Supplementary Dual RNase and β-lactamase Activity of a Single Enzyme Encoded in Archaea

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Table 1. Best blast Hits of ancestor β -lactamase sequence against the NCBI Archaea database.

Orranzid	NCDI II:+ C:	Oreanism Uits	Size (her)	Descriptions	%	Evelue	%
Query Iu	NCDI HILGI	Organism Fills	512e (bp)	Descriptions	Identity	E-value	Q_Cov
	gi 564600023	Methanolobus tindarius	639	Zn-dependent hydrolase	34.62	5.26×10^{-9}	51.17
	gi 503410340	Methanobacterium lacus	645	Hydrolase	34.23	0.018	57.75
	gi 504218036	Methanocella conradii	624	Hydrolase glyoxylase	33.58	1.17×10^{-6}	53.99
	gi 145282681	Pyrobaculum arsenaticum DSM 13514	621	Beta-lactamase domain protein	33.33	2.14×10^{-7}	52.58
	gi 375160102	Pyrobaculum oguniense TE7	621	Zn-dependent hydrolase glyoxylase	32.52	8.80×10^{-5}	52.58
	gi 2495897	Methanocaldococcus jannaschii DSM2661	618	Probable metallo-hydrolase MJ0296	32.17	1.76×10^{-4}	50.70
Ancestor	gi 1008838121	<i>Thermoplasmatales archaeon</i> SM1-50	651	Hypothetical protein	32.14	0.28	54.46
class B	gi 82617404	uncultured archaeon	645	Hypothetical protein	32.12	1.82×10^{-5}	53.05
β-lactamase	gi 1008853468	Thermoplasmatales archaeon DG-70	606	Hypothetical protein	31.78	0.011	51.17
	gi 851372756	Methanocaldococcus bathoardescens	567	MBL fold metallo-hydrolase	31.58	1.05×10^{-4}	50.70
β-lactamase	gi 851225341	Methanosarcina barkeri	641	MBL fold hydrolase (MetbaB)	31.54	4.88×10^{-5}	51.17
	gi 851312700	Methanosarcina barkeri	641	MBL fold hydrolase	31.54	2.85×10^{-5}	51.17
	gi 490731539	Methanocaldococcus villosus	540	Hypothetical protein	31.53	1.87×10^{-5}	50.70
	gi 1001921197	<i>Methanolobus</i> sp. T82-4	672	Hypothetical protein AWU59_1880	31.39	2.10×10^{-6}	53.99
	gi 505138611	Methanomethylovorans hollandica	645	Zn-dependent hydrolase glyoxylase	31.39	6.56×10^{-8}	54.46
	gi 501690724	Methanosphaerula palustris	696	MBL fold hydrolase	31.37	0.002	56.34

	gi 18160175	Pyrobaculum aerophilum str. IM2	624	Possibly metallo-beta-lactamase superfamily	30.89	2.21×10^{-6}	52.58
	gi 494814289	Candidatus Nitrosoarchaeum koreensis	611	Zn-dependent hydrolase	30.83	0.029	54.46
	gi 504866623	Methanolobus psychrophilus	641	MBL fold hydrolase	30.77	1.73×10^{-5}	51.17
	gi 816389003	Lokiarchaeum sp. GC14_75	648	Metallo-beta-lactamase L1	30.77	3.64×10^{-8}	59.62
	gi 851262085	Methanosarcina horonobensis	641	MBL fold hydrolase	30.77	1.56×10^{-4}	51.17
	gi 502745672	Methanocaldococcus sp. FS406-22	558	MBL fold metallo-hydrolase	30.70	5.98 × 10-5	50.70
	gi 170934313	Pyrobaculum neutrophilum V24Sta	615	Beta-lactamase domain protein	30.65	1.60×10^{-6}	53.05
	gi 757124828	Thermococcus paralvinellae	717	Zn-dependent hydrolase	30.61	0.002	55.87
	gi 756792592	Candidatus Nitrosopumilus piranensis	1404	Rhodanese domain-containing protein	30.56	2.45×10^{-5}	63.38
	gi 851287001	Palaeococcus ferrophilus	609	Glyoxalase	30.51	8.05×10^{-4}	50.70
	gi 973113610	<i>Methanocalculus</i> sp. 52_23	603	Beta-lactamase domain protein	30.40	8.24×10^{-6}	51.17
	gi 973162189	Methanomicrobiales archaeon 53_19	603	Beta-lactamase domain protein	30.40	6.11×10^{-6}	51.17
	gi 524837456	Methanoculleus sp. CAG:1088	641	Putative uncharacterized protein	30.37	0.035	50.70
	gi 851257745	Methanosarcina	600	Hypothetical protein	30.37	0.13	59.62
	gi 700303882	Thermococcus eurythermalis	681	Hydrolase	30.28	2.08×10^{-4}	52.11
	gi 735015437	archaeon GW2011_AR11	714	Beta-lactamase protein	30.25	0.23	50.70
	gi 15623131	Sulfolobus tokodaii str. 7	600	Putative hydrolase	30.23	7.15×10^{-6}	54.46
	gi 500271928	Metallosphaera sedula	606	MBL fold metallo-hydrolase	30.23	6.05×10^{-4}	53.52
	gi 919520712	Sulfolobus tokodaii	597	Hypothetical protein	30.23	6.99×10^{-6}	54.46
	gi 851163782	Geoglobus acetivorans	615	Zn-dependent hydrolase	30.17	1.29×10^{-4}	50.70
	gi 851219309	Candidatus Methanoplasma termitum	576	MBL fold metallo-hydrolase	30.08	0.032	52.58
	gi 494104154	Methanotorris formicicus	618	Hypothetical protein	30.08	4.44×10^{-6}	53.05
	gi 329138039	Candidatus Nitrosoarchaeum limnia	1398	Rhodanese domain-containing protein	30.07	6.43×10^{-7}	63.38
	gi 500766631	Methanoregula boonei	696	MBL fold metallo-hydrolase	30.07	0.055	56.34
Ancestor	gi 445600523	Natronococcus amylolyticus DSM10524	1974	β-lactamase	31.07	1.66×10^{-13}	52.02
class C	gi 1011504614	Methanogenium cariaci	834	Hypothetical protein	30.80	7.49×10^{-12}	57.68
β-lactamase	gi 919167542	Methanosarcina sp. WH1	1959	Hypothetical protein	30.63	1.45×10^{-17}	75.74
	gi 919167113	Methanosarcina sp. WWM596	1959	Hypothetical protein	30.63	1.82×10^{-17}	75.74

Archaea		Phyre ² Investigator Database												
Protein Sequences Size (aa)		Best Protein Hit	Hit ID	Confidence	Coverage	Comments	3D Structure							
Class B β-lactamase	213	Crystal structure of New Delhi Metallo-β-lactamase (NDM-1) form <i>Klebsiella pneumoniae</i>	c3rkjA	100%	94%	201 residues (94%) have been modelled with 100% confidence by the single highest scoring template								
Class C-like β- lactamase	653	Structure of the octameric penicillin-binding protein (PBP) homologue from <i>pyrococcus abyssi</i>	c2qmiH	100%	66%	430 residues (64%) have been modelled with 100% confidence by the single highest scoring template								

Table 2. Three-Dimensional (3D) Structure Comparison with Available and Characterized Proteins from the Phyre² Investigator Database.

	M. Barkeri Class B	<i>Methanosarcina</i> sp. Class C-Like	Elizabethkingia GOB-13 ¹³
β -lactams			
Ampicillin	R	R	R
Ampicillin/sulbactam	R	R	R
Penicillin	R	R	R
Piperacillin	R	R	R
Piperacillin/tazobactam	R	R	R
Cefoxitin	R	R	R
Ceftriaxone	R	R	R
Ceftazidime	R	R	R
Imipenem	R	R	R
Meropenem	R	R	R
Aztreonam	R	R	R
Non β -lactams			
Gentamicin	R	R	S
Ciprofloxacin	R	R	R
Amikacin	R	R	S
Trimethoprim-sulfamethoxazole	R	R	R

Table 3. Antibiotic Resistance Pattern of *Methanosarcina* Compared to *Elizabethkingia* GOB-15.

Nd - not determined. R, resistance; S, Susceptible. *Methanosarcina* isolates were culture on SAB medium (Khelaifia *et al.* PLoS One 17;8(4) (2013). 13 - Opota et al., IJAA 49, 93-97 (2016).



Figure S1. Phylogenetic Tree of Class B β -lactamases: The phylogenetic tree was inferred using the approximate Maximum Likelihood method under the JTT matrixbased model. The analysis involved 174 amino acid sequences from class B β -lactamases. Evolutionary analysis was conducted in FastTree and visualized in FigTree. The existing clades are labeled as b-1 to b-4.



Figure S2. Phylogenetic tree of Class C-like β -lactamases: The phylogenetic tree was inferred using the approximate Maximum Likelihood method under the JTT matrix-based model. The analysis involved 151 amino acid sequences from the class C-like β -lactamases. Evolutionary analysis was conducted in FastTree and visualized in FigTree. The existing clades are labeled as c-1 and c-2.

								His118 _{Asp120} ↓ ↓					His196 ↓								His263 ↓										
VIM-18 Pseudomonas aeruginosa	s	Р	L	Α	Q	Α	v	н	F	Ĥ	D	D	R	v	G	G	Α	Α	Ĥ	s	т	L		v	T.	Р	G	H	G	L	Р
VIM-20 Pseudomonas aeruginosa	s	Ρ	L	Α	Q	Α	v	н	F	н	D	D	R	v	G	G	A	Α	н	s	т	L		v	Т	Р	G	н	G	L	Р
VIM-35 Klebsiella oxytoca	s	Ρ	L	A	Q	A	v	н	F	н	D	D	R	v	G	G	Α	Α	н	s	т	L		v	Т	Р	G	н	G	L	Ρ
VIM-38 Pseudomonas aeruginosa	s	Р	L	A	Q	A	v	н	F	н	D	D	R	v	G	G	Α	Α	н	s	т	L		v	Т	Р	G	н	G	L	Р
NDM-1 Klebsiella pneumoniae	Q	Q	м	Е	Q	Α	v	н	Α	н	Q	D	к	М	G	G	Р	G	н	т	s	Т		Т	v	м	s	н	s	A	Р
NDM-2 Escherichia coli	Q	Q	м	Е	Q	Α	v	н	Α	н	Q	D	к	М	G	G	Р	G	н	т	s	Т		Т	v	м	s	н	s	Α	Р
NDM-4 Escherichia coli	Q	Q	М	Е	Q	Α	v	н	Α	н	Q	D	к	М	G	G	Р	G	н	т	s	Т		Т	v	м	s	н	s	Α	Р
KHM-1 Citrobacter freundii	D	s	L	Ρ	к	s	Т	н	F	н	т	D	s	т	G	G	Α	G	н	т	Р	L		v	v	Р	G	н	G	к	v
IMP-11 Acinetobacter baumannii	Α	s	L	Ρ	к	s	Т	H	F	н	s	D	s	т	G	G	Р	G	н	т	Q	v		v	v	Р	s	н	s	D	Т
IMP-4 Pseudomonas aeruginosa	Е	Р	L	Р	к	s	Т	н	F	н	s	D	s	т	G	G	Р	G	н	т	Р	L		v	v	Р	s	н	s	Е	Α
IMP-13 Pseudomonas monteilii	A	A	L	Ρ	к	т	Т	н	F	н	s	D	s	т	G	G	Ρ	G	н	т	Q	L		v	v	s	s	н	s	Е	к
BlaB-10 Elizabethkingia meningoseptica	Q	Q	Ν	Ρ	к	Ν	Т	н	s	н	D	D	R	A	G	G	к	G	н	т	Α	v		v	v	Α	G	н	D	D	w
IND-14 Chryseobacterium indologenes	A	Q	v	к	Р	v	F	н	s	н	D	D	R	A	G	G	Е	G	н	т	Α	v		v	Т	Р	G	н	D	Е	w
IND-15 Chryseobacterium indologenes	Α	Q	v	к	Р	۷	F	н	s	н	D	D	R	A	G	G	Е	G	н	т	v	v		v	Т	Р	G	н	D	Е	w
gi 851312700 Methanosarcina barkeri	м	Е	v	R	Y	Т	v	н	с	н	Y	D	н	A	Α	т	Ρ	G	н	s	к	Т		L	Y	Р	G	н	G	A	Ρ
gi 851225341 Methanosarcina barkeri	м	Е	v	R	Υ	T.	v	н	с	н	Y	D	н	A	Α	т	Р	G	н	s	к	Т		L	Y	Р	G	н	G	Α	Ρ
gi 851262085 Methanosarcina horonobensis	м	Е	v	R	Y	Т	v	н	с	н	Y	D	н	т	A	т	Р	G	н	s	к	Т		L	Y	s	G	н	G	A	Р
GOB-16 Elizabethkingia meningoseptica	Е	Ν	М	Ρ	к	L	L	Q	Α	н	Y	D	н	т	G	н	Ρ	G	н	т	к	с		w	v	Α	s	н	Α	s	Q
GOB-4 Elizabethkingia meningoseptica	Е	Ν	М	Ρ	к	L	L	Q	Α	н	Y	D	н	т	G	н	Р	G	н	т	к	с		w	v	Α	s	н	Α	s	Q
GOB-15 Elizabethkingia meningoseptica	Е	Ν	т	Ν	Е	L	L	Q	Α	н	Y	D	н	т	G	н	Р	G	н	т	к	с		w	v	Α	s	н	Α	s	Q
GOB-18 Elizabethkingia meningoseptica	Е	Ν	М	Ρ	к	L	L	Q	A	н	Y	D	н	т	G	н	Ρ	G	н	т	к	с		w	v	Α	s	н	Α	s	Q

Figure S3. Protein sequences alignment of bacterial and archaeal class B β-lactamases. Known and conserved residues of bacterial metallo-β-lactamases are highlighted with yellow color.



Figure S4. Evaluation of the archaeal MetbaB enzyme activity on nitrocefin at different pH. **(A)** The monitoring of the nitrocefin degradation by MetbaB enzymes during 30 minutes under the four different pH conditions. **(B)** The MetbaB activity test on the chromogenic cephalosporin substrate in liquid medium.



Figure S5. The antibiotic susceptibility testing of a recombinant *E. coli* BL21 mutant containing this Archaeal β-lactamase (*metbaB* gene). GC: Growth control. Bacterial growth is indicated by red color observed in each well.



Figure S6. Phylogenetic relation cheap of different MBL fold proteins including β-lactamases, nucleases, ribonucleases, glyoxalase, and identified enzymes from human, bacteria, archaea, and giant virus.



Figure S7. Monitoring the imipenem hydrolysis by the *E. meningoseptica* metallo- β -lactamase GOB-13 and MetbaB enzyme by LC-MS. Time depended production of the metabolite of imipenem (i.e. imipenemoic acid) is measured here using a unhydrolyzed cilastatine substrate. Both enzymes hydrolyze efficiently imipenem through the increase accumulation of its metabolite.



Figure S8. Glyoxalase II activity assay. D-lactate production was monitored for 40 min following absorbance variations at 450 nm. The GloII positive control was provided in the "Glyoxalase II Activity kit" from BioVision. The negative control was the reagent background control (same mixture with no active enzyme).

				S ⁶⁴ XXK motif	Y ¹⁵⁰ XN motif		
				↓ ↓	t		
				<u> </u>	<u> </u>		
ACC-1 Klebsiella pneumoniae	NIPGMSVAV	YGLAAK QPVT	ENTLFEV	' G <mark>S</mark> L S <mark>K</mark> T	A G T H R V <mark>Y</mark> S <mark>N</mark> I	GLLG KV	PAD
ACC-2 Hafnia alvei	NIPGMSVAV	YGLAAK QPVT	ENTLFEV	΄ G <mark>S</mark> L S <mark>K</mark> T	A G T H R V <mark>Y</mark> S <mark>N</mark> I	GLLG KV	PAD
ACT-3 Enterobacter asburiae	AIPGMAVAV	FGKADV KPVT	PQTLFEL	<mark>с s</mark> i s <mark>к</mark> т	P G T T R L <mark>Y</mark> A <mark>N</mark> T	GLFG NV	РКА
ACT-12 Enterobacter cloacae	SIPGMAVAV	FGKADV TPVT	AQTLFEL	<mark>с s</mark> i s <mark>к</mark> т	P G T T R L <mark>Y</mark> A <mark>N</mark> A	GLFG NV	РКА
MOX-4 Aeromonas caviae	RIPGMAVAV	YGVADR VGVS	EQTLFEI	<mark>с s</mark> v s <mark>к</mark> p	P G S H R Q <mark>Y</mark> S <mark>N</mark> P	GLFG NV	РКQ
FOX-5 Klebsiella pneumoniae	RIPGMAVAV	YGVANR QRVS	EQTLFEI	с <mark>s</mark> vs <mark>к</mark> т	A G T H R Q <mark>Y</mark> S <mark>N</mark> P	GLFG QV	PES
FOX-7 Enterobacter cloacae	RIPGMAVAV	YGVANR QRVS	EQTLFEI	с <mark>s</mark> vs <mark>к</mark> т	A G T H R Q <mark>Y</mark> S <mark>N</mark> P	GLFG QV	PES
DHA-7 Enterobacter cloacae	DIPGMAVAV	YGFADI QPVT	ENTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G D M R L <mark>Y</mark> A <mark>N</mark> S	GLFG TV	PES
CMY-12 Proteus mirabilis	AIPGMAVAV	WGKADI HPVT	QQTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> S <mark>N</mark> S	GLFG TV	PQN
CMY-17 Escherichia coli	AIPGMAVAV	WGKADI HPVT	QQTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> A <mark>N</mark> S	GLFG TV	PQN
CMY-19 Klebsiella pneumoniae	RIPGMAVAV	YGVANR ASVS	EQTLFDI	с <mark>s</mark> vs <mark>к</mark> т	P G S H R Q <mark>Y</mark> S <mark>N</mark> P	GLFG NV	РКQ
CMY-20 Escherichia coli	AIPGMAVAV	WGKADI HPVT	QQTLFEL	<mark>сs</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> A <mark>N</mark> S	GLFG TV	PQN
CMY-39 Citrobacter freundii	AIPGMAVAV	WGKADI HPVT	QQTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> A <mark>N</mark> S	GLFG KV	PQS
CMY-48 Citrobacter freundii	AIPGMAVAI	WGKADI HPVT	QQTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> A <mark>N</mark> S	GLFG TV	PQS
CMY-64 Escherichia coli	AIPGMAIAV	WGKADI HPVT	QQTLFEL	с <mark>s</mark> vs <mark>к</mark> т	P G A K R L <mark>Y</mark> A <mark>N</mark> S	GLFG TV	PQN
OCH-6 Ochrobactrum anthropi	KIPGMAVAI	YGVASK QKVT	EDTIFEI	с <mark>s</mark> vs <mark>к</mark> т	A G T Q R R <mark>Y</mark> S <mark>N</mark> P	GLFG NV	PES
OCH-8 Ochrobactrum anthropi	KIPGMAVAI	YGVASK QKVT	EDTIFEI	с <mark>s</mark> vs <mark>к</mark> т	A G T Q R R <mark>Y</mark> S <mark>N</mark> P	GLFG NV	PES
gi 1011504614 Methanosarcina cariaci	NIPGAVVAV	YGYADI SPVN	EETLFHV	` G <mark>S</mark> I T <mark>K</mark> L	P G T V S S <mark>Y</mark> S <mark>N</mark> Y	TLAA SY	PEA
gi 919167542 Methanosarcina sp. WH1	NVSGATVAV	YGYADI QPVS	NQTLFRV	΄ G <mark>S</mark> V S <mark>K</mark> L	P G E L T A <mark>Y</mark> S <mark>N</mark> Y	ALAA DQ	PLP
gi 919167113 Methanosarcina sp. WWM596	NVSGATVAV	YGYADI QPVS	NQTLFRV	΄ G <mark>S</mark> V S <mark>K</mark> L	P G E L T A <mark>Y</mark> S <mark>N</mark> Y	ALAA DQ	PLP
WP 052727337 Methanosarcina siciliae	PVPGATVAV	YGYADI QPVV	NQTLFRV	΄ G <mark>S</mark> V S <mark>K</mark> L	P G E L T A <mark>Y</mark> S <mark>N</mark> Y	ALAA DQ	PLP

Figure S9. Protein alignment sequences of bacterial and archaeal class C-like β-lactamase proteins. Known and conserved motifs of bacterial class C β-lactamases are highlighted with yellow color.



Figure S10. Degradation of nitrocefin by MetbaB and the archaeal class C-like β -lactamases was monitored for 10 min following absorbance variations at 486 nm. Enzymes were respectively kept at concentrations of 10 μ M and 4 μ M for this assay. The negative control displayed here was the same reagent mixture without any active enzyme.



Figure S11. Putative evolution scenario of enzymatic activities of MBL fold proteins. (+): positive activity; (-) : negative activity.

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