

**Homology search result of glutaminase from *Bacillus amyloliquefaciens*
versus toxic protein**

November 13, 2015
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Conclusion

The homology search between glutaminase enzyme protein and toxic protein using MvrDB was executed. Result indicated that two kind of protein was hit (details were shown below).

However, the glutaminase enzyme protein is completely digested in the digestion tests using a simulated digestive fluid (Annex 1), therefore, the protein is no hazard under the oral ingestion route.

Method

Homology search was carried out using MvirDB (<http://mvirdb.llnl.gov/>) which is provided by the Lawrence Livermore National Laboratory since it was more specific. The database includes not only toxic protein but all the enzyme expressed by drug resistance gene or protein expressed by pathogenic bacteria. The search was carried out using default parameter except E value (changed from 0.5 to 0.2).

Result

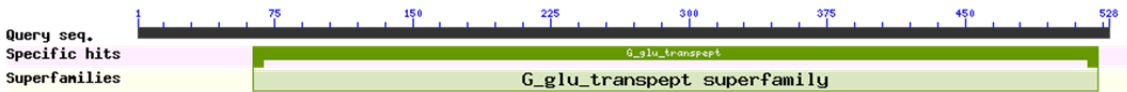
Two proteins were hit. Both proteins were derived from *Bacillus anthracis*.

Blast DB ID	Expects	% Identities
3615 vfid 4694 vsiid 5258 ssid capD belongs to the gamma-glutamyl-transpeptidase family and may be involved in anchoring the capsule to the peptidoglycan.	6.00E-64	32.9
2587 vfid 2913 vsiid 3350 ssid pXO2-55 [Bacillus anthracis]	3.00E-60	32.6

• capD belongs to the gamma-glutamyl-transpeptidase family and may be involved in anchoring the capsule to the peptidoglycan.

		User		Virulence					
Virulence	Literature	Designated	Entered By	Factor	Short Description	Long Description	Update	Protein	Nucleotide
Factor ID	Name	Name		Type			Date	Sequences	Sequences
3615	GBAA_pX O2_0063		kat swan	virulence protein		required for			
					capsule				
					capD belongs to the	biosynthesis in B.			
					gamma-glutamyl-tran	anthracis. capD			
		capD dep,			speptidase family and	may be required	Jan 6		
		BXB0063,			may be involved in	for polyglutamate	2010	Yes	Yes
		pXO2-55			anchoring the	anchoring to the	05:09PM		
					capsule to the	peptidoglycan [J			
					peptidoglycan.	Bacteriol. 2005			
					Nov;187(22):7765-	72.]			

• Highly conserved domain of capD



Gamma-glutamyltranspeptidase

• Result of the homology search between the sequence of glutaminase and capD.

RecName: Full=Capsule biosynthesis protein CapD; Contains: RecName: Full=Capsule biosynthesis protein CapD

large chain; Contains: RecName: Full=Capsule biosynthesis protein CapD small chain; Flags: Precursor

Sequence ID: [sp|Q51693.2|CAPD_BACAN](#) Length: 528 Number of Matches: 1

Related Information

[Identical Proteins](#)-Identical proteins to Q51693.2

Range 1: 51 to 522 [GenPept](#) [Graphics](#) Next Match Previous Match [First Match](#)

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
245 bits(625)	5e-770	Compositional matrix adjust.	185/538(34%)	274/538(50%)	75/538(13%)	

Features: **Query: glutaminase, Sbjct: capD**

Query	51	VATAHPLASQIGADVLLKKGGNAIDAAVAIQFALNVTEPMMSGIGGGFMMVYDAKTKDTT	110
		V+ +HPLA + G VLK GG+A+DAA+ + + L V E SGIGGGG M++ +K K+ T	
Sbjct	51	VSASHPLAVEEGMKVLKNGGSAVDAAIVVSYVLGVVELHASGIGGGGMLII-SKDKE-T	108
Query	111	IIDSRRAPAGATPDMFLDENGKAIPFSERVTKGTAVGVPGLKGLKALDKWGTRSMKQ	170
		ID RE P + N K +GVPG + G+E D +G+ M +	
Sbjct	109	FIDYRETP-----YFTGNQKP-----HIGVPGFVAGMEYIHDNYGSLPMGE	150
Query	171	LITPSIALASKGFIDSVLADAI-----SDYDKLSHTAAKDVFLLPNGEPLKEGDTLVQK	225
		L+ P+I A KGF +D L + Y DKLS +F PNGEP++ G+TL+Q	
Sbjct	151	LLQPAINYAEGKGFVDDSLTMLDLAKPRIYSDKLS-----IFYPNGEPIETGETLIQT	204
Query	226	DLAKTFTAIFYKGTAFYDGAFTKKLAETVQEFGGSMTEQDIKNFNVTIDEPIWGDYQGY	285
		DLA+T I+ +G K FY+G + +++T + ++ +DIK + V + +P+ G+Y GY	
Sbjct	205	DLARTLKKIQKEGAKGFYEGGVARAISKTAK-----ISLEDIKGYKVEVRKPKGNMYGY	259
Query	286	HIATAPPSSGGVFLQMLNLLDDFKLSQYDIRSWQKY-QLLAETMHLAYADRAAFAGDP	344
		+ TAPP S GV LLQML L + ++ + D+ Y + E +AY DR GDP	
Sbjct	260	DVYTAPPFS-GVTLLQMLKLAEKKEVYK-DVDHTATYMSKMEEISRIAYQDRKKNLGD	317

Query 345 EFVNVPLKGLLPDYINARRQLIDINKVNKKPKAGDPWAYQEGSANYKQVEQPTDKQEGQ 404
 +VN+ +++ YI+ K + GD + E +
 Sbjct 318 NYVNMDPNKMVSDKYISTM-----KNENGDALSEAEHES----- 351

Query 405 TTHFTVADRFGNVVSYYTTTIEQLFGSGIMVPGYGVVLNNELTDFDAVPGGANEVQPNKRP 464
 TTHF + DR G VVS T T+ FG+G G+ LNN+L +F + G N +P KR
 Sbjct 352 TTHFVIIDRDGTVVSSTNTLSNFFGTGKYTAGF--FLNNQLQNFGE--GFNSYEPGKRS 407

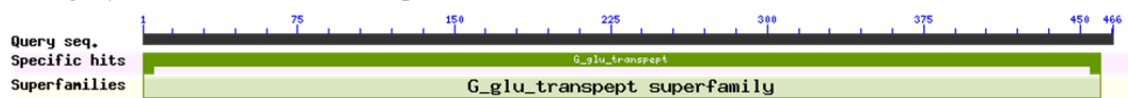
Query 465 LSSMTPTILFKNNEPVLTVGSPGGATIISVLQTIILNKVEYGM-DLKAAVEEPRIYTNSM 523
 + M PT+L K+ E + +GSPGG I +L IL+K +G L+ + E R
 Sbjct 408 RTFMAPTVLKKDGE-TIGIGSPGGNR-IPQILTPILDKYTHGKGLQDIINEYRFTFEKN 465

Query 524 TSYRYEEGVPEEARTKLNEMGHKFGSK--PVDIGNVQSILIDRENGTFTGVADSSRNG 579
 T+Y E + E + +L+ G K P G VQ+++ D + TG D RNG
 Sbjct 466 TAYT-EIQLSSEVKNELSRKGLNVKKKVSPAFFGGVQALIKDERDNVITGAGDGRRNG 522

• pXO2-55

Virulence	Literature	User	Virulence	Short	Long	Update Date	Protein	Nucleotide
Factor ID	Name	Designated	Entered By	Factor	Description	Description	Sequences	Sequences
		Name		Type				
2587	6470206	AAF13660.1	jason smith	virulence protein	pXO2-55 [Bacillus anthracis]	Jan 28 2010 02:16PM	Yes	Yes

- Highly conserved domain of pXO2-55.



Gamma-glutamyltranspeptidase

- Result of the homology search between the sequence of glutaminase and pXO2-55.

pXO2-55 [Bacillus anthracis]

Sequence ID: [gb|AAF13660.1|AF188935.58](#) Length: 466 Number of Matches:

Related Information

[Gene](#)-associated gene details

[Identical Proteins](#)-Identical proteins to WP_010891443.1

Range 1: 3 to 460 [GenPeptGraphics](#) Next Match Previous Match [First Match](#)

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps	Frame
232 bits(591)	6e-730	Compositional matrix adjust.	179/524(34%)	265/524(50%)	75/524(14%)	

Features: **Query: glutaminase, Sbjct: pXO2-55**

Query	65	VLKKGNAIDA A V A I Q F A L N V T E P M M S G I G G G G F M M V Y D A K T K D T T I I D S R E R A P A G A T P	124
		VLK GG+A+DAA+ + + L V E SGIGGGG M++ +K K+T ID RE P	
Sbjct	3	VLKNGGSAVDAAIVVSYVLGVVELHASGIGGGGMLII-SKDKET-FIDYRETT-----	55
Query	125	DMFLDENGKAIPFSERVTKGTAVGVPGTLKGLEKALDKWGTRSMKQLITPSIALASKGFP	184
		+ N K +GVPG + G+E D +G+ M +L+ P+I A KGF	
Sbjct	56	--YFTGNQKP-----HIGVPGFVAGMEYIHDNYGSLPMGELLQPAINYAEKGFK	102

Query 185 IDSVLADAI-----SDYKDKLSHTAAKDVFLPNGEPLKEGDTLVQKDLAKTFTAICYKGT 239
 +D L + Y DKLS +F PNGEP++ G+TL+Q DLA+T I+ +G
 Sbjct 103 VDDSLTMRDLAKPRIYSDKLS-----IFYPNGEPIETGETLIQTDLARTLKKIQKEGA 156

Query 240 KAFYDGAFTKKLAETVQEFGGSMTEQDIKNFNVTIDEPIWGDYQGYHIATAPPPSSGGVF 299
 K FY+G + +++T + ++ +DIK + V + +P+ G+Y GY + TAPPP S GV
 Sbjct 157 KGFYEGGVARAISKTA-----ISLEDIKGYKVEVRKPVKGNMGYDVYTAPPPFS-GVT 210

Query 300 LLQMLNLLDDFKLSQYDIRSWQKY-QLLAETMHLAYADRAAFAGDPEFVNVPLKGLLNPD 358
 LLQML L + ++ + D+ Y + E +AY DR GDP +VN+ +++
 Sbjct 211 LLQMLKLAEKKEVYK-DVDHTATYMSKMEEISRIAYQDRKKNLGDPNYVNMDPNKMVSDK 269

Query 359 YINARRQLIDINKVNKKPKAGDPWAYQEGSANYQVEQPTDKQEGQTTHFTVADRFGNV 418
 YI+ K + GD + E + TTHF + DR G VV
 Sbjct 270 YISTM-----KNENGDALEAEHES-----TTHFVIIDRDGTVV 303

Query 419 SYTTTIEQLFGSGIMVPGYGVVLNNELTDFDAVPGGANEVQPNKRPLSSMTPTILFKNNE 478
 S T T+ FG+G G+ LNN+L +F + G N +P KR + M PT+L K+ E
 Sbjct 304 SSTNTLSNFFGTGKYTAGF--FLNNQLQNFGE--GFNNYEPGKRSRTFMAPTVLKKDGE 359

Query 479 PVLTVGSPGGATIISSVLQTIILNKVEYGM-DLKAAVEEPRIYTNSMTSYRYEEGVPEEAR 537
 + +GSPGG I +L IL+K +G L+ + E R T+Y E + E +
 Sbjct 360 -TIGIGSPGGNR-IPQILTPILDKYTHGKSLQDIINEYRFTFEKNTAYT-EIQLSSEVK 416

Query 538 TKLNEMGHKFGSK--PVDIGNVQSILIDRENGTFTGVADSSRNG 579
 +L+ G K P G VQ+++ D + TG D RNG
 Sbjct 417 NELSRKGLNVKKKVSPAFFGGVQALIKDERDNVITGAGDGRRNG 460

Annex 1

Digestion Tests using a Simulated Digestive Fluid

August 8, 2013
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Amano Enzyme Inc.

1. Sample of Enzyme Protein : Glutaminase from *Bacillus amyloliquefaciens*

2. Method

The test was performed in accordance with the international validation method¹⁾ proposed by ILSI (International Life Science Institute).

Reaction:

1.52 mL of the simulated gastric fluid (SGF, pH1.2 and 2.0) was preheated to 37°C; 0.08 mL of the protein solution was added; and the mixed fluid was incubated at 37°C. To the 0.1mL of incubated fluid, 0.045mL of 200 mM NaHCO₃, pH 11, and then 0.035mL of 4 x SDS-PAGE sample buffer (250 mM Tris-HCl, pH 6.8, 8% SDS, 400 mM Dithiothreitol, 0.04% bromophenol blue, 40% glycerol) were added to stop the reaction. After heating in a boiling water bath for 10 minutes, the mixture was used for SDS-PAGE.

SDS-PAGE:

The sample in each lane is indicated in the table bellow.

Electrophoresis unit, polyacrylamide gel (10-20% gradient gel); migration buffer (10 x Tris/Tricin/SDS) were used. After migration, proteins were stained with a PAGE Blue staining solution and decolored with a 10% acetic acid – 30% methanol solution.

①	Molecular marker
②	Sample +, SGF -, Incubate -
③	Sample +, SGF -, Incubate +
④	0 sec
⑤	30 sec
⑥	2 min
⑦	5 min
⑧	10 min
⑨	20 min
⑩	30 min
⑪	60 min
⑫	Sample -, SGF +, Incubate -
⑬	Sample -, SGF +, Incubate +
⑭	Molecular marker

3. Results (Fig. 1 and 2)

By both pH of simulated gastric fluid, the sample enzyme protein was completely digested to the level which cannot be observed a band in SDS-PAGE after 10 minutes or more..

Fig.1 Results of SDS-PAGE after the digestion test by simulated gastric fluid (pH 1.2)

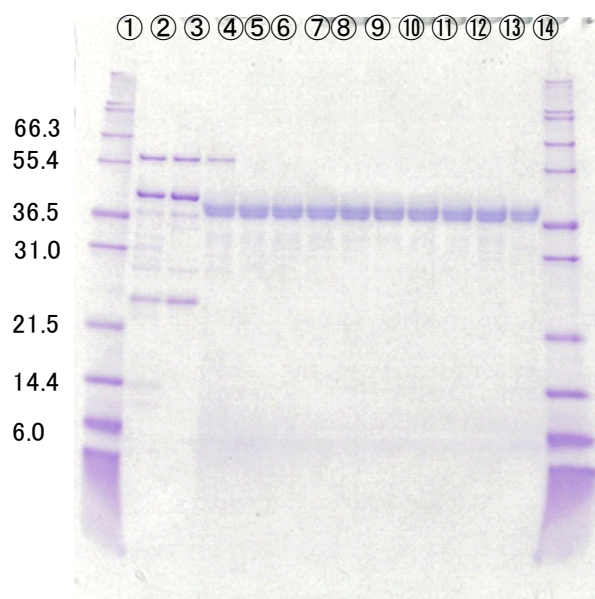
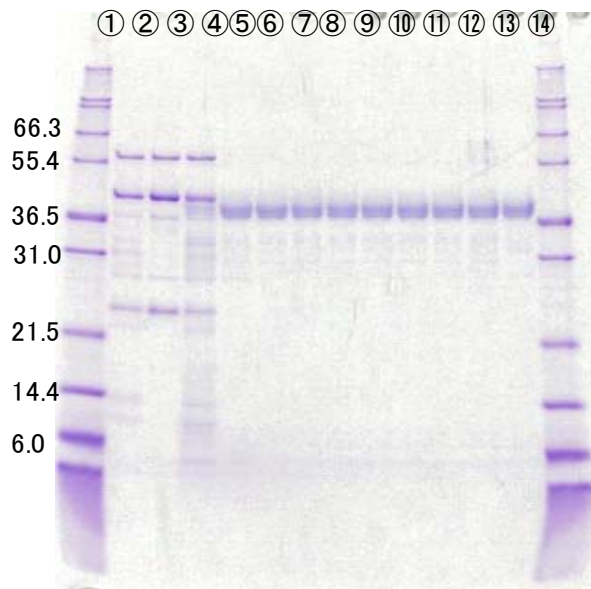


Fig. 2 Results of SDS-PAGE after the digestion test by simulated gastric fluid (pH 2.0)



References:

- 1) Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu TJ, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herouet C, Holsapple M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, Van Ree R, Woolhiser M, & Zawodny J. (2004) A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regul. Toxicol. Pharmacol.*, 39, 87-98.