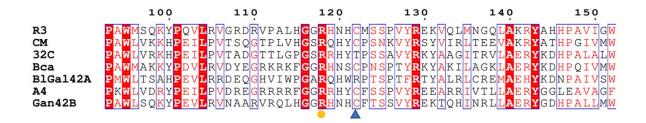
Electronic Supplementary Material (ESI) for RSC Advances. This journal is © The Royal Society of Chemistry 2016

## **Figure S1**

	Domain A 🚞				
		i	ıņ	20	30
R3 CM		MTKFE	.LLSSKISGL	LH <mark>G</mark> ADYNPEQ	WLDHPDVL Wska ti
32C	MGKRFPSGWFSPRVHPPRRQRSP	MTNQATPGI	TASVWNNIEG <mark>I</mark>	GF <mark>G</mark> GD <mark>Y</mark> NPEQ	WPVSVR
Bca BlGal42A	MSASTQHR	AHRWE	PQPLPGNDRKI	WF <mark>G</mark> AD <mark>Y</mark> NPDQ	WPEDVQ
A4 Gan42B	••••••				

	40	50	60	7 <u>0</u>	80	эö
R3	VRDVEMMKEARCN					
CM	LEDMRLMKKANVN					
32C	LEDLELMQEAGVN					
Bca	EEDMRMFNLAGID	VATVNVFSW	AKIQRDEVSYI	)FTWL <mark>D</mark> DIIE	RLTKENIYLC	ATSTGAH
BlGal42A	DEDIRLMKQAGVN	IVSLAIFSW	ANIETSDGNFI	EFDWL <mark>D</mark> RVID	KLYKA <mark>GIAV</mark> DI	LASATASP
A4	KEDARRMREAGLS	HVRIGE <mark>F</mark> AW	ALLEPEPGRLE	EWGWL <mark>D</mark> EAIA	TLAAEGLKVV <mark>I</mark>	GTPTATP
Gan42B	QADLELMKLSHTN	TFTVGVFAW	SALEPEEGVYE	RFEWLDKVFD	DIYRIGGRVII	ATPSGAR



	160	170	180	190	200
R3	HISNEYGGECH	HCDTCQGQFRDW	LKARYV.TLDA	LNKAWWSTFW	SHTYTDWSQLESP
CM	HVNNEYTCHI.SEC				
32C	HVDNELGCHV.SEF				
Bca	HVS <mark>NE</mark> YGGYC				
BlGal42A	HVG <mark>NE</mark> YGCHN.YFD				
A4	QTD <mark>NEYG</mark> CHDTVRCY				
Gan42B	HVSNEYGGECH	HCNLCQEAFREW	LKKKYNHDLDA	LNAAWWTSEW	SHTYTDWSQIESP
				-	

	210	220	230	240	250
R3	SPQGENGV	HGLNLDWRRF	NTDQVTRFCSE	EIRPLKAENP	ALPATTNFMEY
CM	KEMPTFKN	IPAHQ <mark>LD</mark> YKR <b>F</b>	ISDQNLTLFKA	EKKAIRSY <mark>S</mark> K	DIPVMTNLMGL
32C	GVAPSTLN	IPGQQ <mark>LD</mark> FQR <mark>F</mark> I	NSWALMDYYRS	LVAV <mark>LR</mark> EV <b>TP</b>	AVPCTT <mark>N</mark> L <mark>M</mark> ASSA
Bca					DIPVTT <mark>N</mark> L <mark>M</mark> GF
BlGal42A					NIPLTT <mark>N</mark> F <mark>M</mark> VSAS
A4					GKFVTH <mark>N</mark> F <mark>M</mark> GF
Gan42B	SPIGEHTI	HGLNLDWKRF	VTDQTISFFEN	EIVPLRELTP	H <mark>IPITTN</mark> F <mark>M</mark> ADTHDL

	260	270	280	290	300
R3 CM 32C Bca B1Ga142A A4 Gan42B	. HKHVDGFA . TKSMDYFS . YPELDYFK . QNTLDYDD . FTDLDAFA	FAEEMDVVGWD WAKDLDVIAND WAKEMDVVSWD WAHEVDFVSND LAQDLDFASWD	SYPMWHTRQDDIG SYPNPFEEK.P HYLVAADPERH NYPSMDTPF HYFTPGSWH.I SYPLGFTDLMPLPPEE AYPAWHNDWESTA	YPQF IELA SFTA DELA KLRYARTGHPDVAA	LANDLTRS. FSADLTRGI MAHNLMRGL YSASLVDGI FHHDLYRGV
	310	320	330 340	350	360
R3 CM 32C Bca B1Ga142A A4 Gan42B	LKKKPFLVME AGGDPWILME KSGQPFMLME SRKKPWFLME GRGR.FWVME	QAPSAV <mark>NW</mark> RRA HSTSAV <mark>NW</mark> QPR QTPGVQ <mark>NW</mark> QPY QSTSAVNWREI QQPGPVNWAPH	SKLKKPGMHILSSLQA NGAKSPGQMRLWSYEA NQPKMPGEMLRNSLAH NSAKRPGVMRLWSYQA NPRKEPGELIRDSMLH NPSPAPGMVRLWTWEA NKAKRPGMHFLSSMQM	LAHGADGILFFQWF VARGADAVMFFQWF VAHGADTVMFFQLF LAMGADAICYFQWF LAHGAEVVSYFRWF	QSQGGA <mark>BKF</mark> QSFAGS <mark>BKF</mark> RSVGAC <mark>BKY</mark> QSRSGABKF QAPFAQ <mark>B</mark> QM
				Domain B  →	
	370	380		0	420
R3 CM 32C Bca B1Ga142A A4 Gan42B	HSGMVSHNQD HSAMVPHGG. HGAVIEHVGH HSAMLPLAG. HAGLHRPDSA	TNSRIFKEVVQ RDTRVWREVVD EHTRVFRECAE EHSQIYRDVCA PDQG.FFEAKR	LGSILSALAPVAG LGTEMSQLDELVG LGAALQLLAPVRG LGKELQQLGDTILD LGADLDTLSDAGILRS VAEELAALALP VGKALKKMSGIVG	TNYNAEV <mark>AIVFDW</mark> E SRVESRAAIVFDYE ARSEAKVAVMYDWE KLSKARVAIVQDIQ PVAQAPVALVFDYE	NWWALELDA AWWASEIDS NRWALELSS SEWATEHTA AAWIYEVQ.
	420		450	<b>60 170</b>	
R3 CM 32C Bca BlGal42A A4 Gan42B	KPSG.EINYI KPSI.DVRYL GPSI.ALNYV TPTQ.HIREW .PQGAEWSYL	KQMRDLYTIFH DLLRAFHRSLF NEVHKYYDALY TEPLDWFAAFA GLVYLFYSALR	450 AQGIAVDVINADCDLQ ELNIGVDFIHPKEDLSI LRGVSVDMVHPSASLDG KQNIQTDMISVEEDLSI NRGVTADVTPIHAQWD RLGLDVDVVPPGASLR ERDIPVDVITKEHDFSI	NYKLVLSIAQYLVI GYDLVLVCTLYSVI KYKVVIAPVMYMVK TYDAVVIPCVYLFS GYAFAVVPSLPIVF	DDFSAKVKR DEAAANIAA PGFAERVER EEMAERLRT EEALEA
		500	510 -		E 4 0
R3 CM 32C Bca BlGal42A A4 Gan42B	YIKAGGHFLT AAAGGATVLV FVAQGGTFVT FVRNGGKAFV FREAEGPVLF	TFFSGIVDEYD SYFSGITDEKD TFFSGIVNEND TYYSALADEHD GPRSGSKTETF	510 5: LCFLNGFPGPLRPVLG RVYLGGYPGAFKEVLG HVRLGGYPGAFRELLG LVTLGGYPGELRNVMG RLHTEGWPGLIGDVVG QIPKELPPGPLQALLP LAYLGGWHQDLREMFGI	IYVEEFDPMPIGR. VRVEEFHPLLAGS. IWAEEIDALLPGHC VRIEEHCPLGTLFF LKVVRVESLPPGLI	KSQIK QLKLS NEIVL.RQD GMLDHLDVS EVAE

	550	56	o 5	70	580	590
R3 CM 32C Bca B1Ga142A A4 Gan42B	YGETYYTT DGTVS WGGLRGSYSC NGTVV.H .GALGRFPL	ELWKEVIH SIWSEHVH GILCDVIH DL.ADVIDAI GLWREWVEAP	LQGAETIAT LDGAEAFQT AETAEVLAE ADDTTVLAT LKPLLT	YGDDFYAC FTEGYLMC FTGYPLEC YGADYYKC FEADPATGMDC FQDGK YEDDFYAL	QPALTKFGYC VPSLTRRAVC TPVLTRNKFC RAAITVHPYF	KGKTYYMGTK TGAAWYLATF NGQSYYVASS EGGVAYIAGK EGRYLYLAAW
			Dennin Cl			
			Domain C	/		
	600	610	620	630	640	650
R3 CM 32C Bca B1Ga142A A4 Gan42B	LAKDGNMKFI PDRDGIESLV PDADFLQGLI LGRDGISQSL PSPELAGRLL	QTILAESKIQ DRLLAESGVS ANLCEEQGVK PEICAALGFE SALAAEAGLK	PLNQVEIES PVAEADAGV PLLNTPD LDADPRAGD VLSLPE	GVTAARRI ENSKISMTCRS ELTRRRS GVEVAERV VLRVVRE GLRI GVSVQAR	NSSHDYIFLI ADGGSFLFA KNGTSYLFV EDGAIFEFL RRRGTWVFA	NYGQTSEKVK NHTRAAAS NHNAEEMTFD NRTRNTVT NYGPEAVEAP

	660	670 680
R3		GGNLPRKLTLPAFGCQILTRKITQ
CM		GEVSVKANDVKIIKLTK
32C Bca		GTVEAGSVAVIAED GKTISGOATIPARGVMILERA
BlGal42A		DSTDKVTLEPNGVLAFRR
A4		G
Gan42B	L.EEKVKDLFT	GEEIVGEIMLDKYE <mark>V</mark> R <mark>VV</mark> EKRR

Figure S1. Comparison of the β-gal amino acid sequences in GH42 family. The result of ClustalO multiple sequence alignments of  $\beta$ -gals from different sources is shown. Included in the alignments are β-gals from *Rahnella* sp.R3 (R3, psychrophile), *Carnobacterium maltaromaticu*m (CM, psychrophile), Arthrobacter sp. 32c (32C, psychrophile), Bacillus circulans sp. Alkalphilus (Bca, mesophile), Bifidobacterium animalis subsp. lactis Bl-04 (BlGal42A, 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.



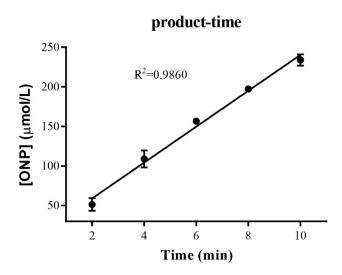


Figure S2. Product vs time plot of R-β-Gal.