNMNDPNCGLP-----YKGVYHLFYQYNP<100>YKDFRDPSTTANTAGSQN----- | QWRLITIGSVR<26>VPGTGMWECVDFPVPVST | 330 | 3.2.1.26 | 32d | <i>Ipomoea batatas</i> |
| Q9XTF3 | 35 HIRPPQNNMNDPNCMPY-----VTGKIHLFYQYNP<107>MENFRDPTTAMWGDPTNP----- | NRWLIAPVARI<34>YDLHMFCEPDPFTTLKQG | 386 | n.d. | 32d | <i>Leishmania major</i> |
| INV1_LYCES | 107 HFQPPQNNMNDPNCGLP-----HGMWYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRLITIGSK<26>VPGTGMWECVDFPVPVSTK | 329 | 3.2.1.26 | 32d | <i>Lycopersicon esculentum</i> |
| Q92119 | 55 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<100>KIQFRDPTTAMWGD----- | GYRWLVGSVR<27>AQGTGMWECDFPVPVSLK | 326 | 3.2.1.26 | 32d | <i>Lycopersicon esculentum</i> |
| Q41799 | 54 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRLITIGSVR<26>VPGTGMWECVDFPVPVSLK | 326 | 3.2.1.26 | 32d | <i>Nicotiana tabacum</i> |
| Q927W9 | 122 HFQPPQNNMNDPNCGLP-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRLITIGSVR<26>VPGTGMWECVDFPVPVST | 331 | 3.2.1.26 | 32d | <i>Oryza sativa</i> |
| Q9F015 | 160 RAMPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>INEFRDPTTAMWGD----- | DKWQVLVGSGL<33>PELGTGMWECVDFPVPVST | 465 | 2.4.1.- | 32d | <i>Paenibacillus macerans</i> |
| INV1_PHAU | 619 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GKWRITIGSK<26>VPGTGMWECVDFPVPVSKK | 333 | 3.2.1.26 | 32d | <i>Phaseolus vulgaris</i> |
| INV1_PEA | 50 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>SSSFRDPTTAMWGD----- | GFWRVLVGSKR<26>ABGTGMWECDFPVPVSLK | 304 | 3.2.1.26 | 32d | <i>Pisum sativum</i> |
| Q43818 | 63 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRLITIGSKD<26>QNTVITSEKLDVFLVHAP | 333 | 3.2.1.26 | 32d | <i>Pisum sativum</i> |
| Q93700 | 279 HVSPQNNMNDPNCMPY-----YKGVYHLFYQYNP<104>PQDFRDPSTTAMWGD----- | DTWMLVGSGL<32>PYLGVWELVFLVPLGKD | 289 | 3.2.1.27 | 32d | <i>Pseudomonas mucidolens</i> |
| Q93173 | 110 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<100>KSFQFRDPTTAMWGD----- | GNWRLVGSVR<27>APGTGMWECDFPVPVSLK | 326 | 3.2.1.26 | 32d | <i>Solanum tuberosum</i> |
| Q43173 | 110 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | QWRLITIGSVR<26>VPGTGMWECVDFPVPVSLK | 329 | 3.2.1.26 | 32d | <i>Solanum tuberosum</i> |
| O81118 | 46 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GNWRLVGSVR<26>VPGTGMWECVDFPVPVSLK | 331 | 3.2.1.26 | 32d | <i>Triticum aestivum</i> |
| Q41604 | 98 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | ASKMLAIGSKD<27>VEGTGMWECVDFPVPVLTN | 329 | 3.2.1.26 | 32d | <i>Tulipa gesneriana</i> |
| Q41605 | 98 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | ASKMLAIGSKD<27>VEGTGMWECVDFPVPVLTN | 329 | 3.2.1.26 | 32d | <i>Tulipa gesneriana</i> |
| INV1_VICFA | 111 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GKWRITIGSK<26>VPGTGMWECVDFPVPVSKK | 332 | 3.2.1.26 | 32d | <i>Vicia faba</i> |
| Q43855 | 50 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>GSVFRDPTTAMWGD----- | GHWRILVGSGL<26>AKRTGMWECDFPVPVSLK | 324 | 3.2.1.26 | 32d | <i>Vicia faba</i> |
| Q43856 | 52 HFQPPQNNMNDPNCGLP-----YKGVYHLFYQYNP<101>ASSFRDPTTAMWGD----- | GKWRITIGSK<26>VPGTGMWECVDFPVPVSKK | 331 | 3.2.1.26 | 32d | <i>Vigna radiata</i> |
| INV1_PHAU | 119 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<099>YKDFRDPSTTAMWGD----- | GNWRLVGSVR<26>VPGTGMWECVDFPVPVSKK | 331 | 3.2.1.26 | 32d | <i>Vigna radiata</i> |
| Q9RLU3 | 660 RAMPQNNMNDPNCMPY-----YKGVYHLFYQYNP<101>INEFRDPTTAMWGD----- | DKWQVLVGSGL<33>PELGTGMWECVDFPVPVST | 465 | n.d. | 32d | <i>Xanthomonas oryzae</i> |
| INV1_MAIZE | 126 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<102>ATAFRDPTTAMWGD----- | GHWMLVGSKR<26>QANTGMWECDFPVPVSLK | 327 | 3.2.1.26 | 32d | <i>Zea mays</i> |
| INV1_MAIZE | 126 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<102>ATAFRDPTTAMWGD----- | GHWMLVGSKR<26>QANTGMWECDFPVPVSLK | 327 | 3.2.1.26 | 32d | <i>Zea mays</i> |
| O81189 | 57 HFQPPQNNMNDPNCMPY-----YKGVYHLFYQYNP<102>ATAFRDPTTAMWGD----- | QWRLITIGSK<26>VPGTGMWECVDFPVPVSLK | 331 | 3.2.1.26 | 32d | <i>Zea mays</i> |
| SACB_BACAM | 75 IESAKGLVDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | KGRKYLVEAST<69>NTVTEIERANVPMKNGK | 120 | 2.4.1.10 | 68a | <i>Bacillus amyloliquefaciens</i> |
| O82854 | 85 SGNLIDLDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | KGRKYLVEAST<69>NTVTEIERANVPMKNGK | 117 | 2.4.1.- | 68a | <i>Bacillus</i> sp. V230 |
| SACB_BACSU | 75 IESAKGLVDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | KGRKYLVEAST<69>NTVTEIERANVPMKNGK | 121 | 2.4.1.10 | 68a | <i>Bacillus subtilis</i> |
| Q97181 | 81 NGNLIDLDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | NGDRYLVEAST<70>NLVTEIERANVPMKNGK | 122 | n.d. | 68a | <i>Clostridium acetobutylicum</i> |
| SACB_BACST | 75 IESAKGLVDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | KGRKYLVEAST<69>NTVTEIERANVPMKNGK | 121 | 2.4.1.10 | 68a | <i>Geobacillus stearothermophilus</i> |
| Q8G3P2 | 261 TTTIPELDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | NGDRYLVEAST<72>HMTVTEIERANVPMKNGK | 265 | 2.4.1.9 | 68a | <i>Lactobacillus reuteri</i> 121 |
| Q8G3V4 | 238 TGMMAHLVDVDSWPLQNA-DGTAANYGVHVFALAGS<122>DYCLDRPHYVD----- | NGDRYLVEAST<72>HMTVTEIERANVPMKNGK | 291 | 2.4.1.10 | 68a | <i>Lactobacillus reuteri</i> 121 |
| Q9Z5E5 | 84 SKGLIDFDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | QKGRYLVEAST<69>NTVTEIERANVPMKNGK | 135 | 2.4.1.10 | 68a | <i>Paenibacillus polymyxa</i> |
| SACB_STRMU | 235 TQIADLDVDSWPLQNA-DGTAANYGVHVFALAGS<121>NHTLDRPHYVD----- | NGDRYLVEAST<72>HMTVTEIERANVPMKNGK | 286 | 2.4.1.10 | 68a | <i>Streptococcus mutans</i> |
| SACB_STRSL | 276 TMRKEIDVDVDSWPLQNA-DGTAANYGVHVFALAGS<129>NHTLDRPHYVD----- | NGDRYLVEAST<72>HMTVTEIERANVPMKNGK | 412 | 2.4.1.10 | 68a | <i>Streptococcus salivarius</i> |
| Q9EVD6 | 164 PATSEVDVDSWPLQNA-DGTAANYGVHVFALAGS<152>GVNFRDPTTAMWGD----- | DTEYVYVFGSG<78>NCVNDQTERPHVYFQDGK | 152 | 2.4.1.- | 68b | <i>Actinomyces naeslundii</i> |
| Q8VW87 | 101 DMSNEGVVMDVDSWPLQNA-DGTAANYGVHVFALAGS<138>PNNFRDPTTAMWGD----- | PGTYVYVFGSG<78>NCVNDQTERPHVYFQDGK | 194 | n.d. | 68b | <i>Arthrobacter</i> sp. K-1 |
| Q97179 | 60 KLTAPMLVDVDSWPLQNA-DGTAANYGVHVFALAGS<119>YSFRDPTTAMWGD----- | TKDYLVEAST<66>VGVNDQTERPHVYFQDGK | 112 | 2.4.1.10 | 68b | <i>Clostridium acetobutylicum</i> |
| SACB_ERNAM | 35 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<125>YVNFDRDPTTAMWGD----- | DGKLYVYVFGSG<56>VGVNDQTERPHVYFQDGK | 118 | 2.4.1.10 | 68b | <i>Erwinia amylovora</i> |
| SACB_ACCDI | 124 PVPNDVDSWPLQNA-DGTAANYGVHVFALAGS<142>PNNFRDPTTAMWGD----- | PGTYVYVFGSG<78>NCVNDQTERPHVYFQDGK | 173 | 2.4.1.10 | 68b | <i>Gluconacetobacter diazotrophicus</i> |
| SACB_PSESG | 35 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<125>YVNFDRDPTTAMWGD----- | DGKLYVYVFGSG<56>VGVNDQTERPHVYFQDGK | 118 | 2.4.1.10 | 68b | <i>Pseudomonas syringae</i> pv. <i>glycinea</i> |
| SACB_RAHQ | 35 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<125>YVNFDRDPTTAMWGD----- | DGKLYVYVFGSG<56>VGVNDQTERPHVYFQDGK | 118 | 2.4.1.10 | 68b | <i>Rahnella aquatilis</i> |
| SACB_ZYMMO | 37 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<114>PNDFRDPTTAMWGD----- | DGKLYVYVFGSG<56>VGVNDQTERPHVYFQDGK | 135 | 2.4.1.10 | 68b | <i>Zymomonas mobilis</i> |
| INV2_ZYMMO | 37 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<116>LNNFRDPTTAMWGD----- | DGKLYVYVFGSG<56>VGVNDQTERPHVYFQDGK | 127 | 3.2.1.26 | 68b | <i>Zymomonas mobilis</i> |
| XYLB_BACOV | 5 RYLYPGDVMVDSWPLQNA-DGTAANYGVHVFALAGS<097>GVSYIDPFAVDDGD----- | GNVYLYVGGWL<60>DTEREFTEASWMMHYNCK | 91 | 3.2.1.37* | 43a | <i>Bacteroides ovatus</i> |
| XYLB_CLOS | 7 PLYSPVYVDSWPLQNA-DGTAANYGVHVFALAGS<102>DNGQDFPFAVDDGD----- | GERTY-----DTEREFTEASWMMHYNCK | 254 | 3.2.1.37* | 43a | <i>Clostridium stercoarum</i> |
| XYLB_PRRUR | 3 KTVPSVYVDSWPLQNA-DGTAANYGVHVFALAGS<098>GVSYIDPFAVDDGD----- | GEIVYVGGWL<59>DOPHTEASWMMHYNCK | 87 | 3.2.1.37 | 43a | <i>Prevotella ruminicola</i> |
| ABNA_ASPNG | 23 GACSGVCTTHDPSGLKIRK-----GDYTYLSTFSGK<089>YVNFDRDPTTAMWGD----- | GTIYVYVGGWL<25>PSTHTEASWMMHYNCK | 117 | 3.2.1.99 | 43b | <i>Aspergillus niger</i> |
| Q9KBP7 | 3 AFSTFEASVHDSVSIK-----EDTYVYVGGWL<110>HNPALDPTTAMWGD----- | GKLMVYVGGWL<26>GNHSHRIEAPYIHYDIET | 540 | n.d. | 43b | <i>Bacillus halodurans</i> |
| XYIA_BACSU | 27 KPIFEVGGWL<109>HNPALDPTTAMWGD-----GKLMVYVGGWL<26>GNHSHRIEAPYIHYDIET | GKLMVYVGGWL<26>GNHSHRIEAPYIHYDIET | 235 | n.d. | 43b | <i>Bacillus subtilis</i> |
| P94522 | 37 PMSSEVPIVMDVDSWPLQNA-DGTAANYGVHVFALAGS<114>PNDFRDPTTAMWGD----- | DGKLYVYVGGWL<56>VGVNDQTERPHVYFQDGK | 135 | 2.4.1.10 | 43b | <i>Bacillus subtilis</i> |
| P94540 | 27 AGCAKQDVHDSWPLQNA-DGTAANYGVHVFALAGS<116>LNNFRDPTTAMWGD----- | DGKLYVYVGGWL<56>VGVNDQTERPHVYFQDGK | 127 | 3.2.1.99* | 43b | <i>Cellvibrio japonicus</i> |
| Identifier | block I | block II | block III | EC# | GH | organism |
| Q97LI4 | 37 ALNTSRVSHVDSIAQA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GNLWLYVGGWL<37>GGYGTGSGSYIVYDKAT | 399 | n.d. | 43b | <i>Clostridium acetobutylicum</i> |
| Q9K6P5 | 5 QNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKVLN-MW<33>GTDITLQTEAPHLKYDGK | 330 | n.d. | 43c | <i>Bacillus halodurans</i> |
| XYNB_BACPU | 3 TNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GKYYLYVGGWL<131>GPNALDPCVYKDD | 339 | 3.2.1.37 | 43c | <i>Bacillus pumilus</i> |
| O52729 | 3 TNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GKYYLYVGGWL<131>GPNALDPCVYKDD | 337 | 3.2.1.37 | 43c | <i>Bacillus</i> sp. KK-1 |
| P94489 | 3 TNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GKYYLYVGGWL<131>GPNALDPCVYKDD | 337 | n.d. | 43c | <i>Bacillus subtilis</i> |
| XYLB_BUTFI | 4 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GKYYLYVGGWL<131>GPNALDPCVYKDD | 332 | 3.2.1.37* | 43c | <i>Butyrivibrio fibrisolvens</i> GS 113 |
| O30426 | 54 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GKYYLYVGGWL<131>GPNALDPCVYKDD | 1111 | 3.2.1.37* | 43c | <i>Caldicellulosiruptor saccharolyticus</i> |
| Q9A928 | 61 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GERWLVGSGL<28>DVEGFSRPGKLMKRGK | 304 | n.d. | 43c | <i>Caulobacter crescentus</i> |
| Q9A9J1 | 6 RNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GRKVLN-MW<33>GTDITLQTEAPHLKYDGK | 338 | n.d. | 43c | <i>Caulobacter crescentus</i> |
| Q9A9M1 | 3 RNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GRKVLN-MW<33>GTDITLQTEAPHLKYDGK | 317 | n.d. | 43c | <i>Caulobacter crescentus</i> |
| Q97DM1 | 2 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKVLN-MW<33>GTDITLQTEAPHLKYDGK | 338 | n.d. | 43c | <i>Clostridium acetobutylicum</i> |
| Q97TI7 | 42 QNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKVLN-MW<33>GTDITLQTEAPHLKYDGK | 298 | n.d. | 43c | <i>Clostridium acetobutylicum</i> |
| YAGH_ECOLI | 3 TNPVLKGFNDPSICRA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 340 | n.d. | 43c | <i>Escherichia coli</i> |
| Q9CFH1 | 4 QNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 361 | n.d. | 43c | <i>Lactococcus lactis</i> |
| O52675 | 3 QNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 342 | 3.2.1.37* | 43c | <i>Salmonella ruminantium</i> |
| Q92P74 | 5 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 336 | n.d. | 43c | <i>Sinorhizobium meliloti</i> |
| O45071 | 39 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 259 | n.d. | 43d | <i>Bacillus subtilis</i> |
| O30426 | 888 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 242 | 3.2.1.37* | 43d | <i>Caldicellulosiruptor saccharolyticus</i> |
| O52374 | 1322 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 247 | 3.2.1.8 | 43d | <i>Caldicellulosiruptor</i> sp. R698.1 |
| Q9K3P5 | 133 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 247 | 3.2.1.8* | 43d | <i>Caldicellulosiruptor</i> sp. Tok78.1 |
| Q97TI6 | 25 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 236 | n.d. | 43d | <i>Clostridium acetobutylicum</i> |
| Q97TI1 | 67 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 422 | n.d. | 43d | <i>Clostridium acetobutylicum</i> |
| XYND_PAEPO | 38 NNPILITATFSTFE<089>NNSGDFDPSLPHDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 377 | 3.2.1.8 | 43d | <i>Paenibacillus polymyxa</i> |
| Q45134 | 84 VSTGTVKGVDSVIRKSA-----GKYYLYVGGWL<131>GPNALDPCVYKDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 99 | 3.2.1.55 | 43e | <i>Butyrivibrio fibrisolvens</i> H17c |
| Q9M2X0 | 132 GRKYYLYVGGWL<131>GPNALDPCVYKDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 127 | n.d. | 43f | <i>Ustilago maydis</i> |
| Q9KQ80 | 7 WNPFIETGRADPFLIKD-----GSDYVYVGGWL<131>GPNALDPCVYKDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 119 | n.d. | 43g | <i>Arabidopsis thaliana</i> |
| Q9L412 | 3 WNPFIETGRADPFLIKD-----GSDYVYVGGWL<131>GPNALDPCVYKDD----- | GRKYYLYVGGWL<131>GPNALDPCVYKDD | 112 | n.d. | 43g | <i>Bacillus halodurans</i> |
| AXHA_ASPNG | 43 TPKSGWATKADPTDVVS-----NGKHIVYASTD<084>STGAIQTVIGGD----- | TNNYLFAGGAD<26>GATNDLFEAVQVYTVVGG | 108 | 3.2.1.55 | 62 | <i>Aspergillus niger</i> |
| Q9VUX6 | 43 TPKSGWATKADPTDVVS-----NGKHIVYASTD<084>STGAIQTVIGGD----- | TNNYLFAGGAD<26>GATNDLFEAVQVYTVVGG | 108 | 3.2.1.55 | 62 | <i>Aspergillus niger</i> |
| AXHA_ASPUT | 43 TPKSGWATKADPTDVVS-----NGKHIVYASTD<084>STGAIQTVIGGD----- | TNNYLFAGGAD<26>GATNDLFEAVQVYTVVGG | 108 | 3.2.1.55 | 62 | <i>Aspergillus tubigenensis</i> |
| XYNC_PSEFL | 337 PKNPWSIKIDSTYKY-----NDTHYVYASTD<077>PNGALDPTTAMWGD----- | THCYLYVGGWL<28>NGNYSYLFEEAANVYKLGQ | 58 | 3.2.1.55 | | |

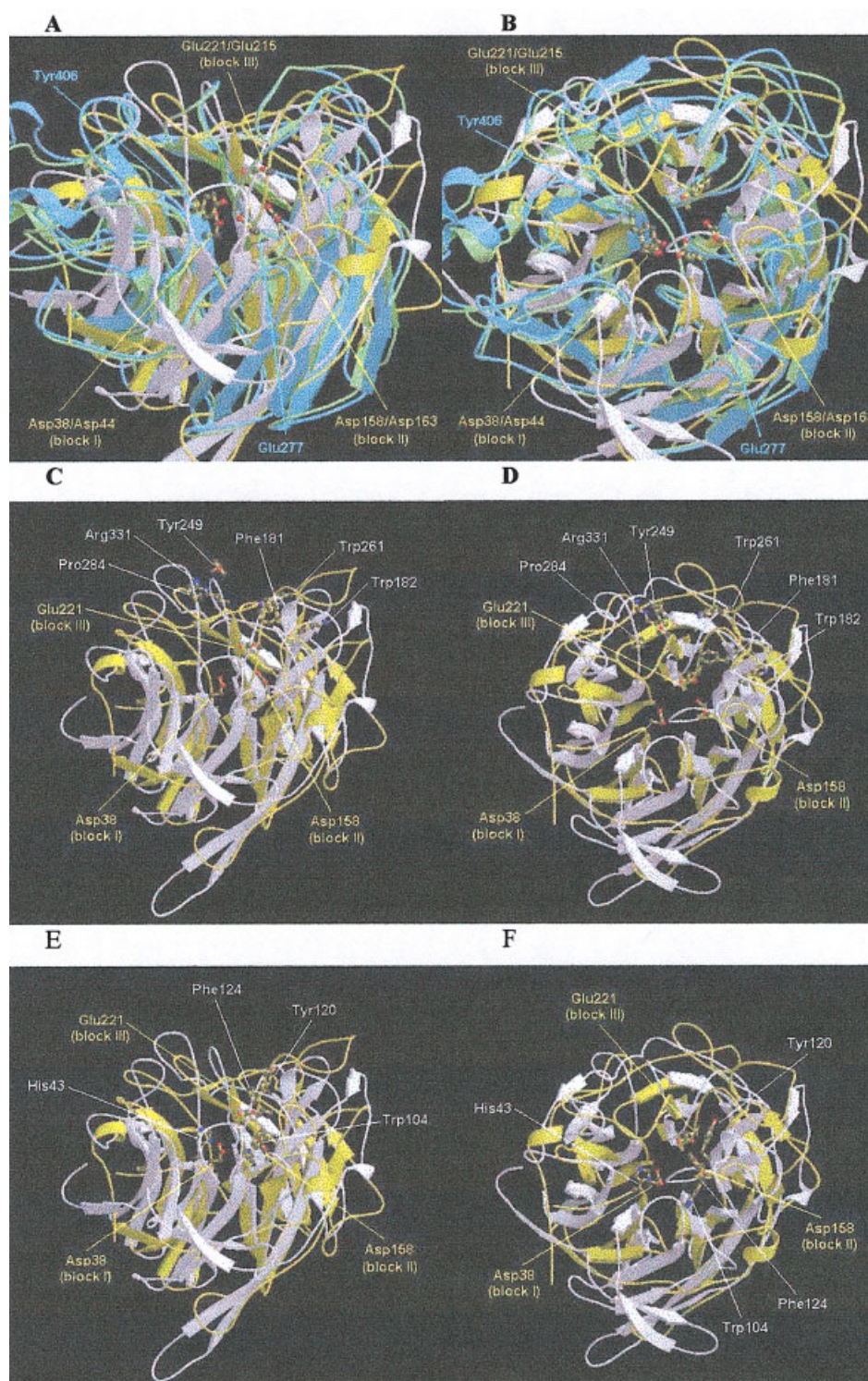


Fig. 2. Structural comparison of *C. japonicus* α -L-arabinanase Arb43A (PDB code: 1gyd) in yellow, influenza B virus neuraminidase (PDB code: 1nscA) in green, neuraminidase of subtype N9 from an avian influenza virus (PDB code: 5nn9) in cyan, and *Trypanosoma rangeli* trans-sialidase (PDB code: 1mz5) in white. Side (A) and top (B) view represent the catalytic acidic residues Asp38/Asp44 (block I), Asp158/Asp163 (block II), Glu221/Glu215 (block III) in *C. japonicus* and *B. subtilis* arabinanases, and the nucleophilic base Glu277+Tyr406 in neuraminidase N9. Side (C) and top (D) view represent the functional residues far from the active site: Arg331 from *B. subtilis* levansucrase; Trp261 from *Z. mobilis* levansucrase; Tyr249 and Pro284 from *T. cruzi* trans-sialidase; and Phe181 and Trp182 from *B. subtilis* endo-1,5- α -L-arabinanase; while side (E) and top (F) view represent the functional residues close to the active site: Tyr120 from *T. cruzi* trans-sialidase; His43, Trp104, and Phe124 from *B. subtilis* endo-1,5- α -L-arabinanase. The figure was prepared using the MOLSCRIPT program of Per Kraulis.²⁷ Structural similarities observed in the β -propeller topologies and the different functions hosts by this architecture are reviewed.³⁵

TABLE I. Fold Recognition Results for GH32, GH62, and GH68 Families Using 3D-PSSM, INBGU, SAM-T02, and mGenTHREADER

| Enzyme | 3D-PSSM | | mGenTHREADER | | INBGU | | SAM-T02 | |
|--|--------------|-------|--------------|-------|--------------|-------|--------------|--------|
| | PDB | Score | PDB | Score | PDB | Score | PDB | Score |
| <i>G. diazotrophicus</i> levanase Q9RBJ1 (GH32) | <u>1eur</u> | 0.845 | <u>1gydB</u> | 0.602 | <u>1gyhA</u> | 51.3 | <u>1gyhA</u> | 0.011 |
| | <u>1nscA</u> | 1.110 | <u>3sil</u> | 0.601 | <u>1cwvA</u> | 8.4 | <u>1cruA</u> | 0.136 |
| | <u>1gyhA</u> | 1.410 | <i>1l0qA</i> | 0.595 | <i>1gof</i> | 6.3 | <u>1qbiA</u> | 0.137 |
| | <i>1erjA</i> | 1.470 | <u>1c9uA</u> | 0.591 | <i>1flgA</i> | 3.7 | <u>1c9uA</u> | 0.299 |
| | <u>1eut</u> | 1.600 | <i>1qksA</i> | 0.583 | <i>1l0qA</i> | 3.6 | <u>1dim</u> | 0.308 |
| | <u>2sli</u> | 1.650 | <i>1gof</i> | 0.541 | <u>1eut</u> | 2.9 | <u>2sil</u> | 0.445 |
| | <u>3sil</u> | 1.660 | <u>1e07A</u> | 0.531 | <u>1eur</u> | 2.4 | <u>3sil</u> | 0.475 |
| | <i>1gxra</i> | 2.960 | <u>2er7E</u> | 0.527 | <i>1jjuB</i> | 2.2 | <u>1kit</u> | 0.479 |
| | <i>1eeeA</i> | 3.340 | <i>1a12A</i> | 0.520 | <i>1g72A</i> | 2.2 | <i>1h2xA</i> | 0.618 |
| | <i>1k8kC</i> | 3.380 | <u>1k0sA</u> | 0.517 | <u>1c9uA</u> | 2.2 | <u>1dfcA</u> | 0.663 |
| | | | | | | | | |
| <i>G. diazotrophicus</i> levansucrase SACB_ACEDI (GH68) | <u>1gyhA</u> | 0.555 | <u>1by5A</u> | 0.564 | <u>1gyhA</u> | 95.5 | <u>1gyhA</u> | 0.0001 |
| | <u>1nscA</u> | 0.729 | <u>1bf2</u> | 0.540 | <i>1gof</i> | 8.0 | <u>1gydB</u> | 0.046 |
| | <u>1dlpA</u> | 1.160 | <i>1gof</i> | 0.534 | <u>1c9uA</u> | 8.0 | <u>1gyeB</u> | 0.187 |
| | <i>1gof</i> | 1.420 | <u>1sat</u> | 0.526 | <u>1cwvA</u> | 5.0 | <i>1a12A</i> | 0.770 |
| | <u>2sli</u> | 1.770 | <u>2mprA</u> | 0.516 | <u>1ihmA</u> | 4.0 | <i>1qhuA</i> | 0.119 |
| | <u>1dnv</u> | 1.900 | <u>1fepA</u> | 0.514 | <u>1k32A</u> | 3.3 | <i>1pex</i> | 1.352 |
| | <u>1eur</u> | 2.540 | <u>1a0tP</u> | 0.508 | <i>1gof</i> | 2.8 | <u>117jA</u> | 1.647 |
| | <u>1qjvA</u> | 2.880 | <u>1c9uA</u> | 0.503 | <u>113wA</u> | 2.7 | <u>1nltA</u> | 1.661 |
| | <u>1pooA</u> | 3.000 | <u>1air</u> | 0.499 | <u>1gh7A</u> | 2.5 | <u>1mz5A</u> | 1.865 |
| | <u>1bwmA</u> | 3.030 | <u>1uok</u> | 0.490 | <i>1jx5A</i> | 2.2 | <u>1axiB</u> | 1.983 |
| | | | | | | | | |
| <i>S. cerevisiae</i> invertase INV2_YEAST (GH32) | <u>1gyhA</u> | 0.107 | <u>1gydB</u> | 0.709 | <u>1gyhA</u> | 37.5 | <u>1gyhA</u> | 0.156 |
| | <u>3sil</u> | 0.220 | <u>1kit</u> | 0.602 | <i>1gof</i> | 9.0 | <u>1eur</u> | 0.508 |
| | <u>1eut</u> | 0.227 | <u>1by5A</u> | 0.562 | <u>1cwvA</u> | 6.4 | <u>1dim</u> | 0.563 |
| | <u>1bihA</u> | 0.608 | <u>1kmoA</u> | 0.558 | <i>1jx5A</i> | 4.2 | <u>3sil</u> | 0.866 |
| | <i>1gog</i> | 0.612 | <i>1erjA</i> | 0.526 | <u>1dfcA</u> | 2.8 | <u>2sil</u> | 0.875 |
| | <u>1eur</u> | 0.633 | <u>2sli</u> | 0.517 | <u>1e1aA</u> | 2.8 | <u>1eut</u> | 0.913 |
| | <u>1cruA</u> | 2.530 | <i>1jofA</i> | 0.506 | <i>1k8kC</i> | 2.7 | <u>1euu</u> | 1.017 |
| | <i>1gof</i> | 3.310 | <i>1bpoA</i> | 0.500 | <u>1eur</u> | 2.5 | <u>1dfcA</u> | 1.109 |
| | <u>1fnf</u> | 3.720 | <u>1crzA</u> | 0.500 | <i>1l0qA</i> | 2.2 | <u>1htyA</u> | 1.262 |
| | <u>2sli</u> | 4.670 | <i>1gof</i> | 0.495 | <u>1mz5A</u> | 2.1 | <u>1hxxA</u> | 1.331 |
| | | | | | | | | |
| <i>C. japonicus</i> arabinofuranosidase XYNC_PSEFL (GH62) | <u>1tl2A</u> | 0.637 | <u>1gydB</u> | 0.604 | <u>1gyhA</u> | 28.9 | <u>1gyhA</u> | 0.517 |
| | <u>1ilmB</u> | 2.030 | <u>1tl2A</u> | 0.527 | <u>1tl2A</u> | 13.9 | <u>1n1tA</u> | 0.930 |
| | <u>1a65A</u> | 2.320 | <u>1qfmA</u> | 0.519 | <u>1lpxA</u> | 5.4 | <u>1mz5A</u> | 0.997 |
| | <u>1aozA</u> | 3.130 | <u>1m7xA</u> | 0.518 | <u>1hwmB</u> | 3.5 | <u>117jA</u> | 1.958 |
| | <u>1l7lA</u> | 3.450 | <u>1bihA</u> | 0.506 | <u>1dlpA</u> | 2.5 | <i>1gen</i> | 2.167 |
| | <u>1hwpB</u> | 3.760 | <i>1gof</i> | 0.504 | <u>1dlpA</u> | 2.5 | <i>1gen</i> | 2.380 |
| | <u>1bxfA</u> | 5.180 | <u>1bjbA</u> | 0.497 | <u>1efaA</u> | 1.9 | <u>1mmuA</u> | 2.437 |
| | <u>1bp3B</u> | 5.260 | <i>1flgA</i> | 0.493 | <i>1gof</i> | 1.5 | <u>1nszA</u> | 2.647 |
| | <u>1kacA</u> | 5.880 | <u>1qinA</u> | 0.486 | <u>1hcxA</u> | 1.4 | <u>1tl2A</u> | 2.943 |
| | <u>1qclA</u> | 5.970 | <u>1by5A</u> | 0.480 | <u>1lrxB</u> | 1.2 | <u>1ebpA</u> | 3.214 |
| | | | | | | | | |

Note: The β -propeller topologies are represented by 4-bladed haemopexin (1qhuA), gelatinase A (1gen), and collagenase-3 (1pex); 5-bladed tachylectin-2 (1tl2A) and Arb43A (1gydB, 1gyhA); 6-bladed tricorn protease (1k32A), bacterial sialidase (2sil, 3sil, 1kit, 1eur, 1eut, 1euu, 1dim), influenza B virus neuraminidase (1nscA), leech (2sli) and trypanosomal (1mz5A, 1n1tA) trans-sialidases, diisopropyl fluoro phosphatase (1elaA), phytase (1pooA), TolB protein (1crzA), LRP1 protein (1lpxA), human low-density lipoprotein receptor (1lrxB), and glucose dehydrogenase (1cruA, 1c9uA, 1qbiA); 7-bladed Tup1 protein (1erjA), galactose oxidase (1gof, 1gog), RCC1 protein (1a12A), clathrin heavy chain (1bpoA), prollyl oligopeptidase (1qfmA, 1h2xA), groucho/TLE1 (1gxra), Arp2/3 complex (1k8kC), quinoheomoprotein amine dehydrogenase (1jjuB), protein-binding YVTN (1l0qA), integrin α -IIB (1jx5), and muconate lactonizing enzyme (1jofA); 8-bladed quinoprotein alcohol dehydrogenase (1eeeA), ethanol dehydrogenase (1flgA), methanol dehydrogenase (1g72A), and cytochrome *cd1* (1qksA). Bold and underlined letters represent the 5- and 6-bladed β -propellers, while italics represent 7- and 8-bladed ones.

presented in this work. The enzymatic function of furanoside hydrolysis has evolved independently on at least three structural frameworks: (β/α)₈-barrel (GH51), β -propeller (GH43), and right-handed β -helix (GH91) architectures.

Similar Architectures in Retaining and Inverting Enzymes

Calculation of the distances between the oxygen atoms of the carboxyl groups of the nucleophilic base Asp38 (block I) and the proton donor Glu221 (block III) in the

TABLE II. Three Key Acidic Residues of Enzymes Acting on Pyranoside and Furanoside Substrates With a Canonical Reaction Mechanism of Inversion or Retention of the Anomeric Configuration

| | | Fold | Mechanism | (p.d., n.b., r.p.) | PDB | Ref. |
|--|------|---|-----------|------------------------|------|------|
| <i>Enzymes acting on pyranoside substrates</i> | | | | | | |
| α -glucuronidase A (GlcA67A) | GH67 | (β/α) ₈ -barrel | Inverting | Glu292, Asp365, Glu393 | 1GQI | [30] |
| α -amylase (TAKA-amy) | GH13 | (β/α) ₈ -barrel | Retaining | Glu230, Asp206, Asp297 | 2TAA | [31] |
| trypanosomal sialidases | GH33 | β -propeller | Retaining | —, Tyr343+Glu231, — | 1MZ5 | [32] |
| <i>Enzymes acting on furanoside substrates</i> | | | | | | |
| α -L-arabinanase 43A (Arb43A) | GH43 | β -propeller | Inverting | Glu221, Asp38, Asp158 | 1GYD | [14] |
| levansucrase (LsdA) | GH68 | β -propeller* | Retaining | Glu401, Asp135, Asp309 | — | [29] |
| invertase (Suc2) | GH32 | β -propeller* | Retaining | Glu204, Asp23, Asp152 | — | [28] |
| α -L-arabinofuranosidase (AbfA T-6) | GH51 | (β/α) ₈ -barrel | Retaining | Glu175, Glu294, — | — | [7] |

Note: *Predicted architecture based on 6-bladed β -propeller. p.d., proton donor; n.b., nucleophilic base; r.p., residue modulating pKa or the orientation of the proton donor; —, functional residue not identified.

TABLE III. Different Combinations of Nucleophilic Base and Proton Donor for GH Clans With (β/α)₈ Barrel and β -Propeller Architectures

| Clan | Fold | GH families | Nucleophile/base | Proton donor |
|------|---|---|------------------|--------------|
| GH-A | (β/α) ₈ -barrel | GH1, GH2, GH5, GH10, GH17, GH26 GH30, GH35, GH39, GH42, GH51 GH53, GH59, GH72, GH79, GH86 | Glu | Glu |
| GH-D | (β/α) ₈ -barrel | GH27, GH36 | Asp | Asp |
| GH-H | (β/α) ₈ -barrel | GH13, GH70, GH77 | Asp | Glu |
| GH-K | (β/α) ₈ -barrel | GH18, GH20 | C-2 | Glu |
| GH-E | β -propeller | GH33, GH34, GH83 | Tyr+Glu | Not known |

Note: All GH clans contain families that retain the anomeric configuration at the cleavage point. C-2 is carbonyl oxygen of C-2 acetamido group of substrate.

crystal structure of Arb43A¹⁴ gives 5.90 Å between oδ1 Asp38 and oε2 Glu221, and 8.26 Å between oδ2 Asp38 and oε1 Glu221. Both values are in agreement with the structural requirements for inverting (GH43) and retaining (GH32, GH68) enzymes.^{9,10} The mechanism of substrate hydrolysis for GH62 is still unknown. We propose the inverting mechanism of hydrolysis for GH62 on the basis of substrate similarity with GH43 family (α -L-arabinofuranosidase activity).

Crystal structures of two *C. japonicus* (*Pseudomonas cellulose*) glycosidases solved by Nurizzo et al.: α -L-arabinanase Arb43A¹⁴ and α -glucuronidase GlcA67A,³⁰ together with the recently determined 3D structure of *G. stearothermophilus* T-6 α -L-arabinofuranosidase (AbfA T-6) by Hoevel et al.,⁷ provide examples of GH families where the substrate, the mechanism of hydrolysis, and even the combination of nucleophilic base and proton donor residues are not conserved respect to GH families or clans, having similar architectures. GH families sharing (β/α)₈-barrel architectures and three carboxylates at the active site can operate with inverting (GlcA67A, GH67) and retaining (GH-clans A, D, H, and K) hydrolysis of pyranoside and furanoside substrates.^{7,30,31} A similar scenario is observed for GH families 32, 33, 43, 62, and 68 acting on pyranoside and furanoside substrates. These enzymes share a β -propeller architecture and operate with inverting (GH43 and GH62) and retaining (GH32, GH33, and GH68) mechanisms of hydrolysis (Tables II and III).

For the enzymes, there is no clear correlation between the Enzyme Commission (EC) classification and the protein architecture. Enzymes with the same EC number may exhibit different folds and vice versa. Indeed, many GH families are polyspecific (containing at least two EC numbers).² A small cluster of catalytic residues that can be placed on almost any architecture determines the enzyme function. Figure 2(A and B) show the superposition of the proton donor residue Glu221 in the protein Arb43A and the nucleophilic base Glu277 in neuraminidase N9 [Protein Data Bank (PDB) code: 5nn9]. Based on the similar location of the active site in the β -propeller architecture of GH43 and clan GH-E, and the other observations presented in this section (Tables II and III), we suggest that some GH families with similar 3D structures, and acting on pyranoside and furanoside substrates, would operate with a canonical reaction mechanism of inversion/retention of anomeric configuration using three key acidic residues.

Distinct Binding Sites for the Acceptor Saccharide in Glycosyl Transfer Reaction

Some retaining GH catalyzes glycoside transfer reactions in addition to glycosyl hydrolysis, for example, the sialyltransferase reaction of trypanosomal trans-sialidases (GH33) and the fructosyltransferase reaction of bacterial levansucrases (GH68).^{1,2} Also, at high concentrations of substrate, the *S. cerevisiae* invertase (GH32) can transfer the fructosyl moiety to primary alcohols such as

methanol and ethanol, and to monosaccharides.¹² According to the sequence–structure compatibility searches presented here, and the published crystal structure, these GH families share a β -propeller architecture.

Previous site-directed mutagenesis studies have revealed a functional role for Tyr120, Tyr249, and Pro284, in the sialyltransferase reaction of *Trypanosoma cruzi* trans-sialidase,³² Arg331 in the fructosyl transfer reaction of *B. subtilis* levansucrase,²⁰ and Trp261 in the fructosyl transfer reaction of *Z. mobilis* levansucrase.³³ On the other hand, mutation of His43, Trp104, Phe124, Phe181, and Trp182 (His13, Trp74, Phe94, Phe151, and Trp152 in mature protein) to alanine in the *B. subtilis* endo 1,5- α -L-arabinanase (GH43) affected the efficient binding and hydrolysis of arabinofuranose and long-chain arabinans.¹⁵

After a structural superposition of the 5-bladed (GH43) and 6-bladed (GH33) β -propellers, the analysis of the location of these aromatic, hydrophobic, and polar residues, revealed that (1) Arg331 and Trp261 are located in different positions far from the active site; (2) Tyr249 and Pro284 are close in space to Arg331; (3) Phe181 and Trp182 are far from the active site in a different position than Tyr249, Trp261, Pro284, and Arg331; (4) His43 is neighbor of the catalytic Asp44 (block I); (5) Trp104 is close in space to the catalytic Glu277 of neuraminidase N9; and (6) Phe124 is in close vicinity to Tyr120, both close to the active site.

Positions far from the active site are involved in sialyl and fructosyl transfer reactions, as well as in the hydrolysis of long-chain glycans, while positions close to the active site are important for the orientation of substrates and the efficient hydrolysis of short glycans [Fig. 2(C–F)]. Thus, based on the structural compatibility between GH68 and the 6-bladed β -propeller of sialidase/neuraminidase, we propose that the β -propeller architecture accommodates distinct binding sites for the acceptor saccharide in glycosyl transfer reaction.

NOTE

The crystal structure of *Bacillus subtilis* levansucrase has been recently published [36]. This structure (PDB codes: 1OYG (apo) and 1PT2 (complex)) reveals a five-bladed β -propeller with three strictly conserved acidic residues at the active site.

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