

TABLE S1. Primers used in the study.

Primer name	Sequence (5' - 3')	Target gene
PEDO-1F	GAGTGGTCGGCTGCTTATGC	<i>bla</i> _{PEDO-1}
PEDO-1R	CGGTAACAATACTGGGCATA	
PEDO-2F	GGTCTCAAGCTACAGAGCCA	<i>bla</i> _{PEDO-2}
PEDO-2R	TATCATGGAGCTCAAAGTAC	
PEDO-3F	ACACCACATACAATACCTAC	<i>bla</i> _{PEDO-3}
PEDO-3R	TATGGTCTAGAGATTCCCTG	
MSI-1F	CGCGCGCATGGTGCTGCAGC	<i>bla</i> _{MSI-1}
MSI-1R	ACGGCATAGGCCTTGCAGGC	
SPG-1F	ATCCGCGCTCGCCATGACAG	<i>bla</i> _{SPG-1}
SPG-1R	CGCTTGCTGCTTCGCCAGCG	
CPS-1F	TAATCCGGCAGAATGGTCAA	<i>bla</i> _{CPS-1}
CPS-1R	CTGACTTGCATGCGAAGCTA	
ESP-1F	TCCTGACTTTGACACAAGCG	<i>bla</i> _{ESP-1}
ESP-1R	CTCCAGATAATCATCTTCCA	

8-27F	AGAGTTTGATC/TA/CTGGCTCAG	16S rRNA
1390R	ACGGGCGGTGTGTA/GCAA	
CH1F	AGTGTTTGTCAATCCGCAGAA	<i>bla</i> _{CPS-1} harboring DNA fragment (Sangersequencing)
CH1R	TTATATCCAGAAGATTCTGC	
HinCIIIF	CCCCCCTCGAGGTC	pZE21MCS cloned DNA fragments
HinCIIIR	ATCAAGCTTATCGATACCGTC	
pCFF	CTGTTTCTCCATACCCGTT	pCF430 cloned DNA fragments
PCFR	TGCAAGGCGATTAAGTTGG	

TABLE S2. Summary of whole genome sequencing data of metallo- β -lactamase-producing strains described in this study.

Metallo β - lactamase producers	Data before <i>de novo</i> genome assembly			Data after <i>de novo</i> genome assembly					
	No. of	No. of base	Average length of	No. of contigs	No. of bp	Shortest	Longest	Average	Coverage
	reads	pairs (bp)	the reads(bp)		in contigs	contig (bp)	contig	contig length	(%)
		in reads					(bp)	(bp)	
<i>Chryseobacterium</i> <i>piscium</i> strain Stok- 1	1323154	192038699	145.1	226	4366306	177	452220	19319	44
<i>Epilithonimonas</i> <i>tenax</i> strain Stok-2	1034846	148829908	143.8	511	6264367	153	640048	12259	23.8
<i>Pedobacter</i> <i>kyungheensis</i> strain Stok-3	1785304	254636293	142.6	67	4367712	157	870169	65189	38.2

<i>Pedobacter roseus</i>	1247336	181278497	145.3	109	6651001	161	730502	61018	27.3
strain SI-33									
<i>Sphingomonas</i> sp.	2001116	291449206	145.6	295	6040780	185	166087	20477	48.2
strain ALS-13									
<i>Pedobacter</i>	1508152	208729193	138.4	2687	6996300	161	492394	2603	29.8
<i>borealis</i> strain ALS-14									
<i>Massilia oculi</i> strain SB1-3	1341422	191324347	142.6	4494	7228529	161	192127	1608	26.5

TABLE S3. Metallo- β -lactamase-associated proteins

Metallo- β -lactamase	Metallo- β -lactamase associated proteins	Metallo- β -lactamase associated protein homologues (accession no.) ¹	Homologue-harboring bacterial species	Query coverage (%)	Identity (%)
CPS-1	Orfa1	GCN5-related N-acetyl-transferase (KFF00122.1)	<i>Chryseobacterium formosense</i>	100	91
	Orfa2	Pirin-related protein (KFF22053.1)	<i>Chryseobacterium</i> sp. CTM	100	92
	Orfa3	NADPH-dependent FMN reductase (KGO09533.1)	<i>Elizabethkingia miricola</i>	99	90
	Orfa4	Osmotically inducible protein OsmC (KFF00117.1)	<i>Chryseobacterium formosense</i>	100	93
ESP-1	Orfb1	Diphosphomevalonate decarboxylase (KFC21403.1)	<i>Epilithonimonas lactis</i>	100	96
	Orfb2	Hypothetical protein (KFC21404.1)	<i>Epilithonimonas lactis</i>	100	90
	Orfb3	ABC transporter (KFC39492.1)	<i>Elizabethkingia meningoseptica</i>	93	37

	Orfb4	Hypothetical protein (KFC23008.1)	<i>Epilithonimonas</i> sp. FH1	100	94
PEDO-1	Ptp1	Phage tail sheath protein (WP_025146708.1)	<i>Sphingobacterium</i> sp. H1ai	95	83
	Ptp2	Phage tail sheath protein (WP_025146707.1)	<i>Sphingobacterium</i> sp. H1ai	100	95
	Pttp	Phage tail tube protein(WP_025146707.1)	<i>Sphingobacterium</i> sp. H1ai	99	96
	Bpp	Phage base plate(WP_025146704.1)	<i>Sphingobacterium</i> sp. H1ai	97	81
	YVTN1	40-residue YVTN family beta-propeller repeat (WP_007413769.1)	<i>Pedosphaera parvula</i>	97	44
	YVTN2	40-residue YVTN family beta-propeller repeat (WP_007413769.1)	<i>Pedosphaera parvula</i>	99	44
	AcrB	Acriflavin resistance protein (WP_029986281.1)	<i>Sphingobacterium</i> sp. H1ai	98	96
	RND	Resistance nodulation cell division protein (WP_015808359.1)	<i>Pedobacter heparinus</i>	93	61

	OMF	Outer membrane protein (WP_015808360.1)	<i>Pedobacter heparinus</i>	100	53
PEDO-2	Orfc1	Transglycosylase (WP_029275226.1)	<i>Pedobacter borealis</i>	100	99
	Orfc2	Hypothetical protein (WP_029275227.1)	<i>Pedobacter borealis</i>	100	99
	Orfc3	Nuclease (WP_029275228.1)	<i>Pedobacter borealis</i>	100	100
	Orfc4	Transcriptional regulator (WP_029275229.1)	<i>Pedobacter borealis</i>	100	99
PEDO-3	Orfd1	GTP-binding protein Der (WP_029274791.1)	<i>Pedobacter borealis</i>	100	98
	Orfd2	GNAT family acetyltransferase (WP_029274790.1)	<i>Pedobacter borealis</i>	99	93
	Orfd3	GNAT family acetyltransferase(WP_025146291.1)	<i>Sphingobacterium</i> sp. H1ai	100	96
	Orfd4	Short-chain dehydrogenase (WP_025146292.1)	<i>Sphingobacterium</i> sp. H1ai	100	92
SPG-1	Orfe1	Phosphopantothenate synthase	<i>Sphingomonas</i> sp.	98	79

	(WP_029936358.1)			
Orfe2	Hypothetical protein (WP_002615847.1)	<i>Stigmatella aurantiaca</i>	84	57
Orfe3	Multidrug DMT transporter permease	<i>Acetobacteraceae</i>	93	72
	(WP_007435308.1)	<i>bacterium</i>		

¹Homologues were generated from GenBank sequence database by using the metallo- β -lactamase-associated proteins as query sequence (blastp). Accession numbers (in brackets) indicate proteins from GenBank that showed maximum identity to the metallo- β -lactamase-associated proteins. See Figure 1 for organization of the operon with genes encoding each metallo- β -lactamase and associated proteins.

TABLE S4. Amino acid sequence identity of ESP-1, CPS-1, PEDO-1, PEDO-2, SPG-1 and MSI-1 and previously described subclass B3 metallo- β -lactamases.

Metallo- β -lactamases	Amino acid sequence identity (%)														
	AIM-1	THIN-B	SMB-1	MSI-1	CAU-1	SPG-1	BJP-1	L1	POM-1	CPS-1	ESP-1	PEDO-2	GOB-1	PEDO-1	FEZ-1
AIM-1		43	40	40	25	29	24	29	30	21	22	19	21	20	20
THIN-B	43		39	37	29	33	28	29	31	19	21	19	21	21	24
SMB-1	40	39		40	29	32	25	27	29	21	22	20	22	21	25
MSI-1	40	37	40		25	29	27	30	26	20	21	19	20	21	23
CAU-1	25	29	29	25		47	38	27	29	27	27	26	26	26	27
SPG-1	29	33	32	29	47		40	30	33	27	28	27	31	30	33
BJP-1	24	28	25	27	38	40		28	31	31	30	31	30	30	30
L1	29	29	27	30	27	30	28		57	20	19	19	20	20	24
POM-1	30	31	29	26	29	33	31	57		22	21	22	21	22	27
CPS-1	21	19	21	20	27	27	31	20	22		82	69	68	56	35
ESP-1	22	21	22	21	27	28	30	19	21	82		70	69	57	57

PEDO-2	19	19	20	19	26	27	31	19	22	69	70		67	57	37
GOB-1	21	21	22	20	26	31	30	20	21	68	69	67		56	37
PEDO-1	20	21	21	21	26	30	30	20	22	56	57	58	56		35
FEZ-1	20	24	25	23	27	33	30	24	27	35	37	35	37	35	

GenBank/EMBL sequence database accession numbers of previously described metallo- β -lactamases: AIM-1, M998375; THIN-B, CAC33832; SMB-1, AB636283; CAU-1, AJ308331; BJP-1, NP772870; L1, ABO60992; POM-1, EU315252; GOB-1, AAF04458; FEZ-1, CAB96921.

TABLE S5. Amino acid sequence identity of PEDO-3 and previously described subclass B1 metallo- β -lactamases.

Metallo- β - lactamase	Amino acid sequence identity (%)														
	IND-1	CGB-1	EBR-1	JOHN-	PEDO-3	BlaB	IMP-	SIM-	KHM-	GIM-	MUS-	TUS-	NDM-	VIM-	SPM-
				1		1	1	1	1	1	1	1	1	1	1
IND-1		83	60	41	40	42	30	28	29	26	40	41	21	24	24
CGB-1	83		58	42	41	43	30	26	30	28	39	40	21	24	26
EBR-1	60	58		39	41	40	30	27	28	28	37	35	22	22	24
JOHN-1	41	42	39		55	47	27	27	30	30	39	42	24	21	25
PEDO-3	40	42	41	55		49	30	30	34	32	41	41	23	23	23
BlaB1	42	43	40	47	49		29	28	29	30	39	39	21	23	24
IMP-1	30	30	30	27	30	29		67	57	44	29	33	27	27	28
SIM-1	28	27	27	27	30	28	67		55	42	29	30	27	26	26
KHM-1	29	30	28	31	34	29	57	55		48	30	32	26	31	25
GIM-1	26	28	28	30	32	30	44	42	48		27	28	25	29	27
MUS-1	40	39	37	39	41	38	29	30	30	27		73	24	25	25

TUS-1	41	40	35	42	40	39	33	30	32	28	73		24	24	24
NDM-1	21	21	22	24	23	21	27	27	25	24	24	24		34	20
VIM-1	24	24	22	21	23	23	27	26	31	29	25	24	34		21
SPM-1	24	26	24	25	23	24	28	26	25	27	25	24	20	21	

GenBank/EMBL sequence database accession numbers of previously described subclass B1 metallo- β -lactamases: IND-1, AF099139; CGB-1, AF339734; EBR-1, AF416700; JOHN-1, AY028464; BlaB1, AF189298; IMP-1, ADI87504; SIM-1, AAX76774; KHM-1, BAH16555; GIM-1, CAF05908; MUS-1, AF441286; TUS-1, AF441287; NDM-1, CAZ39946; VIM-1, CAC35170; SPM-1, CAD37801.