Supporting Information

Single mutations in the ε subunit from thermophilic *Bacillus* PS3 generate a high binding affinity site for ATP

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Table S1: Backbone RMSD of both helical domains in Å if ATP coordinates Mg^{2+} with the Oa and O β atoms.

		Wild type		R103A/R115A						
	whole	1 st helix	2 nd helix	whole	1 st helix	2 nd helix				
Run 1	1.1 +/- 0.3	1.1 +/- 0.3	1.4 +/- 0.3	1.3 +/- 0.3	1.3 +/- 0.3	1.7 +/- 0.5				
Run 2	1.6 +/- 0.4	1.6+/-0.5	1.8 +/- 0.6	1.2 +/- 0.3	1.2 +/- 0.3	1.6 +/- 0.3				
Run 3	1.2 +/- 0.4	1.2 +/- 0.3	1.4 +/- 0.4	1.4 +/- 0.4	1.4 +/- 0.5	1.0 +/- 0.3				
Average	1.3 +/- 0.3	1.3 +/- 0.3	1.5 +/- 0.2	1.3 +/- 0.1	1.3 +/- 0.1	1.4 +/- 0.4				

		R103A		R115A						
	whole	1 st helix	2 nd helix	whole	1 st helix	2 nd helix				
Run 1	1.3 +/- 0.3	1.3 +/- 0.3	1.6 +/- 0.5	2.4 +/- 0.5	2.5 +/- 0.5	1.5 +/- 0.3				
Run 2	1.2 +/- 0.3	1.2 +/- 0.3	1.4 +/- 0.3	1.4 +/- 0.3	1.4 +/- 0.3	1.5 +/- 0.5				
Run 3	1.6 +/- 0.3	1.7 +/- 0.4	2.1 +/- 0.6	0.9 +/- 0.2	0.9 +/- 0.3	1.4 +/- 0.3				
Average	1.4 +/- 0.2	1.4 +/- 0.3	1.7 +/- 0.4	1.6 +/- 0.7	1.6 +/- 0.8	1.5 +/- 0.06				

COM distance [Å]	Wild type	R103A	R115A	R103A/R115A		
Run 1	8.7 +/- 0.3	8.5 +/- 0.3	9.0 +/- 0.3	8.9 +/- 0.3		
Run 2	8.6 +/- 0.4	9.0 +/- 0.2	9.2 +/- 0.3	9.0 +/- 0.3		
Run 3	8.8 +/- 0.3	8.8 +/- 0.4	8.9 +/- 0.3	9.0 +/- 0.3		
Average	8.7 +/- 0.1	8.8 +/- 0.3	9.0 +/- 0.2	9.0 +/- 0.06		

Table S2: Center of mass (COM) distances of the α -helical domains in Å if ATP coordinates Mg^{2+} with the O α and O β atoms. Average distance and standard deviation for this distance was derived by using runs 1 to 3.



Figure S1: Distance of the Mg^{2+} to ATP oxygen atoms, when ATP is bound to the ε subunit from thermophilic *Bacillus* PS3 mutants (R103A and R115A). The top graphs show how Mg^{2+} binds to these oxygen atoms, when the ion is freely distributed. The middle and bottom graphs show the distance distributions, when Mg^{2+} is bound to ATP:O α /O β or ATP:O β /O γ , respectively. It should be noted that two histograms (Mg^{2+} - ATP:O α and Mg^{2+} - ATP:O β) are overlaid if Mg^{2+} is coordinated to ATP:O α /O β (middle row). The results for the R103A and R115A mutants are shown in the left and right coloums, respectively.











- R92:Νε-ATP:Oγ - R92:NHx-ATP:Oγ - R99:NHx-ATP:Oγ - R122:NHx-ATP:Oγ









R92:NHx-ATP:Oβ
R122:Nε-ATP:Oβ
R122:NHx-ATP:Oβ



- R92:Νε-ATP:Ογ - R92:NHx-ATP:Ογ - R99:NHx-ATP:Ογ - R122:NHx-ATP:Ογ



Figure S2: Timelines of relevant interactions of the R103A and R115A single mutants of the ϵ subunit from thermophilic *Baillus* PS3. For each mutant the state in which Mg²⁺ is coordinated by ATP:O α /O β and ATP:O β /O γ is shown on the left and right, respectively



Figure S3: Interactions of the ε subunit R103A (left) and R115A (right) mutants from thermophilic *Bacillus* PS3 with ATP during the simulations when Mg²⁺ is coordinated by ATP:O β /O γ . Dotted lines correspond to the distances observed in the crystal structure of the wild type protein.



Figure S4: Interactions of R103:NHx (black) and R122:NHx (red) with ATP:O γ , respectively of the excluded run of the R115A mutant. In this run, it can be observed that R103 binding to the ligand leads to a release of R122 interactions with ATP.



Figure S5: Energetic contributions of the single windows in both mutants for all TI calculations.



Figure S6: Predicted binding sites of the R103A and R115A mutants of the ε subunit from thermophilic *Bacillus* PS3, in the hypothetical case if the ion is bound to ATP:O β /O γ .

thermophilic Becilius PS3	10 -MKTIHVSVVTPDGPVYE	20 30 40 DDVEMVSVKAKSGELGILPGHIPLVAF	50 60 LEISAARLK-KGGKT	70 - QYIAVSGGF	80 LEVRPDKVTI	ILAQÁA	90 RAEDIDVL	100 RAKAAKERAER	110 RLQ SQQDDID	120 FK <mark>R</mark> AELALK	130 RAMNRLS	140 /AEMK
thermophilic Bacillus PS3 R103A mutant thermophilic Bacillus PS3 R115A mutant thermorphilic Bacillus PS3 R103AR115A mutant	-MKTIHVSVVTPDGPVYE -MKTIHVSVVTPDGPVYE -MKTIHVSVVTPDGPVYE	DDVEMVSVKAKSGELGILPGHIPLVAF DDVEMVSVKAKSGELGILPGHIPLVAF DDVEMVSVKAKSGELGILPGHIPLVAF	PLEISAARLK - KGGKT PLEISAARLK - KGGKT PLEISAARLK - KGGKT	-QYIAVSGGF -QYIAVSGGF	LEVRPDKVTI		RAEDIDVL RAEDIDVL RAEDIDVI	RAKAAKE RAE R RAKAAKE RAE R RAKAAKE RAE R	ALQ SQQDDID RLQ SQQDDID ALQ SQQDDID	FKRAELALK FKRAELALK FKRAELALK	RAMNRLS RAMNRLS RAMNRLS	/AEMK / AEMK
Bacillus pumilus Bacillus zhangzhouensis	-MKTVQVNIVTPDGPVYQ -MKTVQVNIVTPDGPVYE	ADVEMASVRAESGELGILAGHVPMVAF ADVEMVSVRAESGELGILAGHVPMVAF	LKIGAVRLK - NGNST LKIGAVRLK - HSGST	- EL VAVSGGF - EL VAVSGGF	VEVRPDKVTI	I L AQAA	TSKQVDVD TSEKVDVD	RAKSAKQ RAEE RAKSAKQ RAEE	HLNH PNESD HLNH PNESD	VR <mark>R</mark> AELALK VR <mark>R</mark> AELALK	RALNRLN RALNRLN	/AGK
Paenibacillus macerans Caldalkalibacillus thermarum Davidalka IVT 40.04	-MNTLRLNIVTPNGSVYEF -MATVQVDIVTPERKVFQ-	GEADIVIARGVEGELGVMAGHIPLVT	L KTGHVKVN - FHNGN L KTAPVR I K - QGDKE	- EF I AVSDGF - TL I AVSGGF	LEVRPDKVNI	I I VQTA	PASEIDVE	RAKLAKSRAES RAKKAKARHET	HLED DDDNTD	INRAKRALE	RANNELE	/AELQ / /ANSKS
Domibacillus aminovorans Lysinibacillus sphaericus	-MKTENVNIVTPDGPVTE -MKTEQVNIVTPDGPVLE	TNVELLSAKAQSGELGIMAGHVPLVA TEADMVIATTEAGELGILPDHIAMVA	LQIGAVRLVLGDKKS PLKIGGLRVN-KGNST	- EL VAVSGGF - EL VAVSGGF	LEVRSDQVTI		KAENIDIA	RAKEAKMRAER	FLQD KQDQVD KLQS EKDAKE	LKRAELALR	RATNRLN	/YEGKL
Sporosarcina psychrophila Bacillus OxB-1	-MKTIKVNIVTPDGPVVE -MKTMKVNIVTPDGPVCE	TEANMIIAVTETGEIGILPGHIAMVAF TEADMIIAAAESGEIGILPGHIAMVAF	LQIGGLRLQ-KDDST LQIGDLRLK-KDGAT	- EQIAVHGGF - EHVAVHGGF	IEVRPDVVTV	VLAQSA VLAQSA	LASSIDID MAATIDLA	RAKKAAK <mark>L</mark> AEE RAKAAAKRAEE	TLQA KKEGRE KLRA QSDEKE	HE <mark>L</mark> AALDLE HQ I MEYALQ	RALNRIN' RAITRIK'	/YETRKK /YETRL
Bacillus caldotenax Geobacillus stearothermophilus Alinia Iralia	-MKTIHVSVVTPDGPVYE -MKTIHVSVVTPDGPVYE MANTEHEDIVSPEKIAES	DDVEMVSVKAKSGELGILPGHIRLVAF DDVEMVSVKAKSGELGILPGHIPLVAF GEVEOVDVPGVEGDEGVLAGHSPLVAS	LEISAARLK - KGGKT LEISAARLK - KGGKT	-QYIAVSGGF -QYIAFSGGF	LEVRPDKVTI LEVRPDNVTI AEVSDKGLTV		RAEDIDVL RAEDIDVL SLAELDRV	RAKARK - SGRT RAKARK - SGRT	PLQS QQDDID PLQS QQDDID	FKRAELALK FKRAELALK	RAMNRL SI	/AEMK - /AEMK - RITSTOMH
Anourinibacillus migulanus Bacillus halodurans	-MNTIEVEIVTPERVVYR -MPTLHVNVVTPDGKVYE	GDARIVVARGAEGDLGILPNHIPMVTF	LKIAPILVKKHDGAE	- DK I AV SGGN	MEVRKDKVTI	ILAESA	LADEIDVN	RATSAKERAER	RLAE SGREDID RLQQ AKQENID	FKRAQMSLQ FKRAELSLR	RALTRED	/AGK
Bacillus cytotoxicus Bacillus obstructivus	-MKTFPVSIVTPDGPVYE -MKTLKVSIVTPDGPVYD	KEVEMVSVKAESGEMGILPGHIPTVAF SNVEMVSTKAQSGELGILPGHIPMVAF	LKISAVRLK - NGGHT LQIGAVRLK - NGNNT	- DYVAVSGGF - EL VAVSGGF	LEVRPDKVTV	VLAPSA ILAQSA	EANHIDIH KEESIDIE	RANEAKRRAEQ RAKEAKSRAES	RLQD KQANVD RIQS NQADID	FK <mark>R</mark> AELALK QVRAELALR	RAINRLD' RAVNRIN'	/SNMK / YEHRF
Bacillus 103mf Bacillus 103mf Bacillus 1NI A3E	-MKTFPVSTVTPDGPVYE -MKTFPVSTVTPDGPVYE -MKTIKVNVVTPDGPVYE	NEVEMVSVKAESGEMGTLPGHTPTVA NEVEMVSVRAESGEMGTLAGHTPTVAF SAVEMVSTKARGGELGTLPGHTPMVAF	LKISAVRLK-HGGHT LKISAIRLK-NGGHT LFIAALRLK-NGGNT	- DYVAVSGGF - DYVAVSGGF - EEVSINGGF	IEVRPEKVTV	VLSSSA VLAPSA	TANHIDIH	RANEAKRRAEQ RANEAKRRAEQ RAL RAKERAEQ	RMQD KQAHVD RMQD KQAHVD RLRE OKQETMD	FKRAEMALQ FKRAEMALK FRRAELALK	RAINRLD	/SDMK /SNMK
Baoilus 7504-2 Baoilus 7586-K	-MKTIKVSVVTPDGPVYE -MKTVLVNVVTPDGPVYD	SDVEMVSTKALSGELGILPGHIPMVAF ADVEMVSVKAQSGELGILPGHIPMVAF	LQIGAVRLI-TGGNT LEIGAVRLK-QGSGT	- KY LAVSGGL - EL VAVSGGF	LEVRPDQVSI	ILAEAA ILAQTA	VADS I DVD KAED I DVA	RALKAKE <mark>R</mark> AEQ RAEAAKR <mark>R</mark> AEE	RLHA QKQENID RLNH KSDEID	YK <mark>R</mark> AELALQ FK <mark>R</mark> AELALR	RALNRIS ¹ RAINRLN ¹	/AGK / / T K
Bacillus 7884-1 Bacillus 7894-2 Bacillus AE S001701	-MKTIEVSVVTPNGPVYE -MKTIKVSVVTPDGPVYE -MKTMKVSIVTPNGPAVE	SDVEMVITKAQSGELGILPGHIPMVAF SDVEMVSTKAQSGELGILPGHIPMVAF	LAIGVVRMK - KNGKE LQIGAVRLK - NGGKT	ODL I AV SGGF - DF VAV SGGF - DVVAV SGGF	LEVRPDKVTI	ILAQAA ILAQSA	KAEDIDVE QADEIDVE ASTEIDVE	RAL RAKE RAEQ RAVRAKQ RAEQ	RMKE QKVEDID RMNE QKRENID	FRRAELALQ FRRAELALQ	RAINRLT' RAINRIE KAMNRINI	/TGKQ /SERKF
Bacillus AF S006103 Bacillus AF S015802	-MKTIKVSIVTPDGPVFE -MKTLKVNIVTPDGPVYD	SDVEMVSTKATSGDLGISPGHMPLVAF SEVEMVSTKAQSGELGILPGHIPMVAF	LEITSVRLK-KDGKT	- EFVAVSGGF - ELVAVSGGF	LEVRPDQVTI	ILAQSA	LASEIDVE	RAIKAKE RAEQ RAKEAKG RAED	RMKD QHIEHID RMQA QRDDVD	FRRAELALQ FRRAELALK	RAINRLA	/SERRM
Bacillus AFS018417 Bacillus AFS031507	-MKTFPVSIVTPDGPVYE -MKTIRVSIVTPDGPVFD	NEVEMVSVKAESGEMGILPGHIPTVAF SDVEMVSTKATSGDLGISPGHMPLVAF	LKVSAIRLK-NGGHT LEITSVRLK-KDGKT	- DYVAVSGGF - EF I AVSGG I	LEVRPEKVTV	VLAPSA	TASHIDVH TASEIDVE	RANEAKRRAEQ RATRAKERAEQ	RLQD KQAHVD RLKD HQADHTD	FKRAEMALQ	RAVNRLN' RAINRLG'	/SNMK / SERRI
Bacillus AF 5037270 Bacillus AF 5040349 Bacillus AF 5073361	-MKTIKVSVVIPDGPVYE -MKTVLVNVVTPDGPVYE -MKTIKVSIVTPDGPVYE	ADVEMVSTKARSGELGTLPGHTPLVA ADVEMVSVKAQSGELGTLPGHTPNVAF SDVEMVSTKATSGDLGTSPGHMPLVAF	LEIGSVRLK-KDGRI PLQIGAVRLK-KGSST PLEITSVRLK-KGGKT	- EL VAVTGGF - DYVA I SGGF	IEVRPDGVTU	ILAQAA	TAEHIDEA	RAQAAKKRAEE RAQAAKKRAEE RAVKAKERAEO	RLNQ KTDNID RLNQ KTDNID RMRD HQAEHTD	FKRAELALQ FKRAELALQ FRRAELALQ	RAINRLA	/TKF
Bacillus B-jedd Bacillus B14905	-MSTIKVSVVTPDGPVYD -MKTVLVNIVTPDGPVYD	SEVEMVSTKAQSGELGILPGHIPMVAF SEVSMVIAKTTSGEIGVLAGHIPMVAF	LQIGAVRLK-KEGKT	- EL VAVSGGF - DI VAVSGGF	LEVRPDQVTI	ILAQAA ILAPSA	QSSEIDIE VAENIDVQ	RAL RAKE <mark>R</mark> AEQ RAKEAVK <mark>R</mark> AEG	RIKE RQQEHVD RLQG KQDNID	FK <mark>R</mark> AELALQ FK <mark>R</mark> ADLALK	RAINRLAI RALNRINI	/AERRI /HEGNI
Bacillus CHD6a Bacillus EGD-AK10 Bacillus E IAT-18017	-MKTLKVSVVTPDGPVYE -MKTVKVNIVTPDGPVYD -MRTIKVSVVTPDGPVYE	SDVEMVVARAQSGELGVLPGHIPMVAF ADIEMVSVRAESGDLGILPGHIPTVAF SDVEMVSTKAOSGELGVLPGHIPMVAF	LGIGGVRMK - KDGKT LKIGAVRLK - KDGQT	- EQVAVNGGF - EMVAVSGGF	LEVRPDKVTI VEVRPDQVTI	ILAQTAE ILAQAAE	AAEQIDLT TAEGIDKE OSSAIDVE	RAEEAKKRAEQ RAEAARQRAQE RAVRAKERAEQ	RLQS KKDDVD RLNT QSDDTD RLRE ROOENVD	YRRAELALQ IRRAELALQ FRRAELALQ	RAINRIN	/AKRG / /AGK / /AFRRI
Bacillus FJAT-21352 Bacillus FJAT-22090	-MKTIKVNVVTPDGPVYD -MKTLIVNIVTPDGPVYD	AEVEMVSTKAKSGELGIMAGHVPTVAF SEVDMIIAKTASGEIGILPGHIPMVAF	LTIGAVRLK-NGSNT LVIGAVKLK-KDGKS	- DYVAVNGGF - EYVAVNGGF	LEVRPDVVT I VEVRPEKVT I	ILAQSA ILAQSA	RAETIDLA VASDIDVT	RAQAAKA <mark>R</mark> AEQ RAKEAVK <mark>R</mark> AEE	RLQN NDGSND RLQH KQDSTD	AK <mark>R</mark> ANLALK FN <mark>R</mark> AELALK	RAINRIQ RAMNRIN	IAEMR
Bacillus FJAT-20390 Bacillus FJAT-27225 Bacillus FJAT-27228	-MSSFLVEIVTPERKVYA -MRTMKVSVVTPDGPVYE-	ETANMVSVSGVEGELGILPNHIPLVTF SDVEMVSTKAQSGELGILPGHIPMVAF	L R I A P V T I K - RG NSV L D I G A V R L K - K DG N T	- DVVAVNGGF - DVVAVNGGF	LEVRKDKIVI	ILAESA ILAQAA	LATDINIE QSNAIDVE	RAEAAKQ RAQQ	RLAA KQDQVD RLRE RQQENVD	FRRAELALQ	RAMNRLN RAINRLA	/AERRL
Bacilus FJAT-27264 Bacilus FJAT-27264 Bacilus FJAT-27916	-MNTFLLEIVTPDRLVYS -MRTIKVSVVTPGGPVLE	KQVNSLTVRGVEGELGILPGHIPLVTF DEVEMVSTKAKTGELGILAGHIPMVAF	LQVAPLSVK - ADGVT	- ISTAVHGGF - EWVAVSGGF	VEVHKDKVTV	VLAESA	LPKDIDVE	RAEAARERAQR	RLQS-LSKQDDID RLADRQAAID	HRRAELALQ AHRAEMALK	RAVTRIK	/STGKGQQ
Bacillus FJAT-27986 Bacillus HMSC76G11	-MRTIKVSVVTPGGPVLD -MKTVQVNVVTPDGPVVD	EEVEMVSTKAKTGELGILAGHIPMVAF ADVEMVSVKAQSGELGILPGHIPLVAF	LDIGSVRLK - TGNNT	- EWVAVSGGF - EL VAVSGGF	MEVNGEEVTI	ILAQSA	RAEDIDTA TVEDIDLT	RAKQAKA RAER RAQSAKE RAEQ	RLQD RQVEID RLNK STDDID	AH <mark>R</mark> AELALK FK <mark>R</mark> AELALR	RALNRLD	AEQRQR /SQNKI
Bacillus JCM19047 Bacillus JCM 19046 Bacillus JF8	-METIQVSVVTPDGLVYS -METIQVSVVTPDGLVYS -MKTIHVSVVTPDGPVYE	GDAELVVVKTTEGELGIKAKHIPLVS GDAELVVVKTTEGELGIKAKHIPLVS GDVEMVSVKAKSGELGILPGHIPLVAF	LAAGPARFI-RDGRE	- DQVAVSGGF - DQVAVSGGF	IEVRPDQVSI		RPEQIDIE	RAEKAKDRAET	RLND AAGHID RLND AAGHID RLQS QQDDID	KKRAEAALY FRRAELALK	RAVTRLD	/AKKK
Bacillus KCTC13219 Bacillus LF1	-MKTVTVSIVTPDGPAYD -MKTIKVSVVTPDGPVYD	SEVTMVVAKTVSGEIGVLPGHIPMVAF ADVEMVSTKAQSGELGILPGHIPLVAF	LAISAVKLKKQDGST LAIGSVRLK-KDGKT	- DF VAV SGG F - EL TAV SGG F	IEIRPEKVSI LEVRPEKVTI	ILAPSA ILAQTA	AAASIDLA KAEDIDVE	RAKEALN <mark>R</mark> AEG RAIRAKERAEQ	RLEG KKDDID RLHE QHQEHID	FK <mark>R</mark> AELALK FR R AELALQ	RALNRIN' RAVNRLGI	/HEGNI /SGRNL
Bacilus LLU1 Bacilus Leaf406 Bacilus M5-12	-MSTIRVNVVTPDGKVYD -MKTLKVNIVTPDGPVYD -MKTVKVSVVTPDGPVYD	SEVDMVVVRTTEGELGTLPKHTPLVA SEVDMVSTKAQSGELGTLPGHTPMVAF SEVEMTSTKAQSGELGTLPGHTPMVAF	PL TVGAVRLK - KGNSE PLQTGAVRLK - KGGDT PLKTGAVRLK - KGAFT	- EL VAVSGGF - EL VAVSGGF	LEVRPEQVII	ILAEAAE ILAQSAE	TAEAINIE STEDIDLA	RARAAKERAED RAKEAKGRAEG RAQAAKARAEO	RVNS IKKDAID RLQG SRDDVD RLQD KQDNID	F KRAELALK F RRAELSLK AKRAFLALO	RAMNRIN	/SGK
Bacillus MBGLi79 Bacillus MRMR6	-MKTLKVNIVTPDGPVYD -MKTFKVNIVTPDGPVYE	ADIEMVSVRAESGELGILPGHIPTVAF SDVEMVSTKAQSGELGILAGHIPMVAF	LKIAAVRLK - KDGQT LAIGVVRLK - KGGKE	- EL VAVSGG I QDL VAVSGG F	LEVRPDHVT		TSEQIDKA RSSDIDLE	RALAAKRRAEE RALRAKE <mark>R</mark> AEQ	RLQK HTPDVD RLRD SRQEDTD	II <mark>R</mark> AELALK FK <mark>R</mark> AELALQ	RAINRLD'	/AR
Bacillus MUM13 Bacillus MUM_116 Bacillus OG2	-MKTIKVSVVTPDGPVYD -MKTIKVNVVTPDGPVYE -MKTIKVSVVTPDGPVYE	AEVEMVSTKAKSGELGIMAGHIPMVAF SEVEMVSTKAKSGELGILPGHIPLVAF	LQIGSVRLK-NEGHT LEIGSVRLK-KGGST	- DL I AVNGGF - EL VAVSGGF	LEVRPDVVTI	ILAQTA ILAQAA ILAQAA	RAETIDLA NAADIDVD OSEGIDIE	RAQAAKARAEQ RALRAKERAEQ	RLQD STDAID RLQQ AQQEHLD RLAD OKRONID	AKRAELALK FKRAELALQ	RAVNRIN RAINRLA PAVNRIS	AERNF /SEKR
Bacillus OK048 Bacillus OV194	-MKTIKVSVVTPDGPVYE -MKTLEVSVVTPDGPVFE	SDVEMVSTKAQSGELGILPGHIPMVAF GTAEMVSAKAKSGELGVLPGHIPLVAF	LAIGVVRLKKSESKC LTISAVRVH-QDGKT	PDL I AVSGGF	LEVRPDQVTI	LAQSS	KAED I DVE LASE I EME	RAL RAKE RAEQ	RMKE QKVEDID RLAA TNQDDID	FRRAELALQ HKRAELALR	RAVNRLAV	/SQGKM
Bacillus RU2C Bacillus Root147 Guellus Root147	-MKTIHVSVVTPDGPVYD -MKTIHVSVVTPDGPVYE	SEVEMVSAKAQSGELGILHGHIPMVAF SEVEMVSTRAQSGELGILHGHIPMVAF	LOIGAVRLK - KGSSS LOIGAVRLK - KASST	DEVVAVSGGF - EL VAVSGGF	LEVRPDKVTI	ILAQAA	TSDMIDVS TAEDIDVA	RAEEAKNRAET	RLQS KQDDVD RLDS KQDDVD	FKRAELALK VKRAEIALK	RALNRIE	/TKQKM ISQRKF
Bacillus SA5 Bacillus SJS	-MDMIKVSIATPDGPVYE -MKTVQVNVVTPDGPVYD	GDVEMVVVKAQSGELGILPGHVPLVAF AEVEMVSVKAQSGELGILPGHVPNVAF	LTIGAVRLI-QNNEE	- KPVAVSGGF - EL VAVSGGF	LEVRPDKVTI	ILAQSA	MPEKINVE	RAKEAKERAEK	RMND KDID RLNQ KTADMD	MKRAELALA SHRAQLALS	RAMNRID	IAGK
Bacillus T33-2 Bacillus TS-2	-MKTIKVSVVTPDGPVYE -MPTINVNVVTPDGKVYD	SDVEMVSTKAQTGELGILPGHIPMVAF GDVDMVSVRTVDGELGILPKHIPLVAF	LOIGAVRLK - NGNKT LTVGAVRLK - NGSNV	- EYVAVSGGF - EKVAVSGGF	LEVRPDQVTI	ILAQAA	RSDDIDVD	RAL RAKE RAEQ	RLRE RQQENVD RLNE IKQDQID	FR <mark>R</mark> AELSLQ IKRAQLALK	RAMNRIS	/AERKI /ASK
Bacillus V3-13 Bacillus V3-13 Bacillus V5-Bf	-MKTIKGSVVTPDGPVYE -MKTIKGSVVTPDGPVYE -MKTFKVSIVTPDGPVYD	SDVEMVSVVAQSGELGILPGHIPMVA SDVEMVSTKAQSGELGILPGHIPMVAF SEVEMVSTKAQTGELGILAGHIPMVAF	LQIGAVRLK-KGSST LQIGAVRLK-NGGKT LQIAAVRLK-KDGKT	- DF VAV SGGF - EY LAV NGGF	LEVRPDQVTI	ILAQSA	SSSEIDVD	RALRAKERAEE	RLNG QKREEID RLQD NQENID	FKRAQLALQ AKRAELALQ	RAINRIS	/AQRKI
Bacillus X1 Bacillus akibai	-MKTIKVNVVTPDGPVYE -MPTVQVSVVTPDGKVYD	SDVEMVSTKAKSGELGILPGHIPLVAF GDVDMVSVRTVEGELGILPKHIPLVAF	LEIGSVRLK-KGGNT LTVGAVRLK-KGSTV	- EI I AVSGGF - EKVAVSGGF	LEVRPDTVTV	VLAQAA ILAEAA	KASDIDVE LPTGIDVD	RAKRAKE RAEK RALQAKE RAQK	RLRA QQQENID RIES NRQDEID	FK <mark>R</mark> AELALR FKRAELALR	RAINRLA RASNRLD	/SEKK /AGK
Bacillus alcalophilus Bacillus azoloformans Bacillus hadius	-METINVNVVTPDGKVYD -METIQVSVVTPDGAVVE -METEKVSIVTPDGPAVE	GDVDMVSVRTVDGELGILPKHIPLVAF TDVEMVSVKAVSGELGILPGHIPLVAF ADVEMVSAKADSGELGILPGHIPMVAF	PLTIGAVRLK - NGSTV PLTISAVRLK - NGNNT PLDVGAVRINKGAGKE	- DKVAVSGGF - DKVAVSGGF	VEVRPDQVTI		L PSD I DVD L ATS I DI D TAFNI DVA	RAKQAKE RAEK RARAAKE RAER RAKFAKMRAFN	RLEE I KQEQ TD RLQQ AKTDDVD RLSN KODNID	SKRAQLALK FKRAELALK IORAFLALK	RAINRLD	/SGK
Bacillus bataviensis Bacillus beveridgei	-MKTIKVSVVTPDGPVYE -MKTMQANVVTPDGSVFS	SDVEMVSTKAQSGELGILPGHIPMVAF GDVEMVSVKTPDGGLGILPGHLPLVSF	LEIGAVRLK - KDGKT LAIGPVRLK - QSGNI	- EF I AVSGGF - QPVAVNGGF	LEVRPDQVTI	ILAQAA ILAESA	TSEHIDVE LPSEIDIN	RAQRAKE <mark>R</mark> AEQ RARAAKE <mark>R</mark> AER	RLKE QKLEHID RLEE AKKENLD	FR <mark>R</mark> AELAMR FK <mark>R</mark> AEMALK	RAINRLN RAVNRLE	/SEHRI /GESG
Bacillus boroniphilus Bacillus caldolyticus Bacillus camellico	-MKTIKVSVVTPDGPVYE -MKTIHVSVVTPDGPVYE -MKTIKVNIVTPDGPVYS	SDVEMVSTKASTGELGILPGHIPMVAF DDVEMVSVKAKSGELGILPGHIPLVAF SDVEMVITKAOSGELGILPGHIPTVAF	PLOIGAVRLK - NGSNT PLEISAARLK - KGGKT	- EF LAVSGGF - QY LAVSGGF	LEVRPEQVTI	ILAQSA ILAQAA	KSSEIDLE RAEDIDVL	RALKAKE RAEQ	RLHE RKQENID RLQS QQDDID	FKRAELALQ FKRAELALK	RAINRIS ¹ RAMNRLS ¹	/AERRI /AEMK
Bacillus campisalis Bacillus canaveralius	-MKTMKVHVVTPDGPVYD -MKTIKVSVVTPDGPVYE	SEVEMVSTKAQTGELGILPGHIPMVAF SDVEMVSTKAQSGELGILPGHIPMVAF	LOIGAVRLM - KGVNT	- DYVAVSGGF - DFVAVSGGF	LEVRPDTVTI	ILAQTA	KSSEIDIE SSSEIDID	RAVRAKE RAEQ RAVRAKO RAEE	RLRE RQQENID RLHS QKREDVD	FK <mark>R</mark> AELALQ FR <mark>R</mark> AELALQ	RAINRIS RAINRIS	AQRR /AERK
Bacillus caseinilyticus Bacillus celiulosilyticus Bacillus cimulans	-MKTMHTNVVTPDGSVFE -MKTMQANVVTPDGSVYN -MKTIKVNVVTPDGSVVH	GQVEMVSVKTTEGELGIMAGHLPLVSF GQVEMVSAKTTEGELGILPGHLPLVTF SDVEMVLAKAESGELGILAGHLPMVA	LTIGAVRIR-KESKV LDVGAVRLK-HDSKI	-QLIAVSGGF -QLIAVSGGF	MEVRPDEVNI MEVRPDEVNV	ILAESA VLAESA	L PSQIDIA L PSDIDVA	RARAAKE RAER	RLQE AKKANID RLEQ AKKDNID	FKRAELALK FKRAEMALK	RAINRLE	/SSYGNKS /ANK
Bacilus cieusi Bacilus cieusi	-MKTIKVSVVTPDGPVYE -MATIQVSVITPDGAVYE	SDVEMVSTKAQSGELGILPGHIPMVAF GDAELVVVKTVEGELGIKAKHIPLVSF	LEIGAVRLK - KAGTT LAVGPARFI - KEGKE	- EFVAVSGGF - EQVAVSSGF	LEVRPDQVTI	ILAQSA	QASE I DI D RPEQ I DTE	RAVRAKERAEQ	RLQE SKQDNVD RLAS EHVD	FKRAELALQ RKRAEAALQ	RAINRIS	/VERKL VAKHKGA
Bacillus coagulans Bacillus coahultansis Dealtas actual	-MRTFEASVVTPDGPVYN -MKTLQVNIVTPDGPVYD	AEVEMVIAKAKSGEIGILYGHVPLVAF AQVEMVSTKAQSGELGILPGHIPMVAF	LDIGVVRFK - NGDQE	- KVVAVSGG I - EL VAVTGGF	LETRSNQVTI	ILAQAA	TPEQIDIR TAESIDVA	RAEEAKQ RAEK	RLHQ SHQDDVD RLQA KQDNVD	FKRAELALK FSRAELALK	RAMNRID'	/YRHFSR
Bacillus cucumis Bacillus daliensis	-MKTVKVSVVTPDGPVYD -MKTMQTNVVTPDGSVFD	SEVEMVSTKAKSGELGILPGHIPLVAF	LAISAVRLK-KGGNT LAIGAIRLK-KDKDV	- DF VAVSGGF - RL I AL SGGF	LEVRPDQVTI	ILAGTA	LASDIDVE	RALRAKERAEQ	RMQE QRAEHTD RLEE AKRDNID	FRRAELALQ	RAINRLA	/SEKKL
Bacillus deserti Bacillus endophyticus	-MKTLQVSVVTPDGPVYD -MKTMNVSVVTPDGPVYS	ADAQMVSTKAQSGDLGVLPGHIPMVAF EDVEMVIAKTQGGELGVLPGHIPMVSF	LEIGSVRVK-KDGST LKIGAVKLN-TPSGT	- EF LAVSGGL	LEVRPDKVTI	ILAQSA	KADNIDLA TAGAIDIK	RAKAAAA RAEK RAEEAKQRAEE	RLQD KSDHVD RLAN KQADLD	ARRAELSLR VORAELALR	RAINRISI	. AEKKF /GSRNV
Bacillus galactosidilyticus Bacillus ginsengihumi	-MKTLQVHIVTPDGPVFD -MRTYKVSIVTPDGPVYS	GDVEMITTKAESGELGILPGHIPUVAF SEVEMIIAKAKSGEIGILYGHIPUVAF	LOIGAVRLN-KGTET	- EF VAVNGGF - EL VAVSGG I	VEVQQEKVTI	ILAQTA	KADSIDIA	RAEEAKKRAES	RLQG SQDDVD RLQ SNHDDVD	FKRAQLALK FKRAELALK	RAMNRID	/YKHH ·····
Bacillus glycinifermentans Bacillus gobiensis	-MKTLKVNIVTPDGPVYD -MKTLKVNIVTPDGPVYE	AD I EMVSVRAE SGELGIL PGHIPTVAF AD I EMASVRAE SGELGIL PGHIPTVAF	LKIAAVRLK-KDGQT LQIGAVRLK-KDAEL	- EL VAVSGG I - KL VAVTGGF	LEVRPDHVTI	ILAQTA ILAQAA	TSEE IDKE TAEN IDVE	RALAAKRRAEE	RLQK HSPDID RLNK ASEEID	IVRAELALK LVRAELALK	RAINRLE RAINRLD	/ A K
Bacillus hisashii Bacillus hisashii Bacillus homeokine	-MKTVSVSIVTPDGRVTD -MKTVSVSIVTPDGPVYE -MKTIKVSVVTPDGPVYE	SDVEMVSVRTTEGELGTLPRHTPLVA SDVEMVSTKAASGELGTLPd HVPMVAF SDVEMTSTKAESGELGTLAGHVPMVAF	LAIGAVRLK-KGITV LAIGAVRLK-KDGHT LOIGAVRLK-KANQT	- DL VAVSGGF - DL I AVNGGF	LEVRPDGVTI	ILAQAA	NANEIDID	RAUEAKKRAEU RAIRAKORAEE RAKRAKDRAEO	RLRK QKQSEID RLRK QKRENVD	YRRAELALS FKRAELALQ	RALNRIN	/AQNAR
Bacillus koreensis Bacillus kruiwichiae	-MKTIHVSVVTPDGPVYN -MPTIQVSVVTPDGKVYD	ADVEMVSARAQSGELGILPGHVPLVAF GDVDMVSVRTIEGELGILPKHIPLVAF	LKIGAVRLK-KGGST LTVGAVRLK-KGSTV	- EL VAVSGGF - EKVAVSGGF	LEVRPDNVTI	ILAQAA	KADQIDIV LPTGIDVD	RAEEAKKRAES RAREAKERAQK	RLGA QKDHVD RIDS ARADEID	FK <mark>R</mark> AELALN FK <mark>R</mark> AELALK	RAINRIE	/SQRKM /AGK
Bacillus Inioralis Bacillus Iciaeleuriae Bacillus manificonansis	-MKTVLVNVVTPDGPVYD -MKTIKVSVVTPDGPVYD -MKTFPVSIVTPDGPVYE	ADVEMVSVKAHSGELGILPGHIPMVAF AEVEMVSAKAKSGELGIMAGHVPMVAF KEVEMVSVKAESGEMGILPGHIPTVAF	LEIGAVRLK-QGSGT LDIAAVRLK-KEGGT LKISAVRLK-SGGDT	- DY LA LKGGF - DY VAVSGGF	LEVRPDKVTI	ILAQTA ILAQPA VLAPSA	o AETIDLV TASHIDEH	RARSAKERAEE RARSAKERAEK RANEAKRRAEG	RLNQ KTDEID RLQE KPDSID RLQD KQANVD	FKRAELALR Ih <mark>R</mark> AELALK FKRAELALO	RAINRLD' RAVNRIN RAVNRLD	/TK I^ENK VSNMK
Bacillus methanolicus Bacillus nakamurai	-MKTIKVSVVTPDGPVYE -MKTVKVNIVTPDGPVYD	SDVEMVSTKAQSGELGILPGHIPMVAF ADIEMVSVRAESGDLGILPGHIPTVAF	LQIGAVRLK-KGGKT LKIGVVRLK-KDGQT	- EYVAVSGGF - EL AAVSGGF	LEVRPDQVTI	ILAQAA ILAQSA	TSEQIDVE TAEGIDKE	RAKRAKE RAEE RALAAKKRAED	RLRA QNQDN I D RLNK RSDDTD	FK <mark>R</mark> AELALK IRRAELALQ	RAINRIS ¹ RAVNRLD ¹	/AEKKL
Bacillus neutsonii Bacillus okhansis Bacillus oleivorans	-MKTIKVNVVTPDGPVHE -MPTIQVSVVTPDGKVYD -MKTVNVHIVTPDGPVYF	SDVEMVIAKAESGELGILAGHIPMVAF GAVDMVVVRTVEGELGILPNHIPLVAF ADVEMVSTKAKSGELGILPGHIPTVAF	PL KIGAVQL K - KDKTS PL TVGAVRL N - KGTTV PL AIGAVRI K - KGAOT	- EKVAVTGGF - EKVAVTGGF	VEVRPDQVTI VEVRPDQVTI	ILAGSA	LPSDIDVD KAFDIDVA	RAL RAKERAEK RAKEAEKRARO RAFOAKKRAFF	RIES OKNEHVD RIES AKTDAID RINO SKODNID	FHRAELALK FKRAELALR FTRAFLALK	RAMNRIS RATNRLD RAVNRIN	/AGK
Becillus persicus Bacillus pseudalcaliphilus	-MNTMKVNVVTPDGPVYE -MPTVNVNVVTPDGKVYD	SDVEMVSTKAQTGELGIMPGHIPMVAF GDVDMVSVRTVDGELGILPKHIPLVAF	LOIGAVRLK - KGGNT LTVGAVRLK - KGSSV	- EFVAVSGGF - EKVAVSGGF	LEVRPDTVTV	VLAQTA ILAEAA	KSDNIDVE LPSDIDVD	RAMRAKE RAEQ RAKQAKE RAEQ	RLRE KQQENVD RLGE I RQDQ I D	FR <mark>R</mark> AELALQ SK <mark>R</mark> AQLSLQ	RAINRIS RAMNRLD	/ AQGKL / SGQ
Bacillus pseudolimus OF4 Bacillus salsus Bacillus salsus	-MSTIRVNVVTPDGKVYD -MKTVKVSVVTPDGPVYE -MKTIOANVVTPDGSVES	GDVDMVVVRTVEGELGILPKHIPLVAF ADVEMVVTRAQSGELGILPGHIPMVAF GDVEMVSVKTREGGLGILPGHIPIVSF	L TVGAVRLK - KGNSE LQIGVVRMK - KATNT	- EQVAVSGGF - EL VAVSGGF	LEVRPDQVTI	ILAEAA ILAQTAE	L PSA I DVD TAEQ I DAR	RARAAKE RAES RAEEAKQ RAEQ	RLNS TQDAVD RLQA KQDDVD RLAE AKKENID	FKRAELALK FRRAELSLK	RAINRLD' RAINRLN'	/TGK /TGNKY
Bacillus smithii Bacillus solimangrovi	-MKTMKVTVVTPDGPVHE MSTMKVSVVTPDGPVYE	SEAEMVSTRATSGELGILPGHIPLVAF TDVEMISVKALSGELGILPGHIPMVAF	LEIGAVRLK-NGNKT	-QWIAVSGGF - HLVAVSGGF	LEVRPDRVTI	ILAQAA	RAEDIDIE	RAQAAKE RAER RAKAAKE RAER	RLRQ ENMD RLKN GPKDNMD	NKRAELALK SKRAELALK	RAVNRIN	YKHK
Bacillus sporothormodurans Bacillus sublemaneus Bacillus thormaseannaise	-MKTLKVSIVTPDGPVYD -MKTIKVSVVTPDGPVYE -MSTVOEDIVTPERTVIS	SNVEMVSTKAQSGELGILPGHIPMVAF SDVEMVSTKASTGELGILPGHIPMVAF	LQIGAVRLK - NGNNT LQIGAVRLK - NGSKY	- EL VAVSGGF - EF LAVSGGF	LEVRPEQUTI	ILAQSA ILAQSA	KAESIDIE ISSEIDLE	RAKEAKSRAEG RALRAKERAEK	RIQS NQPEVD RLHE RRQETHD	RVRAELALR FKRAELALQ	RAINRIN RAINRIS	/FEHRF /AERK I
Bacillus vireti Bacillus wakoensis	-MKTIKVSVVTPDGPVYE -MPTIQVSVVTPDGKVYD	SDVEMVSTKAQSGELGILPGHIPMVA GAVDMVVVRTLDGELGILPNHIPLVA	LOIGAVRLK-KEGNM	- EF LAVSGGF	LEVRPDQVTI	ILAQSA	TASEIDVE	RAL RAKE RAEQ	RMRD QKAEHID RIDS AKADEID	FRRAELALQ	RAINRLA	/SERRML /AGR
Bacillus weihalensis Bacillus wudalanchiensis Bacillus xembermedur	-MKTVLVNVVTPDGPVYD -MKTFKVSIVTPDGPAYE	ADVEMVSVKAQSGELGILPGHIPMVAF SDVEMVSAKAQSGELGILPGHIPMVAF	LRIGAVRLK-QGSST	- EF VAVTGGF PDL VAVSGGF	LEVRPDKITI		RAESIDVA TAENIDIA	RAKEAKKRAEE RAKEAKSRAES	RLNHKTDDID RLSNKQDNVD	FKRAELALQ	RAINRLN	/TK /YEGRV
pacauls xeromermodurans Brevibacilius choshinensis Brevibacilius laterosporus	-MSIFLLEIVTPERKVYA -MSKMTVEVVTPERVVYS -MSKMVIEIVTPERVVYS	GQAEMVIARGVVGDLGILPNHIPLVTF GQAEMVIARGVVGDLGILPNHMPLVSF GEATMVIARGVEGELGILPNHTPIVTF	LKTAPVRIKTEGEKE	- EYTAVNGGF - VVMAVSGGF - TVTAVSGGF	MEVRGDKVT I MEVRGDKVT I	ILAESA ILAETA ILAEAA	LPEQIDIE LPGDIDVE QSTDIDAA	RAQAAKGRAEE RAQAAKGRAEK RAELAKORAFK	RLAS KKREFD RLNE KYPDLD RLNE KFPDLD	FORAERALQ	RAINRIE! RAIARLDI RAVVRLDI	/TK /TK
Brevibecillus reuszeri Fictibecillus macauensis	-MSKMTVEVVTPERVVYS -MKMLEVSVVTPDGPVFE	GQAEMVIARGVQGDLGILPNHMPLVSF GTANMVSAKAKSGELGVMPGHVPLVAF	LKIAPVRIKTEGDKE	- VL MAVSGGF - HYVAVSGGF	MEVRGDKVT	ILAETA VLAQAA	L PGD I DVD L ASD I EVD	RAKAAKA <mark>R</mark> AEK RARAAKE R AEQ	RLAE KYAELD RLA SNQSDLD	VQRAERALQ RKRAELALK	RAMARLD RATNRLE	/TK
Geopacinus Intermogrucosidasius Kyrpidia tusciae Lysinibacillus BF-4	-MKTIKVSVVTPDGPVYE -MSTTILDIVTPERTVYS -MKTVTVNIVTPDGPVYD	ADVEMVSAKAKSGELGILPGHIPLVAF RPVEMVILRGAEGDLGILPGHMPLVTI SDVSMVIVKTTSGEMGILAGHIPMVAF	LEISAVRLK-KEGKT LPIGLVRIK-EEGRW LAVGAVKLKKADNKF	- EYTAVSGGF - SLVALSGGF - ELVAVSGGF	LEVRPDRVTI	ILAUAA VLAEAA ILAPAA	KAEDIDID LPEEIDVA VATDIDVT	RAKAAKERAER RAERARERAER RAKEALARAFO	RLAE KGRSDID RLAE KGRSDID RMAK KOADID	YVRAELALK ATRAELALK	RAINREN RALNRER RAMNRER	AREEAKVSLR
Lysinibacillus macroides Oceanobacillus caeni	MKTVQVNIVTPDGPVYD MKTLTVSVVTPNGPVLE	SEVSMVIAKTTSGEIGVLAGHIPMVAF DEVEMVVCRAESGEIGIMPHHIPIVAF	LAISAVKLK-KENGS LSIAAVRLK-KAGSE	TDIVAVSGGF DKLAVNGGI	IEVRPEKIS MEVSSNKVS	ILAPSA ILAQSA	IAENIDVQ KPSDIDVE	RAKEAVKRAEG RARKAKERAER	RLQS KQDDID RLQS NQDNID	FK <mark>R</mark> ADLALK KL R AELALQ	RALNRIN' RAINRLD	/HEGNI
Paenibacillus lautus Paenibacillus macerans Paenihacillus maceusriensis	-MSTILLEIVTPDRLVYS -MSTFLLEIVTPEHVVFS -MNTFLLEIVTPDH0499	EUVNSVTVRGVEGELGILPGHISFVTF GEVDSLTVRGSEGELGIMRGHIPLVTF NEVNGVSVRGIEGELGILPGHIDE	LUTAPVYVK - TGNQF LOVAPTVAK - KGKET	- TPFAVQGGF - RL IAVHGGF	VEVRKDKIVI IEVQSDKVIV	ILAESA VLAESA	KAEEIDLE LPEEIDVE	RAEAAKERAET	RLHA-KGRODEVD	HRRAEMALQ HRRAELALQ	RAMNRIK	/ I'RN
Paenibacillus polymyxa Paenibacillus solari	-MSTYLLEIVTPERLVYT	EQVNSISVRGSEGDLGILPGHLPLVTF EQVNSVTVRGVEGELGILPGHISFVTF	LKIAPIRIK-IGGQT	- EVIAVNGGF	LEVRKDKVVI	ILAESA	RSEEIDVE	RARAARE RAEL RAEAAKE RAET	RLQS KQEQID RLHA - KGRQDEVD	HRAEVALQ	RALNRLN	ATSIRTNKN. VRHN
Solibecillus isronensis Virgibecillus heicdenilrilicans	-MKTVTVNIVTPDGSVYD -MKTLTVSVVTPDGPVLE	SEVTMVIAKTTSGEIGVLAGHIPMVAF DSFEMVSCKAESGELGILPGHIPLVAF	LTIGSVKLKKADGTT LTISAVRLK-HENRV	- DVAAVSGGF - EHLAVSGGF	IEVRPEKISI MEVRPEKVTI	ILAPSA ILAQSA	VASSIDIA KPSDINVQ	RAKEALARAEG RAKEAKERAER	RLQK KQDDID RLQS KQDDVD	FTRAELSLK FQRAELALK	RAMNRIN	/HEGNI /GQ

Figure S7: Sequence alignment of the whole sequence of ϵ subunits from different bacillic bacteria.