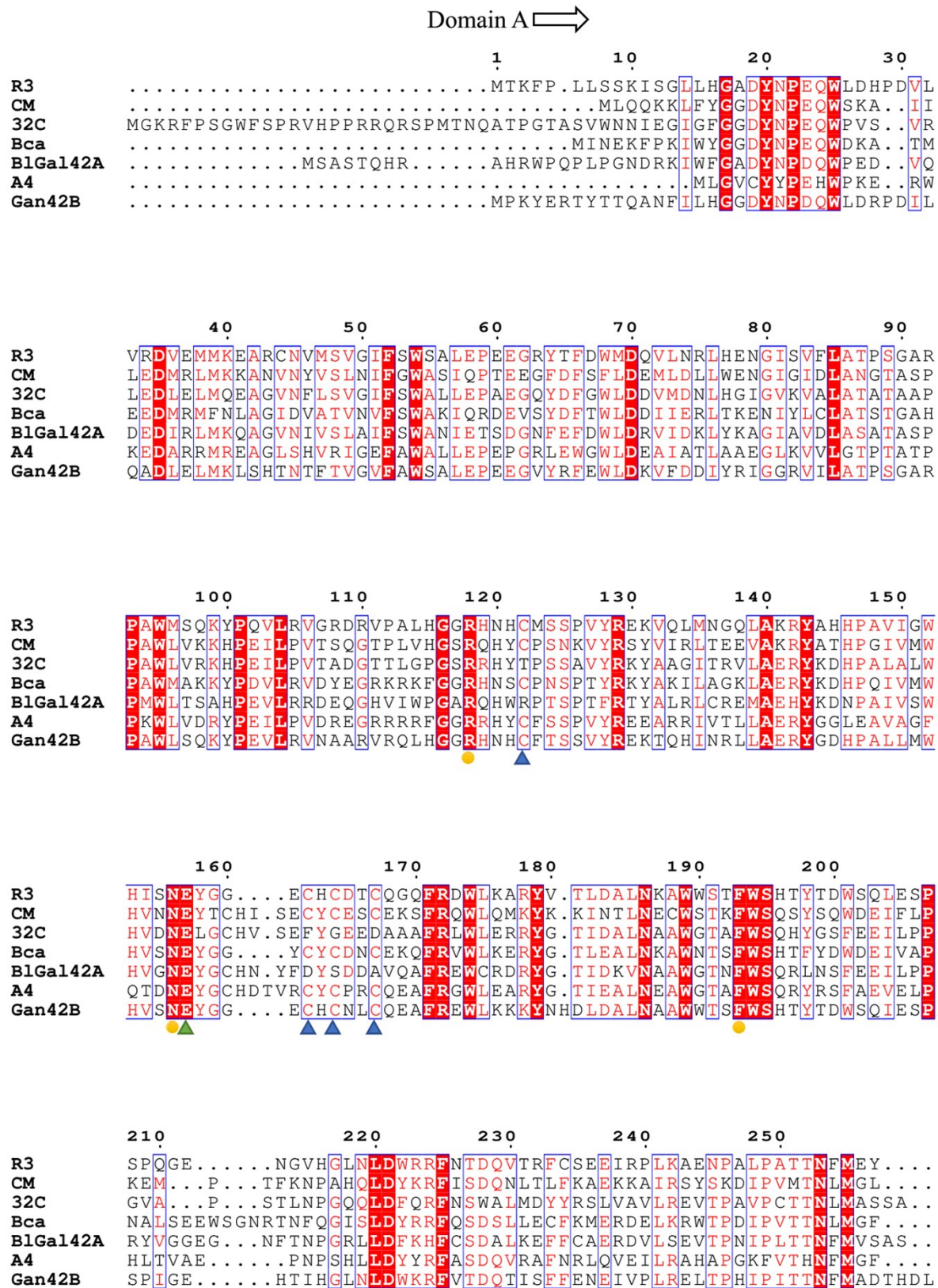


Figure S1



260 270 280 290 300

R3 ..FNDYDYWKLAVGIDFISWDSYPMWHTRQDDIG.....L.AAYTAMYHDLMRITL
 CM ..HKHVDGFAFAEEMDVVGWDSYPNPFEEK.P.....YPQFLANDITRS.
 32C ..TKSMDFYSWAKDIDVIANDHYLVAADPERH.....IELAFSADLTRGI
 Bca ..YPELDYFKWAKEMDVVSWDNYP SMDTPF.....SFTAMAHNLMRGL
 BlGal142A ..QNTLDYDDWAHEVDFVSNHDHYFTPGSWH.I.....DELAYSASLVDGI
 A4 ..FTDLDAFALAQDIDFASWDSYPLGFTDLMPLPPEEKLRYPARTGHPDVAFFHHDLYRGV
 Gan42B IPFQGLDYSKFAKHLDVLSWDAYPAWHNDWESTA.....DLAMKVGFINDLYRSL

310 320 330 340 350 360

R3 KQGKPFVLMESTPSFTNWQPTSKLKKPCGMHILSLQAVAHGADSVQYFQWRKSRGSC^{EKF}
 CM LKKKPFVLMQAPSAVNWRRRANGAKSPGQMLRWSYEALAHGADGILFFQWRQSQQGA^{EKF}
 32C AGGD^{PWILME}HSTSAVNWQPRNQPKMPEMLRNSLAHVARGADAVMFFQWRQSFAGS^{EKF}
 Bca KSGQPFVLMQTPGVQNWQPYNSAKRPGVMRLWSYQAVAHGADTVMFFQLRRSVGAC^{EKY}
 BlGal142A SRKK^{PWFLME}QSTSAVNWREINPRKEPGEIIRD^{SMLHLAMGAD}AICYFQWRQSRSGA^{EKF}
 A4 GRGR.FVWMEQPGPVNWAPHNPSAPAPGMVRLWTWEALAHGAEVVSYFRWRQAPFAQ^{EQM}
 Gan42B K.QQ^{PFLME}CTPSLVNWHKVNAKARPGMHFLSMSQ^{MIAHGSDS}ILYFQWRKSRGSF^{EKF}

370 380 390 400 410 420

R3 HGAVVDHVGHIDTRVGREVAELGSI^{LSALAP}...VAGSRVEAKVAIFD^{WESRW}AMDDAM
 CM HSGMVS^{HNQDTN}SRIFKEVQLGTEMSQLDE...LVGTNYNAEVAIVFD^{WENW}WALELDA
 32C HSAMVPHGG.RDTRVWREVDLGAALQLLAP...VRGSRVESRAAIVFD^{YEA}WAWA^{SE}IDS
 Bca HGAVIEHVGHEHTRVFRECAELGKELQQ^{LGD}..TILDARSEAKVAVMYD^{WENRW}ALELSS
 BlGal142A HSAMPLLAG.EHSQIYRDV^{CALGAD}LDTLSDAGILRSKLSKARVAIVQ^{DIQSE}WATEHTA
 A4 HAGLHRPDSAPDQG.FFEAKRVAEELAA^{LA}...LPVVAQAPVALVFD^{YEA}AWIYEVQ.
 Gan42B HGAVVDH^{DNRTD}SRVFEVAE^{VGKAL}LKKMSG...IVGTNRPAEVAI^{LYD}WENWAL^{LN}DAQ

Domain B ⇨

430 440 450 460 470 480

R3 GPRNAGLHYENTVADHYRALWAQGI^{AVDVI}NADCDLQGYD^{LVIAP}MLYMVRE^{GV}GERISA
 CM KPSG.EIN^{YIKQMR}LDLYTIFHELNIGVDFIHPKEDLSN^{YKLVLS}IAQYLV^{TD}DFSAK^VKR
 32C KPSI.DVR^{YLDL}LRAFHRSLFLRGVSDMVHPSASLDG^{YD}LVLVCT^{LYSV}DEAAAN^{IAA}
 Bca GPSI.ALN^{YVNE}VHKYD^{ALYKQN}IQTDMI^{SVEED}LSKYK^{VVIAP}VMYMK^{PGFA}ERVER
 BlGal142A TPTQ.HIREWTEPLDWFAAFANRGV^{TADV}TPIHAQWD^{TD}YD^{AVVIP}CVYLF^{EEMA}ERLRT
 A4 .PQGA^{EWSY}LGLVYLFYSALRR^{LG}LVDVVP^{PGAS}LRGYAF^{AVVP}SLPIV^{RE}EAL...EA
 Gan42B GFA^{AETKR}YPQT^{LVQHY}RPFWERD^{IPVDVI}TKEHDFSR^{YKLLIAP}MLYLV^{SEET}IARL^{KE}

490 500 510 520 530 540

R3 FVQA^{GGRFV}ATYWSGIVN^{ET}DLCFLNGFPG^{PLRP}VLGIWA^{EED}ISL^{TDEQHNS}VAGVEGN
 CM YIKA^{GGHFL}TTFSSGIVD^{EY}DRVYLG^{YPGA}FK^{EV}LGIV^{VEE}FDPMPIGR...KSQIK
 32C AAAGGATV^{LVS}YFSG^{ITDE}KD^{HVRL}GGYPGA^{FR}ELLG^{VRVEE}FHP^{LLAGS}...QLKLS
 Bca FVAQGGTFV^{TTFSSGIVN}ENDL^{VTL}GGYPGA^{LRN}VMGIWA^{EED}IDL^{LPGHQNE}IVL^{RQD}
 BlGal142A FVRN^{GGKAFV}TYYSALAD^{EHDR}LHTEGWPGLIGD^{VVGV}RIEEH^{CP}LGTLP^{FGML}DHLDVS
 A4 FREAE^{GPVLF}GPRSGSKT^{ET}FIQ^{PKELPP}GPLQAL^{LLPL}KVVR^{VES}LPPGLLEVAE...EA
 Gan42B FVAN^{GGT}LM^{TYISGIVD}ED^{HL}LA^{YLG}GWHQD^{LR}EMFG^{MEPI}ET^{DT}LYPRDRNS^{VHY}...

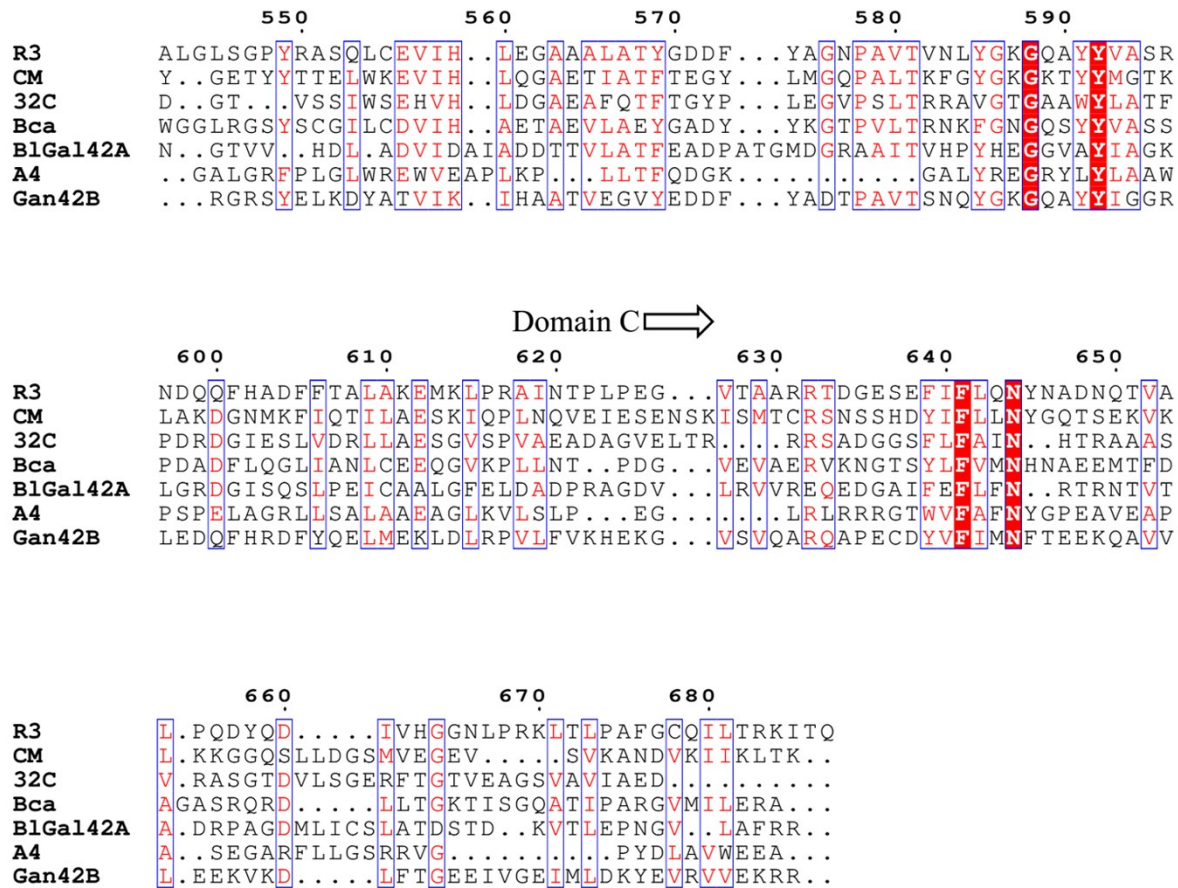


Figure S1. Comparison of the β -gal amino acid sequences in GH42 family. The result of ClustalO multiple sequence alignments of β -gals from different sources is shown. Included in the alignments are β -gals from *Rahnella* sp.R3 (R3, psychrophile), *Carnobacterium maltaromaticum* (CM, psychrophile), *Arthrobacter* sp. 32c (32C, psychrophile), *Bacillus circulans* sp. Alkalphilus (Bca, mesophile), *Bifidobacterium animalis* subsp. *lactis* Bl-04 (B1Gal42A, mesophilie), *Thermus thermophiles* A4 (A4, thermophiles) and *Geobacillus stearothermophilus* (Gan42B, thermophiles). The catalytic residues, metal binding residues, and residues involve in substrate binding are marked by green triangles, blue triangles, and yellow dots, respectively.

Figure S2

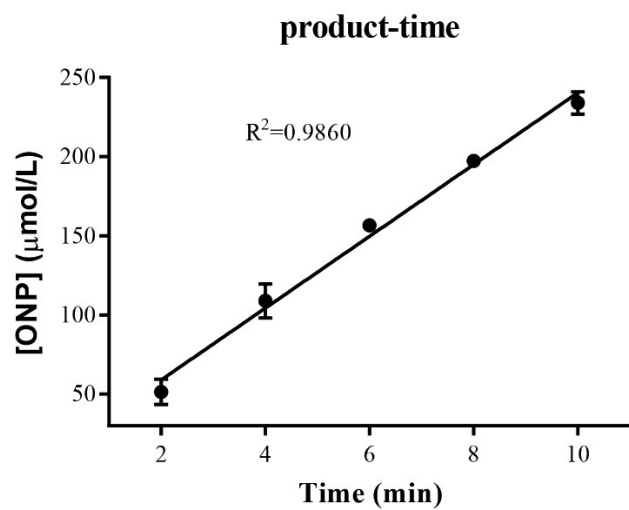


Figure S2. Product vs time plot of R-β-Gal.