

Supporting Information

AN ANTIBIOTIC-RESISTANCE ENZYME FROM A DEEP-SEA BACTERIUM *Marta Toth,[†] Clyde Smith,^{*,‡} Hilary Frase,[†] Shahriar Mobashery,[†] and Sergei Vakulenko^{*,†}*

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Running head: β -lactamase from a deep-sea bacterium

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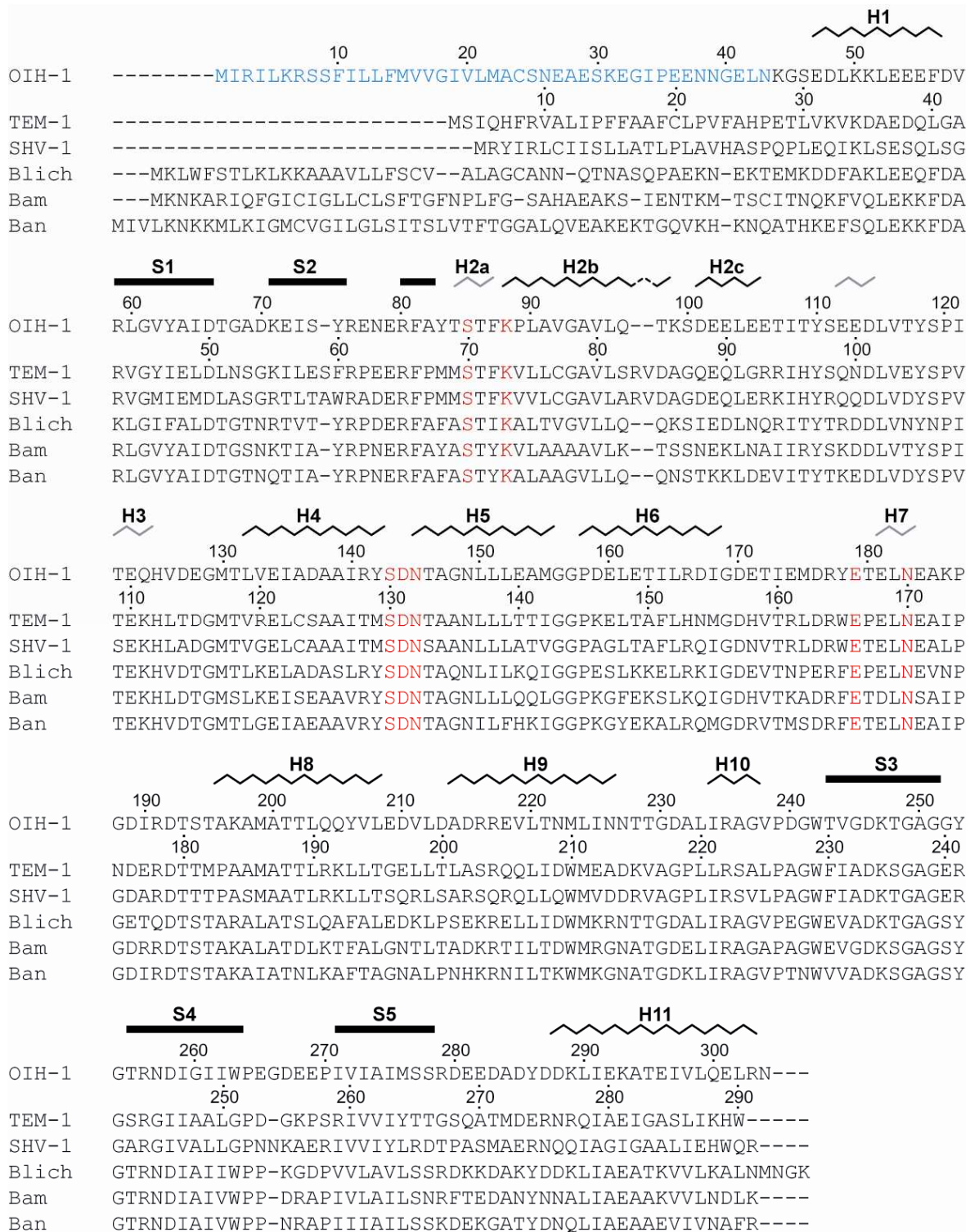
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Caption For Supporting Figure S1

Supporting Fig. S1. Amino acid sequence alignment for OIH-1 β -lactamase with other β -lactamase enzymes. Sources: OIH, *Oceanobacillus iheyensis*; TEM-1, *E. coli.*; SHV-1, *Klebsiella pneumonia*; Blich, *Bacillus licheniformis* B2s; Bam, *B. amyloliquefaciens* FZB42; Ban, *B. anthracis* strain Ames. Conserved amino acid motifs are in red. The residues in blue in the OIH-1 sequence denote the amino acids not observed in the current structure.

Supporting Fig. S1



Supporting Table S1. Amino acid composition and intramolecular interactions of selected β -lactamases and *O. iheyensis* proteins

	β -lactamase enzymes*				<i>O. iheyensis</i> enzymes	
	OIH-1	Blich (1i2s)	TEM-1 (1btl)	SHV-1 (1shv)	DHPS (3d0c)	MCI (3fyy)
Number of residues [†]	274	282	286	286	608	782
Asp [‡]	28 (10)	23 (8)	16 (6)	16 (6)	36 (6)	66 (8)
Glu	38 (14)	23 (8)	20 (7)	15 (5)	54 (9)	48 (6)
Lys	12 (4)	25 (9)	11 (4)	7 (2)	30 (5)	60 (8)
Arg	13 (5)	14 (5)	19 (7)	25 (9)	24 (4)	34 (4)
Asn	12 (4)	17 (6)	8 (3)	7 (2)	36 (6)	30 (4)
Gln	5 (2)	10 (4)	9 (3)	14 (5)	54 (9)	24 (3)
Ile	21 (8)	14 (5)	17 (6)	16 (6)	50 (8)	46 (6)
Leu	24 (9)	28 (10)	33 (12)	36 (13)	48 (8)	86 (11)
Val	14 (5)	14 (5)	15 (5)	17 (6)	60 (10)	58 (7)
Aromatic residues	16 (6)	17 (6)	25 (9)	17 (6)	64 (11)	102 (13)
Total hydrogen bonds [§]	298 (1.09)	303 (1.19)	306 (1.16)	323 (1.22)	1244 (2.04)	783 (1.00)
- main chain/side chain	184 / 114	186 / 117	194 / 112	196 / 127	883 / 361	480 / 303
Salt bridges [¶]	33 / 18	34 / 16	26 / 14	34 / 23	60 / 39	59 / 28
Hydrophobic interactions	218 / 102	216 / 90	215 / 58	214 / 84	559 / 260	341 / 154

* Enzyme source; OIH-1, *Oceanobacillus iheyensis*; Blich, *Bacillus licheniformis* Bs3; TEM-1, *Escherichia coli*; SHV-1, *Klebsiella pneumoniae*; DHPS (dihydropicolinate synthase) and MCI (muconate cycloisomerase) are the only two *O. iheyensis* structures currently in the Protein Data Bank. [†] Residues in the mature enzyme. [‡] The numbers of residues are given as the total number in the sequence followed by their percent abundance in parentheses. [§] The number in parentheses is the ratio of hydrogen bonds to residues in the refined structure. [¶] Salt bridges, interactions $< 6 \text{ \AA} / < 4 \text{ \AA}$. ^{||} Hydrophobic interactions $< 5 \text{ \AA} / < 4 \text{ \AA}$.

Supporting Table 2. Surface properties of selected β -lactamases and *o. Iheyensis* enzymes

	OIH-1	Blich	TEM-1	SHV-1	DHPS	MCI
Number of residues [*]	260	260	263	265	602	782
Water molecules	515	152	199	81	442	578
D + E [†]	62 / 55	43 / 37	36 / 30	31 / 23	90 / 84	110 / 86
K + R [†]	23 / 21	35 / 33	29 / 27	30 / 28	52 / 48	90 / 82
Net charge	-39	-8	-7	-1	-38	-20
Surface Charge	-34	-4	-2	5	-36	-4
Surface acidic/basic ratio	2.75	1.15	1.07	0.85	1.78	1.04
Total surface area (\AA^2)	11160	10820	11575	11110	23620	27080
Acidic surface area (\AA^2)	5070 / 46	3140 / 29	2300 / 20	1665 / 15	6460 / 27	6160 / 23
Basic surface area (\AA^2)	1580 / 14	3200 / 30	2500 / 22	2490 / 22	4230 / 18	7010 / 26
Net charge density ($e/\text{\AA}^2$)	-3.50×10^{-3}	-0.74×10^{-3}	-0.60×10^{-3}	-0.09×10^{-3}	-1.61×10^{-3}	-0.74×10^{-3}
Surface charge density ($e/\text{\AA}^2$)	-3.05×10^{-3}	-0.46×10^{-3}	-0.17×10^{-3}	0.45×10^{-3}	-1.52×10^{-3}	-0.15×10^{-3}
Acidic residues / 10^3\AA^2	4.9	3.4	2.5	2.1	3.6	3.3
Basic residues / 10^3\AA^2	1.9	3.0	2.3	2.4	2.0	3.2

^{*} Number of residues in the refined structure. [†] Total number / number on the surface

Supporting Table 3. Kinetic parameters for the hydrolysis of ampicillin by the oih-1 β -lactamase in the presence of NaCl

NaCl (M)	k_{cat} (sec ⁻¹)	K_{m} (μM)	$k_{\text{cat}} / K_{\text{m}}$ (M ⁻¹ s ⁻¹)
0	94 \pm 3	15 \pm 2	(6.3 \pm 0.9) x 10 ⁶
0.85	97 \pm 3	17 \pm 3	(5.7 \pm 1.0) x 10 ⁶
1.7	97 \pm 2	24 \pm 2	(4.0 \pm 0.4) x 10 ⁶
3.4	91 \pm 2	31 \pm 3	(2.9 \pm 0.3) x 10 ⁶
5.1	89 \pm 2	56 \pm 4	(1.6 \pm 0.1) x 10 ⁶

Supporting Table 4. Surface properties of selected halophilic proteins

	dodecin (1mog)	ferredoxin (1doi)	MDH (2j5k)	NDK (2az1)	FTFT (1ftr)	AmyB (3bc9)	GlucDH (2b5w)	DHFR (1vdr)	CA (1y7w)
Kingdom	archaea	archaea	archaea	archaea	archaea	bacteria	archaea	archaea	plantae
Residues*	804	128	1202	928	1184	585	710	315	274
Water molecules	1176	237	1023	105	706	574	1350	80	265
Resolution (Å)	1.70	1.90	2.00	2.35	1.70	1.35	1.65	2.55	1.86
Oligomeric state	12	1	4	6	4	1	2	2	1
D + E [†]	192 / 192	34 / 34	248 / 232	204 / 186	208 / 192	100 / 82	124 / 112	60 / 58	33 / 30
K + R [†]	48 / 48	6 / 6	92 / 80	84 / 66	84 / 80	45 / 36	58 / 48	28 / 28	17 / 16
Net charge (e)	-144	-28	-156	-120	-124	-55	-66	-32	-16
Surface charge (e)	-144	-28	-152	-120	-112	-46	-64	-30	-14
Surface acidic/basic ratio	4.00	5.67	2.90	2.82	2.40	2.28	2.33	2.07	1.88
Total surface area (Å ²)	30900	6330	41630	32410	38545	23245	27980	15330	11870
Acidic surface area (Å ² / %)	16350 / 53	2935 / 46	17990 / 43	15300 / 47	14460 / 38	6230 / 27	9870 / 35	5510 / 36	2340 / 20
Basic surface area (Å ² / %)	1560 / 5	330 / 5	4530 / 11	3630 / 11	5810 / 15	2280 / 10	5090 / 18	2320 / 15	1050 / 9
Charge density (x 10 ⁻³ e/Å ²)									
- net	-4.66	-4.42	-3.75	-3.70	-3.22	-2.37	-2.36	-2.09	-1.35
- surface	-4.66	-4.42	-3.65	-3.70	-2.91	-1.98	-2.29	-1.96	-1.18
Acidic residues (per 10 ³ Å ²)	6.2	5.4	5.6	5.7	5.0	3.5	4.0	3.8	2.5
Basic residues (per 10 ³ Å ²)	1.6	0.9	1.9	2.0	2.1	1.5	1.7	1.8	1.3
Water molecules/residue	1.46	1.85	0.85	0.11	0.60	0.98	1.90	0.25	0.97

Protein source: dodecin and NDK (nucleoside diphosphate kinase) - *Halobacterium salinarum*; ferredoxin and MDH (malate dehydrogenase) - *Haloarcula marismortui*; FTFT (formylmethanofuran:tetrahydromethanopterin formyltransferase) - *Methanopyrus kandleri*; AmyB (β -amylase) - *Haloferax volcanii*; GlucDH (glucose dehydrogenase) - *Haloferax mediterranei*; DHFR (dihydrofolate reductase) - *Haloferax volcanii*; CA (carbonic anhydrase) - *Dunaliella salina*. * number of residues in the refined structure. † total number / number on the surface.