

**Characterization of a Novel β -L-Arabinofuranosidase in *Bifidobacterium longum*:
FUNCTIONAL ELUCIDATION OF A DUF1680 FAMILY MEMBER**

**Kiyotaka Fujita¹, Yukari Takashi, Eriko Obuchi, Kanefumi Kitahara and Toshihiko
Suganuma**

From the Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890-0065,
Japan.

Running head: Characterization of a novel β -L-arabinofuranosidase.

¹To whom correspondence should be addressed. Tel./Fax: 81-99-285-8639; E-mail:
kfujita@ms.kagoshima-u.ac.jp.

SUPPLEMENTARY FIGURE LEGEND

Supplementary FIG. S1. Multiple sequence alignment of HypBA1 and its homologs. The homologous proteins are shown in Fig. 11. The alignment was created with MUSCLE and BoxShade 3.21. Identical residues and conservative substitutions are highlighted in black and dark gray, respectively. Asterisks indicate the residues selected for site-directed mutagenesis. The protein characterized in this study is enclosed in the box.

BL1 242 ---NIFHDLGFYKPTYFQ---AAEPVRDQQTADGHAVR-----VGYLCTGVAHVGRLL
 BI1 242 ---NIFHDLGFYKPTYFQ---AAEPVRDQQTADGHAVR-----VGYLCTGVAHVGRLL
 BLL1 242 ---NIFHDLGFYKPTYFQ---AAEPVRDQQTADGHAVR-----VGYLCTGVAHVGRLL
 BB 214 ---NIFPDLGFYKPTYFQ---AAEPVRDQQTADGHAVR-----VGYLCTGVAHVGRLL
 BD1 242 ---YIFRDLGFYKPEYFQ---AAEPVRDQQTADGHAVR-----VGYLCTGVAHVGRLL
 BP1 242 ---YIFRDLGFYKPTYFQ---AAQPVREQQTADGHAVR-----VAYLCTGIAHVARI
 BC1 242 ---YIFRDLGFYKPTYFQ---AAQPVREQQTADGHAVR-----VAYLCTGIAHVARI
 BAn 250 GLPAIFPAMETWSHEYTL---TARPIRDQQTAVGHAVR-----VAYLLAGVMQVGRLL
 BC3 250 ----FYTDLHM-PLKYEYV---QDEPILDKQHAEGHAVR-----LLYLAAAVSKVGRLL
 BD2 271 DGRNYEPREQ--NYAYYQ---ADKPVTEQTEALGHAVR-----AAYFYSGVADVARI
 BC2 277 DGRNYEPREQ--NYTYYYQ---ADKPVTEQTEALGHAVR-----AAYFYSGVADVARI
 BAd 270 DGRNYAPREQ--NYAYYQ---ADKPVTEQTEALGHAVR-----AAYFYAGADVARI
 BI2 269 ADANYKPNTDPSRYAYHQ---ANKPVTEQDEAVGHAVR-----AGYFYSGIADVARI
 BL2 269 ADANYKPNTDPNRYAYHQ---ANKPVTEQDEAVGHAVR-----AGYFYSGIADVARI
 BLL2 269 ADANYKPNTDPNRYAYHQ---ANKPVTEQDEAVGHAVR-----AGYFYSGIADVARI
 BP2 233 ERYHVMADR-FKDHAIEDPLAQGEDVLTGMHANTQIPK-----VLCWERLIGAI
 BLL4 337 RSEFLKASAFEDTDKLDNCGAGVDLNNLHANQHHPQ-----FVGYAKDAAMGDADI
 BLL3 598 KQTVLTAAHLEDETALFQKLANGQDPLNGLHANTTIPKLTGAMQRYVAYTEDEDLYNS-L

*E322

BL1 288 LGDQG-----LIDTAKREWKNIIVTIRMYVTGAIGSTHV---GESFTYDYDLPN--DTM
 BI1 288 LGDQG-----LIDTAKREWKNIIVTIRMYVTGAIGSTHV---GESFTYDYDLPN--DTM
 BLL1 288 LGDQG-----LIDTAKREWKNIIVTIRMYVTGAIGSTHV---GESFTYDYDLPN--DTM
 BB 260 LGDQG-----LIDTAKREWKNIIVTIRMYVTGAIGSTHV---GESFTYDYDLPN--DTM
 BD1 288 TGDRC-----LLDAVHRMWNISIVGKRMVVTGAVGSTHV---GESFTYDYDLPN--DTM
 BP1 288 TGDQG-----LLDAAHREFWNNIVSKRMVVTGAIGSTHV---GESFTYDYDLPN--DTM
 BC1 288 TGDQG-----LLDAAHREFWNNIVSKRMVVTGAIGSTHV---GESFTYDYDLPN--DTM
 BAn 299 TNDEG-----LLRTGERLWNNIVHKRMYITGGIGSTHV---GEAFTYDYDLPN--DTM
 BC3 294 LNDQK-----MLDTAERLWNIIVKRMVITGAVGSCQV---GESFSFDDDLPN--DLV
 BD2 318 TGEAT-----LLESCETLWRNIVDRKLYITGGIGATHM---GEAFSFDYDLPN--DTA
 BC2 324 TGEAA-----LLESCETLWRNIVDRKLYITGGIGATHM---GEAFSFDYDLPN--DTA
 BAd 317 TGDSD-----LLASCERLWRNIVDRKIYITGGIGATHM---GEAFSFDYDLPN--DTA
 BI2 318 ADDQD-----LADAAERLWRNIVDKKLYVTGGIGGTVD---GEAFSYNYDLPN--DSA
 BL2 318 ADDQD-----LADAAERLWRNIVDKKLYVTGGIGGTVD---GEAFSYNYDLPN--DSA
 BLL2 318 ADDQD-----LADAAERLWRNIVDKKLYVTGGIGGTVD---GEAFSYNYDLPN--DSA
 BP2 280 CNDEQ-----ADAATNTFWDSVVEHRS---VSICAHSV---SEHFHPTDDFSSMIESR
 BLL4 390 DADARAR---YLKAVEGYWGMIVPGRMYAHGGTGE---GEMWGAHTVAG--DI-
 BLL3 657 SADERGKLTSLYLKAAQNFEDIIVVKDHTYVNGGNSQSEHFHVAGELWKDA-TQNG--DQN

*E338

*E366

BL1 336 YG-----ETCASVAMSMFAQQMLDLEPKGEYADVLEKELFNCSIAGISLDGKQYYYVN
 BI1 336 YG-----ETCASVAMSMFAQQMLDLEPKGEYADVLEKELFNCSIAGISLDGKQYYYVN
 BLL1 336 YG-----ETCASVAMSMFAQQMLDLEPKGEYADVLEKELFNCSIAGISLDGKQYYYVN
 BB 308 YG-----ETCASVAMSMFAQQMLDLEPKGEYADVLEKELFNCSIAGISLDGKQYYYVN
 BD1 336 YG-----ETCASVCM SMLSRQMLDLEPKGEYADVLERELFNCAIAGISLDGKQYYYVN
 BP1 336 YG-----ETCASVAMSMFARQMLDLEPNGEYADVLERELFNCAIAGISLDGKQYYYVN
 BC1 336 YG-----ETCASVAMSMFARQMLDLEPNGEYADVLERELFNCAIAGISLDGKQYYYVN
 BAn 347 YG-----ESCASVCMCFVARQMLEHELRGEYADVLEKELFNCAIAGIALDGGKFFFYVN
 BC3 342 YG-----ETCASVAMLFYGKSLMETKPRGSVADVMEKELFNGLVLSGVQLDGTIFYFYVN
 BD2 366 YS-----ESCAAIATAFFARRMLEIQPKSEYADVME SALYNTTLAGMALDGGKFFFYVN
 BC2 372 YS-----ESCAAIATAFFARRMLEIQPKSEYADVME SALYNTTLAGMALDGGKFFFYVN
 BAd 365 YS-----ESCAAIATAFFARRMLEIQPKSEYADVME SALYNTTLAGMALDGGKFFFYVN
 BI2 366 YS-----ETCAAIATAFFARRMLEIAPKAEYADVME SALYNTTLAGMALDGGKFFFYVN
 BL2 366 YS-----ETCAAIATAFFARRMLEIAPKAEYADVME SALYNTTLAGMALDGGKFFFYVN
 BLL2 366 YS-----ETCAAIATAFFARRMLEIAPKAEYADVME SALYNTTLAGMALDGGKFFFYVN
 BP2 327 EG-----PETCNSYNMSKLAERLWLRSGSADYINFYERVLENHLLSTINPKQPGFVYFT
 BLL4 436 -GKRN---AESCAAYNMLKVARYLFFTEQKPAYMDYYERTILNHILGGKSRDLDSGTALT
 BLL3 714 GGYRNFSTVETCNEYNMLKLARILFQVTKDSKYSEYYEHTFINATVASONPETGMTTYFQ

Fig. S1a

BL1 389 ALETTDPDGLD-NPDRHHVLSHRVDWFGCA-----CCPANIARLIASVDRIYTER-----
 BI1 389 ALETTDPDGLD-NPDRHHVLSHRVDWFGCA-----CCPANIARLIASVDRIYTER-----
 BLL1 389 ALETTDPDGLD-NPDRHHVLSHRVDWFGCA-----CCPANIARLIASVDRIYTER-----
 BB 361 ALETTDPDGLA-NPDRHHVLSHRVDWFGCA-----CCPTNIAQLIASVDRIYTER-----
 BD1 389 ALESTPDGLD-NPDRHHVLSHRVDWFGCA-----CCPANIARLIASVDRIYTER-----
 BP1 389 ALETSPDGLD-NPDRHHVLSHRVDWFGCA-----CCPANVARLIASVDRIYTER-----
 BC1 389 ALETSPDGSD-NPDRHHVLSHRVDWFGCA-----CCPANVARLIASVDRIYTER-----
 BAn 400 PLEADVQATENNPDRRHVLLERAQWFGCA-----CCPSNIARLIASVDRIYTER-----
 BC3 395 PLEADPAASKGNPTKAHLLTRRAGWEDCA-----CCPANLGRLLTSLDQYLYTVS-----
 BD2 419 PLEVVPEACHRDERKEHVKPVRQKWFCA-----CCPPNIARMVESVQOYAYTVA-----
 BC2 425 PLEVVPEACHRDERKEHVKPVRQKWFCA-----CCPPNIARMVESVQOYAYTVA-----
 BAd 418 PLEVVPEACHRDERKAHVKPVRQKWFCA-----CCPPNIARIVEDVQOYAYTIG-----
 BI2 419 PLEVNEYACHKDSRLRHVKPVRQKWFCA-----CCPPNIARIVESVQOYAYTVA-----
 BL2 419 PLEVNEYACHKDSRLRHVKPVRQKWFCA-----CCPPNIARIVESVQOYAYTVA-----
 BLL2 419 PLEVNEYACHKDSRLRHVKPVRQKWFCA-----CCPPNIARIVESVQOYAYTVA-----
 BP2 381 PMRSQHYRAYSTPEQECF-----W-----CCVSGLENHARYGRLLIYALQRPAAQ
 BLL4 492 PGNCYMPVNPATQKEY-----GDGNICT-----CCGGTALESHSKYQDSIYFHS-----
 BLL3 774 PMKAGYPKVFGITGTDY-----DADWFGGAIGEYWCCTGTGIENFAKLNDSFYF-----

BL1 438 -----DGGKTVLSSHQFIAN--KADF--ASGLTVEQR---S
 BI1 438 -----DGGKIVLSSHQFIAN--KADF--ASGLTVEQR---S
 BLL1 438 -----DGGKTVLSSHQFIAN--TAEF--ASGLTVEQR---S
 BB 410 -----DGGKTVLSSHQFIIN--KAEF--ASGLTVEQR---S
 BD1 438 -----DGGKTVLSSHQFIAN--EATF--DSGLYVVQR---S
 BP1 438 -----DGGRTVLAHQFIAN--QASF--DSGLHVEQR---S
 BC1 438 -----DGGRTVLAHQFIAN--QASF--DSGLHVEQR---S
 BAn 450 -----EDERMIAAHQFIAN--DAEF--FDDVRVKQE---S
 BC3 445 -----NDGKTVYAHQFVAN--KTEF--EDGFTIEQTQAGD
 BD2 469 -----DDASTLYVHLYMGGVVS AKLGGSDVSLEVR---A
 BC2 475 -----DDASTLYVHLYMGGVVS AKLGGSDVSLEVR---A
 BAd 468 -----DSSSTLYVHLYMGGVHARLSGTDVRLDVM---S
 BI2 469 -----EDGGTLFTHLYMGGVAKAELNGTAVELDVT---A
 BL2 469 -----EDGGTLFTHLYMGGVAKAELNGTAVELDVT---A
 BLL2 469 -----EDGGTLFTHLYMGGVAKAELNGTAVELDVT---A
 BP2 425 DSADSAAAGFASSAAETGNTVSNNAEAETRLLVNLVIDS--TFDCPEQCLRTTORAARI
 BLL4 537 -----TDNKELYVNLFTAS--TLDWTDITGLKIAQE---T
 BLL3 823 -----TDENNVYVNMFWSS--TYTDTRHNLITITQT---A

BL1 466 DFPWDSHVEYTVSLPASA-----ADSSVRFGLRIPGW--SLGS----YTTLTVNGKPAVG
 BI1 466 DFPWDSHVEYTVSLPASA-----ADSSVRFGLRIPGW--SLGS----YTTLTVNGKPAVG
 BLL1 466 NFPWDGHVEYTVSLPASA-----TDSSVRFGLRIPGW--SRGS----YTTLTVNGKPAVG
 BB 438 DFPWNCHVEYTVSLPASA-----TDSSVRFGLRIPGW--SLGS----YALTVNGKSAVA
 BD1 466 DMPWSGHVEFEVNLAEGA-----QPVRFGVRIPSW--SANA----YALAVDGEPECK
 BP1 466 DFPWNCHIEYMVELPAEA-----AD-SVRFVRIPTW--SADS----YALTCDGVAVKT
 BC1 466 DFPWNCHIEYMVELPAEA-----AD-SVRFVRIPTW--SADS----YALTCDGVAVKT
 BAn 478 DFPRECVVRFVTVDVPEGA-----DPVIEKVRIPSW--SPE----YRLTVDGVDTVG
 BC3 476 EYPWSCDLTFHVSNEGL-----DKKVAVRIPQW--SKD----YTLEVNGEAVEL
 BD2 500 GMPWNGAGAITVTLPESSD---EGQVPEPFALALRIPAW--AGGESAA-DSIHAAGEKDSR
 BC2 506 GMPWNGAGAITVTLPESSD---EGQVPESFALALRIPAW--AGGESAA-DSIHATGEKDSR
 BAd 499 DMPWSCKGSAVGFDAAGDSASDASKDAVFTIALRIPAW--AGGETAS-DAVTVRGRDDIS
 BI2 500 NLPWYGDGKAVVRLGNDAAAGASAQAPARFTLAERLIPGW--VGEESAAAAAITATGEPESG
 BL2 500 NLPWQGDGKAVVRLGDDAAGTSAQAPARFTLAERLIPGW--VGDESAAAAAITATGESESG
 BLL2 500 NLPWQGDGKAVVRLGDDAAGTSAQAPARFTLAERLIPGW--VGDESAAAAAITATGESESG
 BP2 483 EDGVDYTVTFITLESTAEHVPDTPGGLRETTFLFRPWWAEHYGVMEATCAVCTLDPARTN
 BLL4 566 NYPEEETSTISITAAPKS-----AVTERIRIPAW--SKG----AKIEVNGKAIDG
 BLL3 852 NVPKTEDVTFEVSGETCSA-----NLKLRVPDWAITNG-----VKLVVDGTEQAL

Fig. S1b