

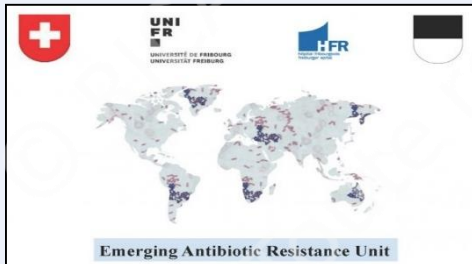
ZHO-1, a novel B1 metallo-beta-lactamases identified

from environmental isolates

Nicolas Kieffer*¹, Julia Guzman Puche², Hyo Jung

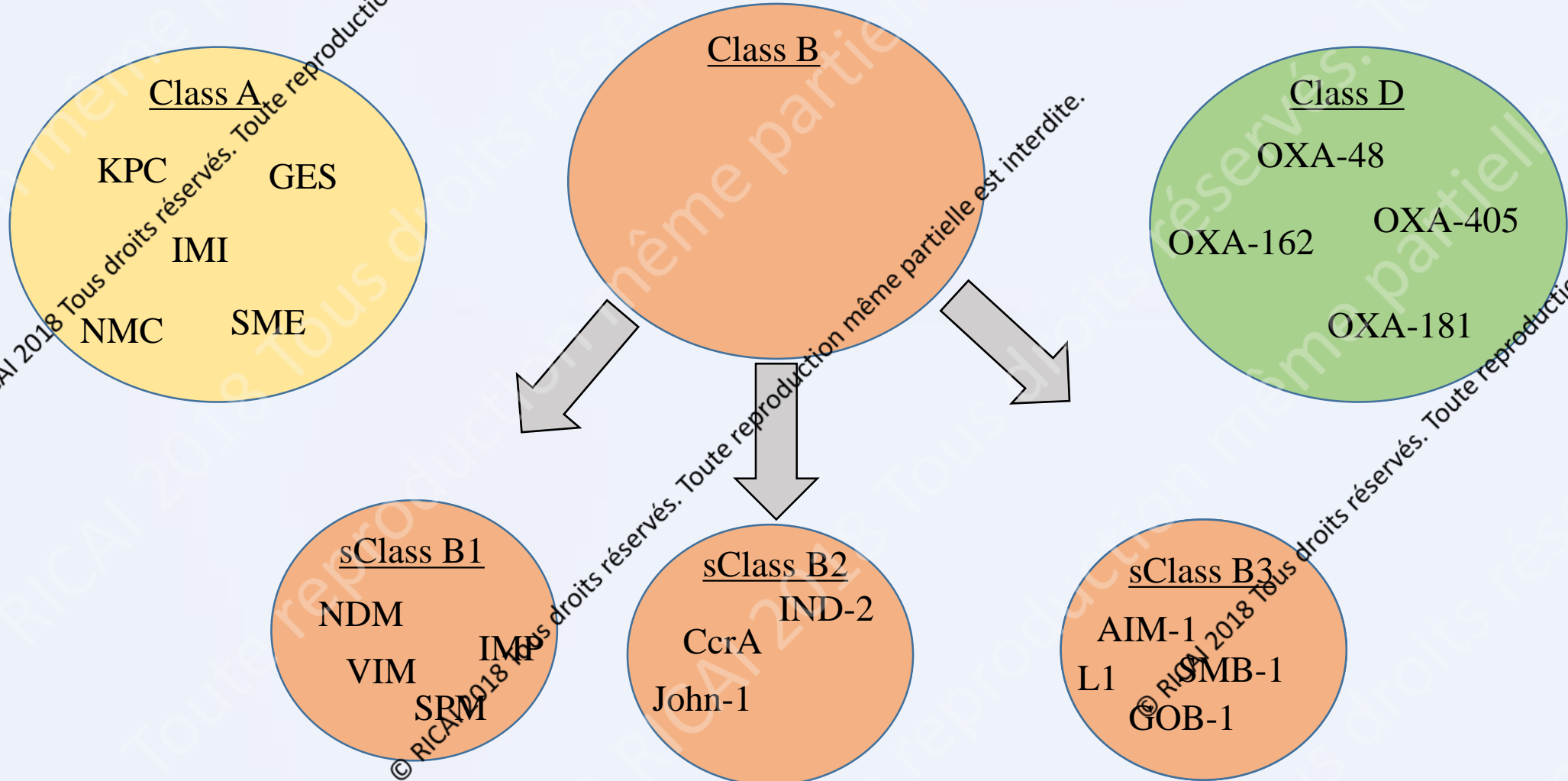
Kang³, Che Ok Jeon³, Laurent Poirel¹ Patrice Nordmann¹

¹Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland, ²University Hospital Reina Sofía-IMIBIC-University of Córdoba, Córdoba, Spain, ³ Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul, 156-756, Republic of Korea



BACKGROUND

- The emergence of carbapenemase-producing bacteria is a major clinical concern



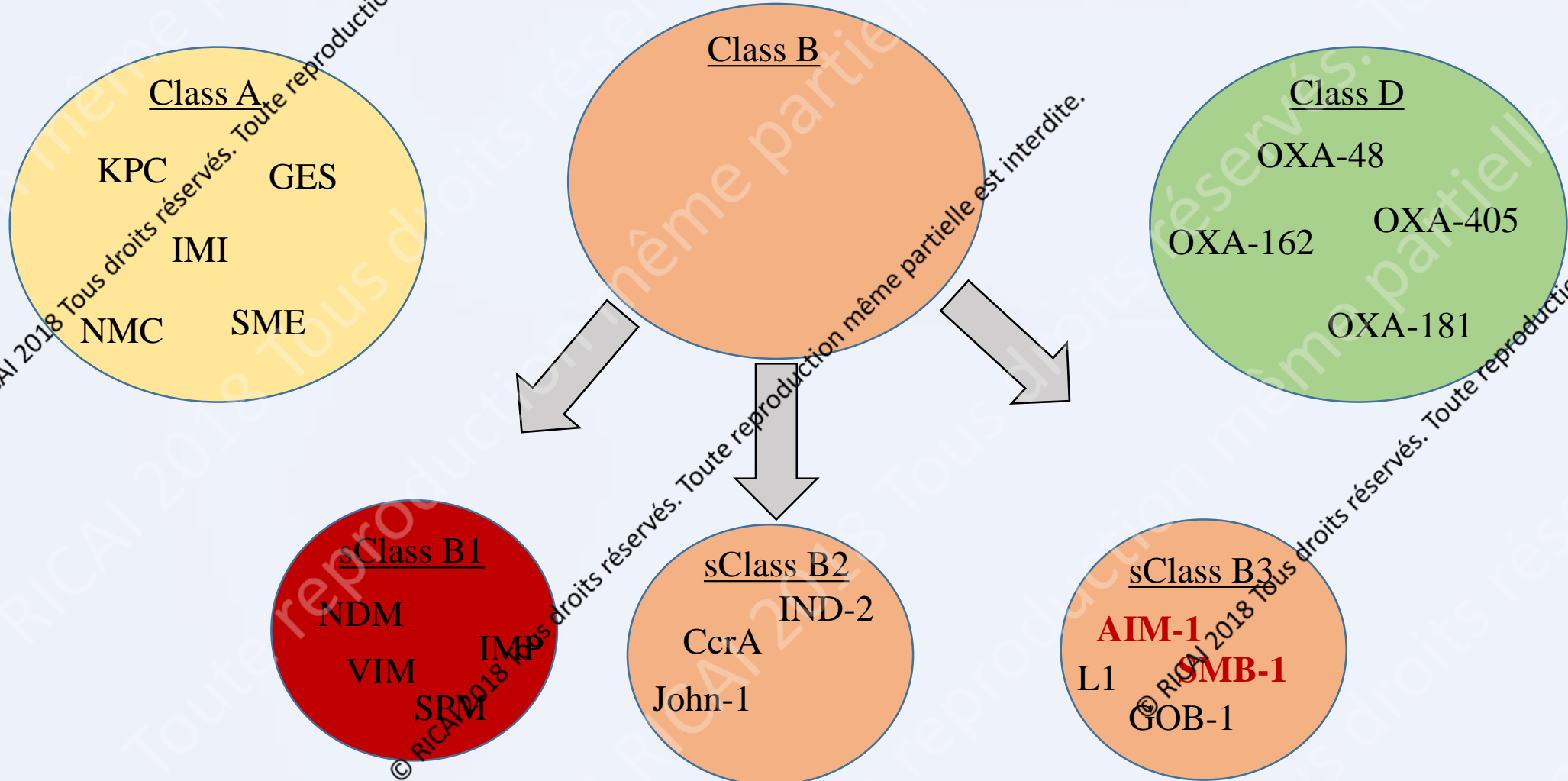
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BACKGROUND

- The emergence of carbapenemase-producing bacteria is a major clinical concern



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BACKGROUND

- Progenitors of carbapenemases : environmental isolates

- bla_{OXA-48} like : *Shewanella* spp.

→ bla_{OXA-48} : *Shewanella oneidensis*

→ $bla_{OXA-181}$: *Shewanella xiamenensis*

- bla_{OXA-23} : *Acinetobacter radioresistens*

→ bla_{NDM-1}

→ bla_{VIM-1}

→ bla_{IMP-1}

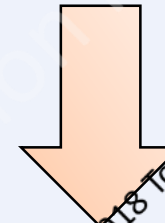
→ bla_{SPM-1}

→ bla_{KPC}

?



In silico analysis (blast NCBI)



Progenitors of carbapenemase?

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 348-351
0066-4804/04/\$08.00+0 DOI: 10.1128/AAC.48.1.348-351.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

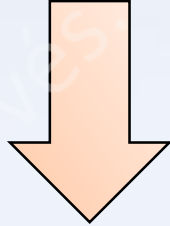
Chromosome-Encoded Ambler Class D β -Lactamase
of *Shewanella oneidensis* as a Progenitor of
Carbapenem-Hydrolyzing Oxacillinase
Laurent Poirel, Claire Héritier, and Patrice Nordmann*

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4405-4407
0066-4804/11/\$12.00 doi:10.1128/AAC.00681-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Origin of OXA-181, an Emerging Carbapenem-Hydrolyzing
Oxacillinase, as a Chromosomal Gene in
Shewanella xiamenensis
Anaïs Potron, Laurent Poirel, and Patrice Nordmann*

BACKGROUND

In silico analysis (blast NCBI excluding all clinical species)



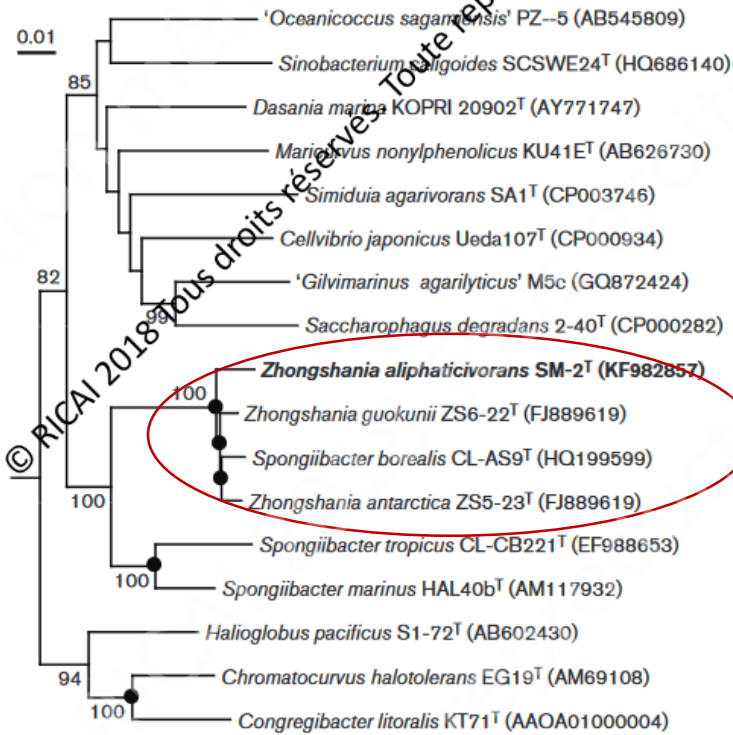
One putative candidate identified

	Max score	Total score	Query cover	E value	Ident	Accession
DIM/SIM/IMP family subclass B1 metallo-beta-lactamase [Zhongshania aliphatica] orans]	504	504	78%	2e-179	100%	WP_082793655.1
metallo-beta-lactamase domain-containing protein [gamma proteobacterium BDW918]	499	499	78%	2e-177	99%	EIF44595.1
DIM/SIM/IMP family subclass B1 metallo-beta-lactamase [gamma proteobacterium BDW918]	490	490	77%	4e-174	98%	WP_036005637.1
subclass B1 metallo-beta-lactamase [Zhongshania aliphatica] orans]	476	476	73%	2e-168	100%	MO67908.1
PST family subclass B1 metallo-beta-lactamase [Pseudomonas sp. TTU2014-096BSC]	293	293	77%	5e-96	57%	WP_058073988.1
subclass B1 metallo-beta-lactamase PST-1 [Pseudomonas stutzeri]	291	291	77%	2e-96	58%	WP_043942497.1

Presentation of *Zhongshania aliphaticivorans*

- *Z. aliphaticivorans*: new species identified in 2013 from low sea tides sediments on the west coast of South Korea.

N. LO, H. J. Kang and C. O. Jeon

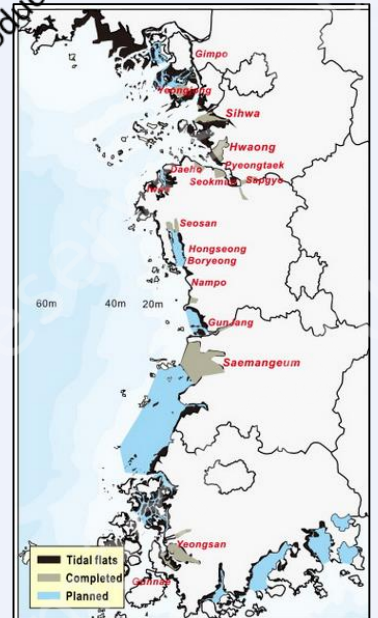


- Gram negative rod
- Halophile (grows on Marine Agar)
- Facultatively anaerobic, motile
- Aliphatic hydrocarbon-consuming bacteria : putative pollutant-degrading bacteria

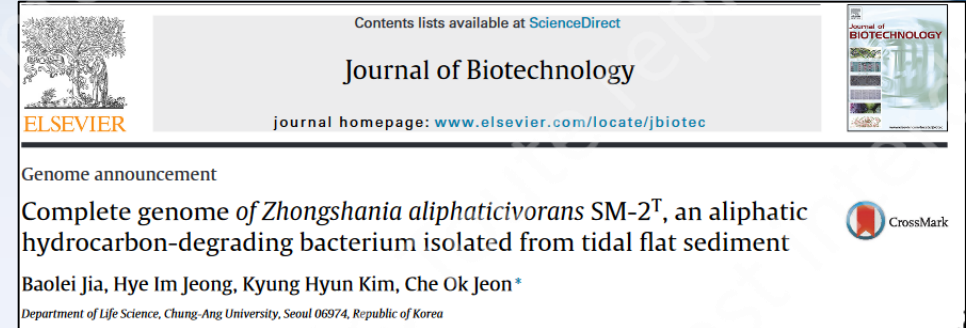
Zhongshania aliphaticivorans sp. nov., an aliphatic hydrocarbon-degrading bacterium isolated from marine sediment, and transfer of *Songiibacter borealis* Jang et al. 2011 to the genus *Zhongshania* as *Zhongshania borealis* comb. nov.

Naysim LO, Hyo Jung Kang and Che Ok Jeon

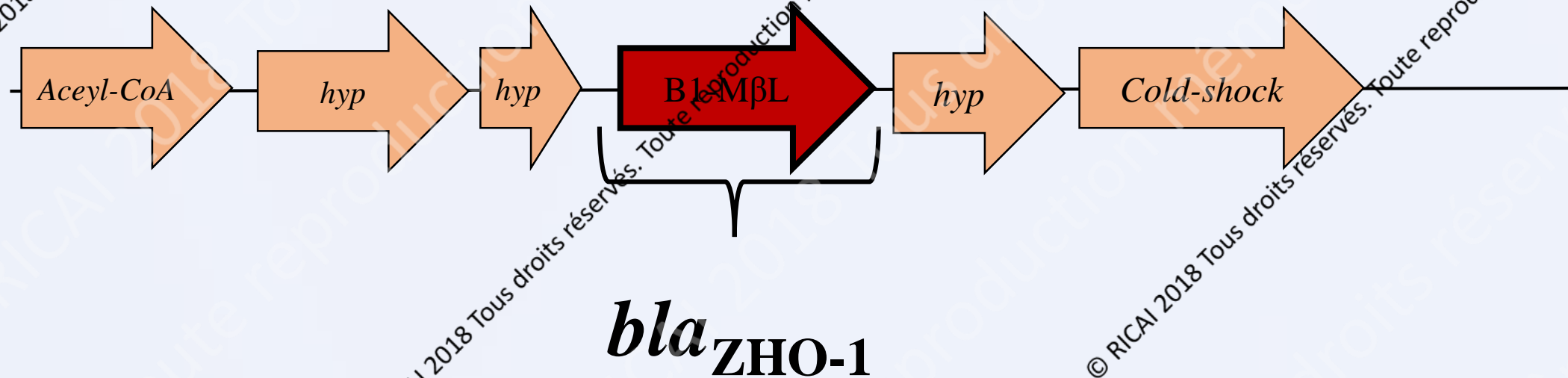
Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul, 156-756, Republic of Korea



2- Presentation of *Zhongshania aliphaticivorans*

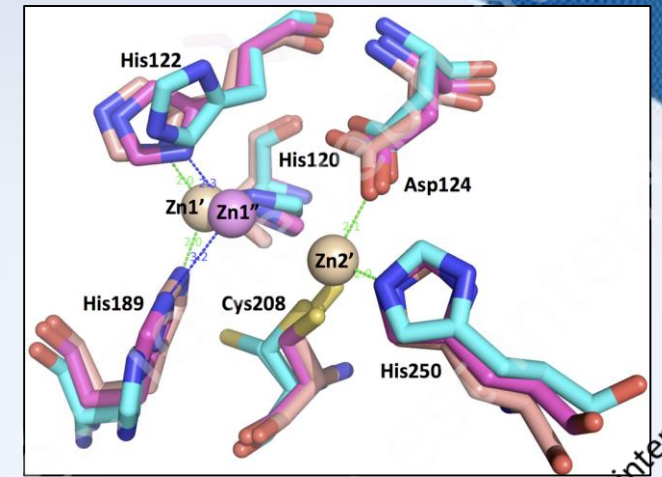


- Genome published recently (2016)
- No mobile element associated with the M β L gene either



ZHO-1 metallo- β -lactamase

- 51% amino acid identity with DIM-1 IMP-1 and KHM-1, respectively
- Protein of 246 amino acids



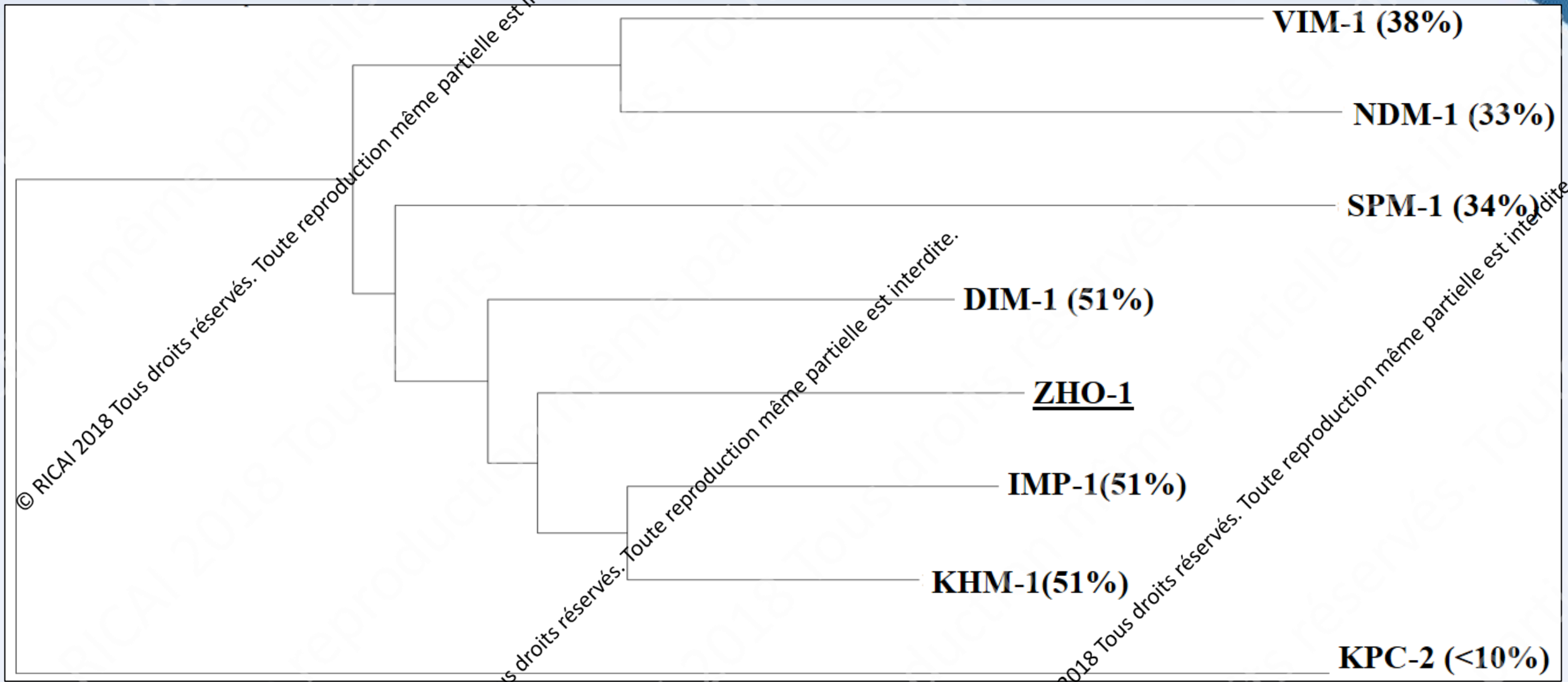
Kim et al. 2011

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ZHO-1 metallo-β-lactamase



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ZHO-1 metallo- β -lactamase

	1								
ZHO-1	-----M	RP ^R IF ^I VALL ^L LT ^T	LT ^L LM ^L SHA ^A QV ^V	-----W	-----	-----A ^A SEEL	P ^P PL ^L K ^K I ^I Q ^Q Q ^Q LT ^T D	S ^S V ^V Y ^Y L ^L H ^H I ^I S ^S H ^H K ^K V ^V	
IMP-1	-----M	SK ^S LS ^L -----V ^V	F ^F I ^I FL ^L FC ^C SI ^I A	-----T	-----	-----A ^A AE ^E SL	P ^P DL ^L K ^K I ^I E ^E KL ^L DE	G ^G V ^V Y ^Y V ^V H ^H T ^T S ^S F ^F EE	
SPM-1	-----M	NS ^N PK ^K SR ^R AL ^L EG	FM ^F GAF ^F CL ^L LL ^L V	-----	-----	-----A ^A GAP ^P LS	A ^A K ^K SS ^S D ^D H ^H V ^V D ^D LP	Y ^Y N ^N L ^L T ^T AT ^T K ^K I ^I D ^D S	
VIM-1	-----M	L ^L K ^K VI ^I SSI ^I LV ^V	MT ^M AS ^A VMA ^A VAS	PL ^P AHS ^H GE ^E PS ^S G	-----	-----E ^E Y ^Y PT ^T V ^V NE ^E IP ^P V	GE ^G V ^V R ^R LY ^Y Q ^Q I ^I AD	G ^G V ^V W ^W SH ^H I ^I AT ^T OS	
NDM-1	MEL ^M PN ^P IM ^I HP ^H V	AK ^A LSTA ^L LAA ^A	L ^L MLS ^L GC ^G MP ^M PG	-----	-----	-----E ^E IR ^R PT ^T IG	Q ^Q OM ^O ET ^E GD ^G OR ^R F	G ^G DL ^D V ^V FR ^F QL ^Q AP	
	71								
ZHO-1	V ^V D ^D G ^G F ^F GL ^L V ^V DS ^S N	GL ^G IV ^I -LIG ^I SE	AY ^A IV ^I D ^D TP ^T W ^W ST	Q ^Q D ^D TE ^E FL ^L LOW ^W I	N ^N -A ^A Q ^Q G ^G F ^F TL ^L KS	V ^V V ^V ST ^T H ^H F ^F H ^H ED ^D R	T ^T AG ^A IE ^E Y ^Y LN ^N AN		
IMP-1	V ^V NG ^N W ^W GV ^V PK ^K H	GL ^G VV ^V -LV ^L NAE	AY ^A LI ^L D ^D TP ^T F ^F TA	K ^K D ^D TE ^E K ^K L ^L V ^V TF	V ^V -E ^E R ^R GY ^Y K ^K I ^I KG	S ^S I ^I SS ^S H ^H F ^F H ^H SD ^D S	I ^I GG ^G I ^I E ^E W ^W LN ^N SR		
SPM-1	--R ^R DF ^D Y ^Y SS ^S N	VL ^V VA ^A K ^K ML ^L D ^D GT	VV ^V IV ^I V ^V SS ^S PF ^F EN	L ^L GT ^G T ^T LM ^L D ^D W ^W V	A ^A K ^K TM ^T K ^K P ^P K ^K V ^V V	A ^A IN ^I TH ^H F ^F HL ^L D ^D G	T ^T GG ^G NE ^N I ^I Y ^Y KK ^K M		
VIM-1	F ^F D ^D G ^G -AV ^A Y ^Y PS ^S	GL ^G IV ^I -RD ^R G ^G DE	L ^L LL ^L ID ^I TAW ^A GA	K ^K NTA ^N ALL ^A AE ^A I	E ^E K ^K O ^O I ^I GL ^L P ^P V ^V IN	AV ^A ST ^S H ^H F ^F H ^H DD ^D R	V ^V GG ^G V ^V D ^D VL ^L RAA		
NDM-1	M ^M PG ^P F ^F G ^G AV ^A AN	GL ^G IV ^I -RD ^R G ^G GR	VL ^V V ^V VD ^D TAW ^A TD	D ^D Q ^Q TA ^T Q ^Q IL ^L N ^N WI	K ^K Q ^Q E ^E IN ^N L ^L P ^P VAL	AV ^A V ^V TH ^H A ^A H ^H Q ^Q DK	M ^M GG ^G M ^M D ^D AL ^L HAA		
	141								
ZHO-1	A ^A I ^I PT ^T AS ^A ART	N ^N K ^K IL ^L Q ^Q R ^R Q ^Q GR ^R P	LA ^L ANT ^A FN ^N K	-----	-----	-----	-----		
IMP-1	S ^S I ^I PT ^T Y ^Y ASE ^A ELT	N ^N ELL ^L KK ^K D ^D G ^G K ^K V	QA ^Q AT ^A NS ^N FS ^F SG	-----	-----	-----	-----		
SPM-1	GA ^G ET ^E W ^W SS ^S DL ^L T	K ^K OL ^O R ^R LE ^L EN ^N KK	DR ^D I ^I K ^K AA ^A E ^E F ^F Y ^Y K	N ^N ED ^E L ^L K ^K RR ^R IL ^L S	S ^S H ^H V ^V P ^P AD ^A N ^N V ^V F	D ^D L ^L K ^K OG ^O K ^K V ^V F	-----		
VIM-1	GA ^G V ^V AT ^A Y ^Y AS ^A P ^P ST	RR ^R LA ^L E ^E A ^A E ^E GN ^N E	IP ^I TH ^H S ^S LE ^L G	-----	-----	-----	-----		
NDM-1	GI ^G AT ^A Y ^Y AN ^A ALS	N ^N QL ^Q AP ^A Q ^Q EG ^E M ^M V	AA ^A QH ^Q SL ^L TF ^F A	-----	-----	-----	-----		
	211								
ZHO-1	F ^F Y ^Y P ^P GA ^A G ^G HA ^H Q ^Q D	N ^N V ^V V ^V WL ^L PE ^P Q ^Q K	LL ^L F ^F GG ^G CL ^L IRA	NA ^N A ^A TS ^T LS ^L NT ^N S	DA ^D VL ^L SA ^S W ^W SA ^S S	VE ^V EEL ^L Q ^Q S ^S RY ^Y AD	A ^A K ^K LV ^V VP ^P GH ^G D		
IMP-1	F ^F Y ^Y PG ^P PG ^P H ^H TP ^T D	N ^N V ^V V ^V WL ^L PER ^P K	IL ^I F ^F GG ^G CF ^C IK ^K P	Y ^Y ---G ^G LN ^N LG	DA ^D NI ^I E ^E AW ^W PK ^K S	A ^A K ^K LL ^L K ^K S ^S Y ^Y G ^G K	A ^A K ^K LV ^V VP ^P SH ^S S		
SPM-1	S ^S FP ^F GA ^A HS ^H PD	N ^N V ^V V ^V Y ^Y FP ^F KK ^K K	LL ^L F ^F GG ^G CM ^C IK ^K P	K ^K ---E ^E LG ^L YL ^L G	DA ^D NV ^V K ^K AW ^W P ^P DS	AR ^A RL ^L KK ^K F	AK ^A IV ^V IP ^P GH ^H E		
VIM-1	F ^F Y ^Y PGA ^A AH ^H STD	N ^N L ^L V ^V V ^V Y ^Y V ^V PS ^P AN	V ^V LY ^L GG ^G CA ^C V ^V HE	LS ^L ST ^S S ^S AG ^A N ^N VA	DA ^D DL ^L AE ^E W ^W PT ^T S	VE ^V RI ^I Q ^Q K ^K H ^H Y ^Y PE	A ^A E ^E V ^V VI ^I PG ^P H ^H GL		
NDM-1	F ^F Y ^Y PG ^P PG ^P H ^H TS ^S D	N ^N IT ^I VG ^V ID ^D GT ^T D	IA ^I F ^F GG ^G CL ^L IK ^K D	SK ^S AK ^K SL ^L GN ^N LG	DA ^D AD ^D TE ^E H ^H Y ^Y A ^A AS	AR ^A AF ^F GA ^A AF ^F PK	AS ^A M ^M IV ^V M ^M SH ^S A		
	281								
ZHO-1	V ^V G ^G D ^D V ^V SL ^L LE ^L HT	R ^R V ^V LA ^L TV ^V GO ^O AV	SK ^S *-----	-----	-----	-----	-----		
IMP-1	V ^V GD ^G AS ^A LL ^L KL ^L T	LE ^L Q ^Q AV ^V K ^K GL ^L NE	S ^S KK ^K PS ^S K ^K PS ^S *	-----	-----	-----	-----		
SPM-1	W ^W GG ^G PE ^P M ^M V ^V N ^N KT	I ^I K ^K VA ^V E ^E K ^K AV ^V GE	M ^M RL ^L *-----	-----	-----	-----	-----		
VIM-1	P ^P GG ^G LD ^L LL ^L Q ^Q HT	AN ^A V ^V V ^V K ^K AH ^H K ^K NR	SV ^S AE ^A *-----	-----	-----	-----	-----		
NDM-1	P ^P DS ^D R ^R AA ^A I ^I TH ^H T	AR ^A M ^M AD ^D K ^K LR ^R *-	-----	-----	-----	-----	-----		

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ZHO-1 metallo- β -lactamase

	1																										
ZHO-1	-----M	RP	IFVALLLT	LT	LM	SHAQV	---	W	---	ASEEL	PPLKI	QQLTD	SVYLH	ISHKV													
IMP-1	-----M	SKLS	---	VF	FIFL	CSIA	---	T	---	AAESL	PDLKI	EKLDE	GVYVH	TSFEE													
SPM-1	-----M	NSPK	SRALRG	FM	GAF	CLLLV	---	AGAPLS	AKSS	DHVDLP	YNLT	TATKIDS	DVFV	VD---													
VIM-1	-----M	LKVI	SSIVGV	MT	ASV	MAVAS	PLAHS	GPEPSG	EYPT	VNEIPV	GEVRL	YQIAD	GVW	SHIATOS													
NDM-1	MELPN	IMHPV	AKLST	ALAAA	LMLS	GCMPG	---	EIRPTIG	QQMET	TGDRF	GDLV	FRQLAP	NVW	QHTSYLD													
	71																										
ZHO-1	VDG	FGLV	DSN	GLV	-	LIGSE	AYIV	DTP	WST	QDTE	FLL	OWI	N-	AQGF	TLKS	VVST	HFH	DR	TAGIE	YLNAN							
IMP-1	VNG	WVVP	PKH	GLVV	-	LVNAE	AYLI	DTP	PFTA	KDTE	KL	VTF	V-	ERGY	KIKG	SISS	HFS	SOS	TGGI	EWLNSR							
SPM-1	---	RDFY	SSN	VLVA	KMLD	DGT	VVIV	SSP	PFEN	LGT	QTL	MDWV	AK	TMK	PKKV	AIN	THF	HLDG	TGGN	EYKKM							
VIM-1	FDG	-AVY	PSN	GLIV	-	RDGDE	LLLI	D	TAWGA	KNTA	ALLAEI	EKOI	GLP	VV	AVST	HFD	DR	VGGV	DVLR	AA							
NDM-1	MPG	F	GAVALN	GLIV	-	RDGGR	VLV	V	D	TAWTD	DQTA	QILNWI	KQE	INL	PVAL	AVV	THA	QDK	MGG	DALHAA							
	141																										
ZHO-1	AIPT	ASART	NKIL	QRQ	GRP	LAANT	FNK	---	---	---	---	---	---	DKF	---	---	SLV	KAH	IEV								
IMP-1	SIFT	YASELT	NELL	KKD	GKV	QATNS	FSG	---	---	---	---	---	---	VNY	---	---	WLV	KNK	IEV								
SPM-1	GAET	WSSDLT	KQLR	LEEN	KK	DRIK	AAEFYK	NEDL	KRR	ILS	SH	V	PADNVF	DLK	Q	GKVF	---	SFS	NEL	VEV							
VIM-1	GVAT	YASPST	RRLA	EAE	GNE	IPTH	SLEG	---	---	---	---	---	---	LSSSG	---	---	DAVR	F	GPVEL								
NDM-1	GIAT	YANALS	NQLA	PQ	EGMV	AAQH	SLTFA	---	---	---	---	---	---	ANGW	VEPA	---	TAPN	F	G	PLKV							
	211																										
ZHO-1	FYPG	A	GHAQD	NVVV	WL	PEQK	LLF	G	CLIRA	NAAT	SL	NTS	DAVL	S	AWSAS	VEEL	Q	SRYAD	AKLV	V	P	GHQD					
IMP-1	FYPG	F	GHQD	NVVV	WL	PERK	ILF	G	GC	F	I	KP	Y-	---	GNLG	DANI	E	A	W	P	K	S					
SPM-1	SFPG	A	HSPD	NVVV	Y	PKK	LLF	G	CM	I	KP	K-	---	ELG	YL	DANV	K	A	W	P	D	S					
VIM-1	FYPG	A	HSPD	NLVV	Y	VPSAN	VLY	G	C	A	VHE	LS	S	S	A	G	N	V	A	DADL	A	E	W	P	T		
NDM-1	FYPG	F	GHQD	NITV	G	IDGTD	I	A	F	G	C	I	KD	S	K	S	L	G	N	L	G	DAD	T	E	H	Y	A
	281																										
ZHO-1	VGDV	S	LLEHT	RVL	A	T	V	G	O	A	V	SK*	---	---	---	---	---	---	---								
IMP-1	VGD	A	S	LLKLT	LE	A	V	K	G	L	N	E	S	K	P	S	K	P	S	N*							
SPM-1	WGG	P	E	M	V	N	K	T	I	K	V	A	E	K	A	V	G	E	M	R	L*						
VIM-1	P	G	L	D	L	L	QHT	A	N	V	V	K	A	H	K	N	R	S	V	A	E*						
NDM-1	P	D	S	R	A	A	I	T	H	T	A	R	M	A	D	K	L	R*	---	---	---						

- Conservation of amino acid residues involved in the zinc ions binding:

→ H₁₂₀, H₁₂₂, H₁₈₉, C₂₀₈, H₂₅₀

Z. aliphaticivorans susceptibility testing

Isolate susceptible to all beta-lactams

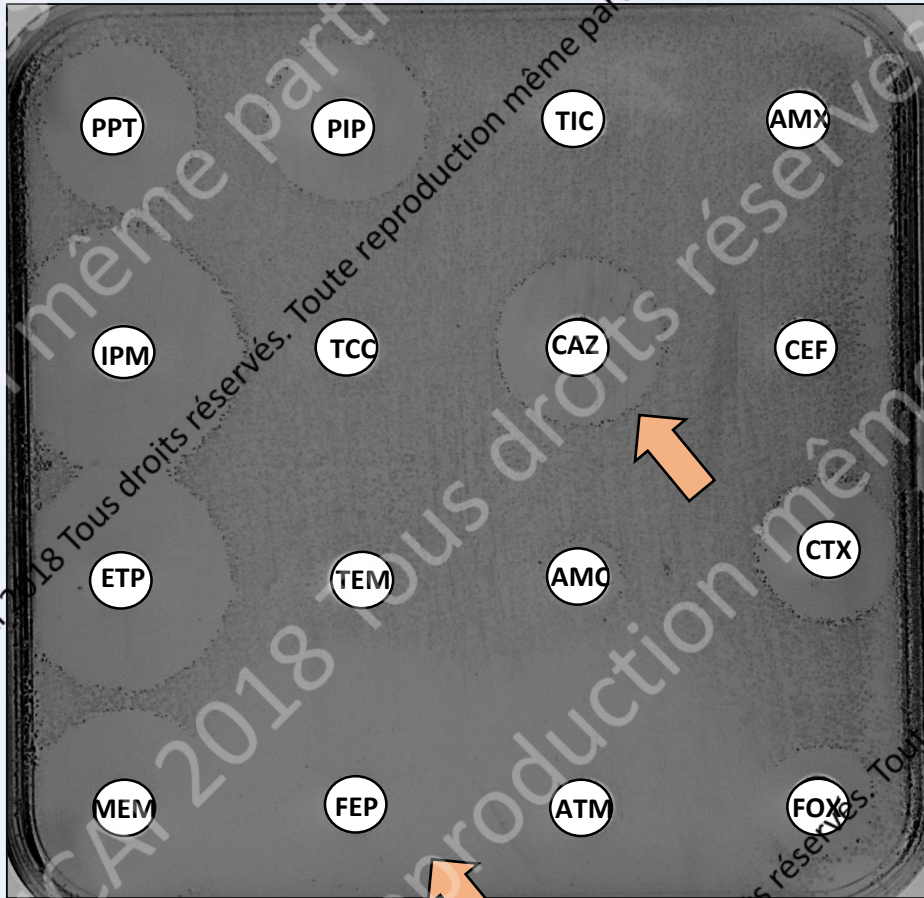
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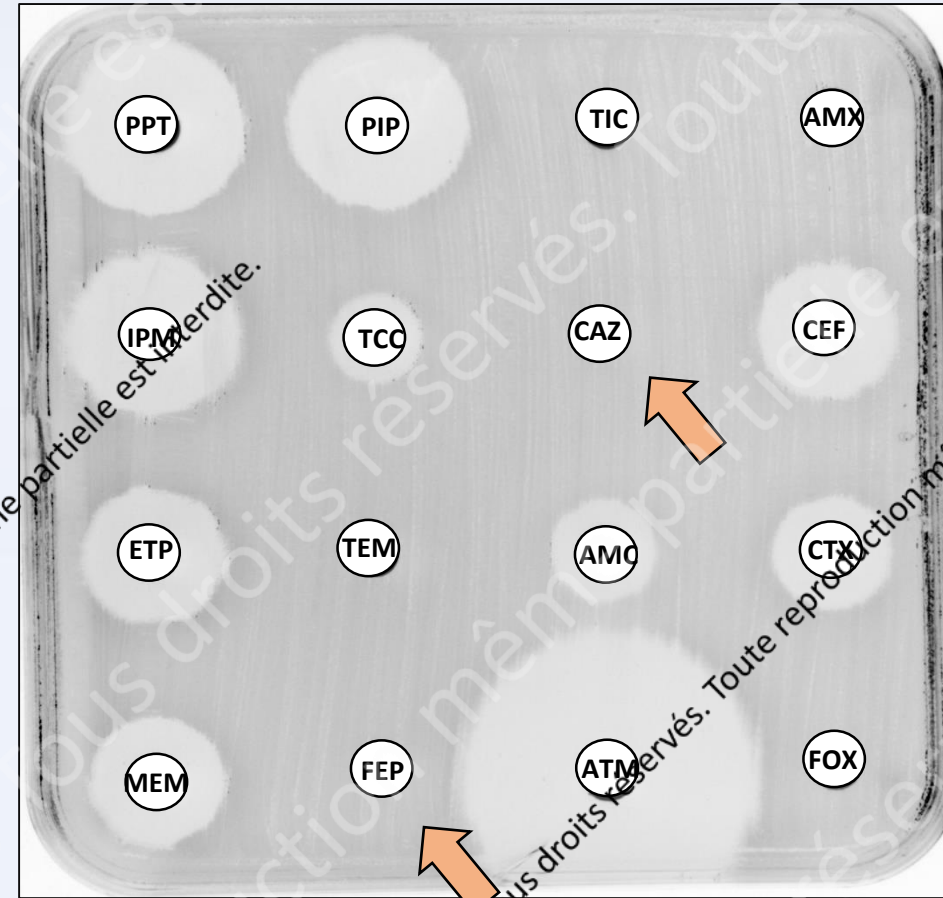
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Resistance phenotype of *E. coli* expressing and ZHO-1

→ Comparison between ZHO-1 and IMP-1-expressing clones



*bla*_{ZHO-1} *E. coli* TOP-10

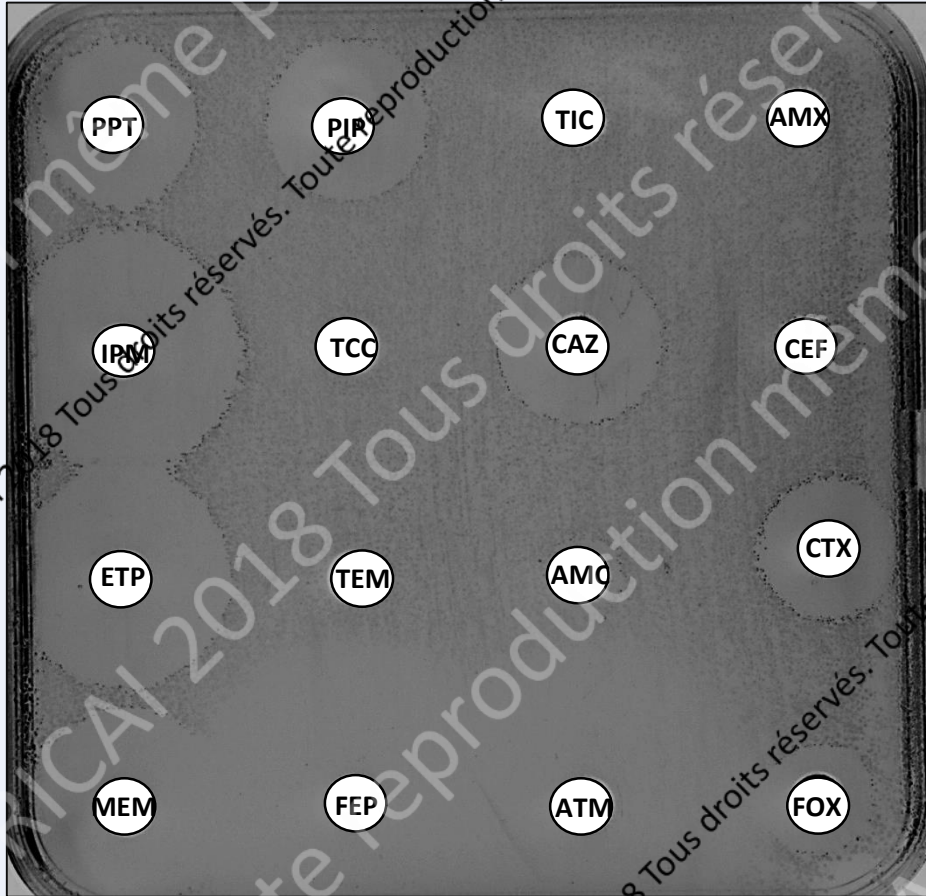


*bla*_{IMP-1} *E. coli* TOP-10

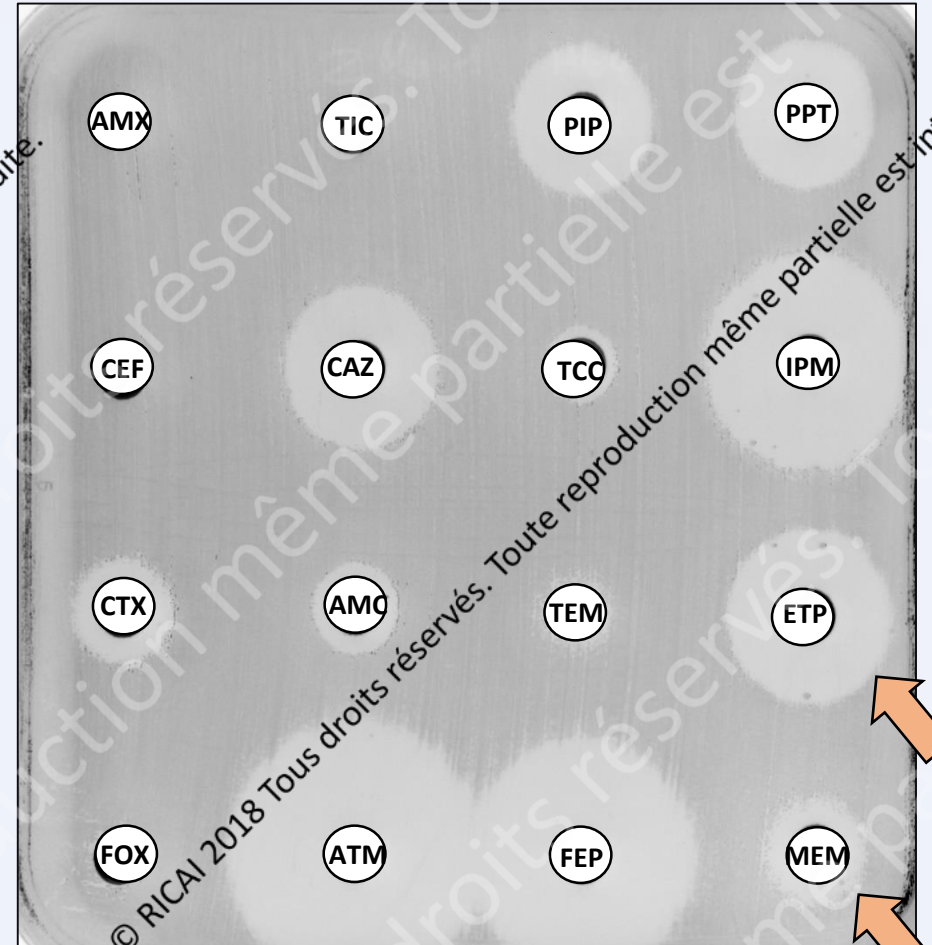
Expression ZHO-1 in porin deficient *E. coli*

Cloning strategy : pTOPO cloning of ZHO-1 in *E. coli* Hb4

E. coli Hb4 : OmpC/OmpF porin-deficient *E. coli*



*bla*_{ZHO-1} *E. coli* TOP-10



*bla*_{ZHO-1} in *E. coli* Hb4

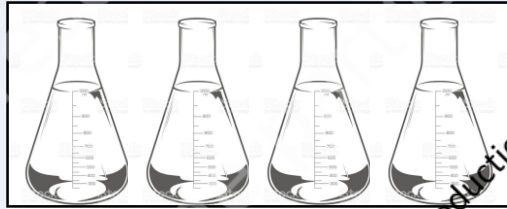
Enzyme purification:

Purification of the enzyme using ÄKTA-prime (GE Healthcare Life Sciences)

- ZHO-1 enzyme:
 - isoelectric point: 6,4
 - 246 aa
 - 26.72 kDa

➔ Purification using Q sepharose column with Tris-HCl pH 8,2 and Tris HCl pH 8,2 ; 1M NaCl

Enzyme purifications:



4L of culture



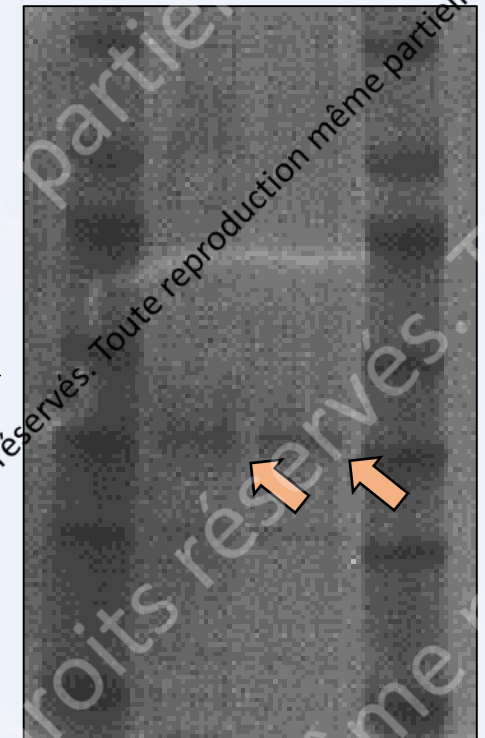
Sonication



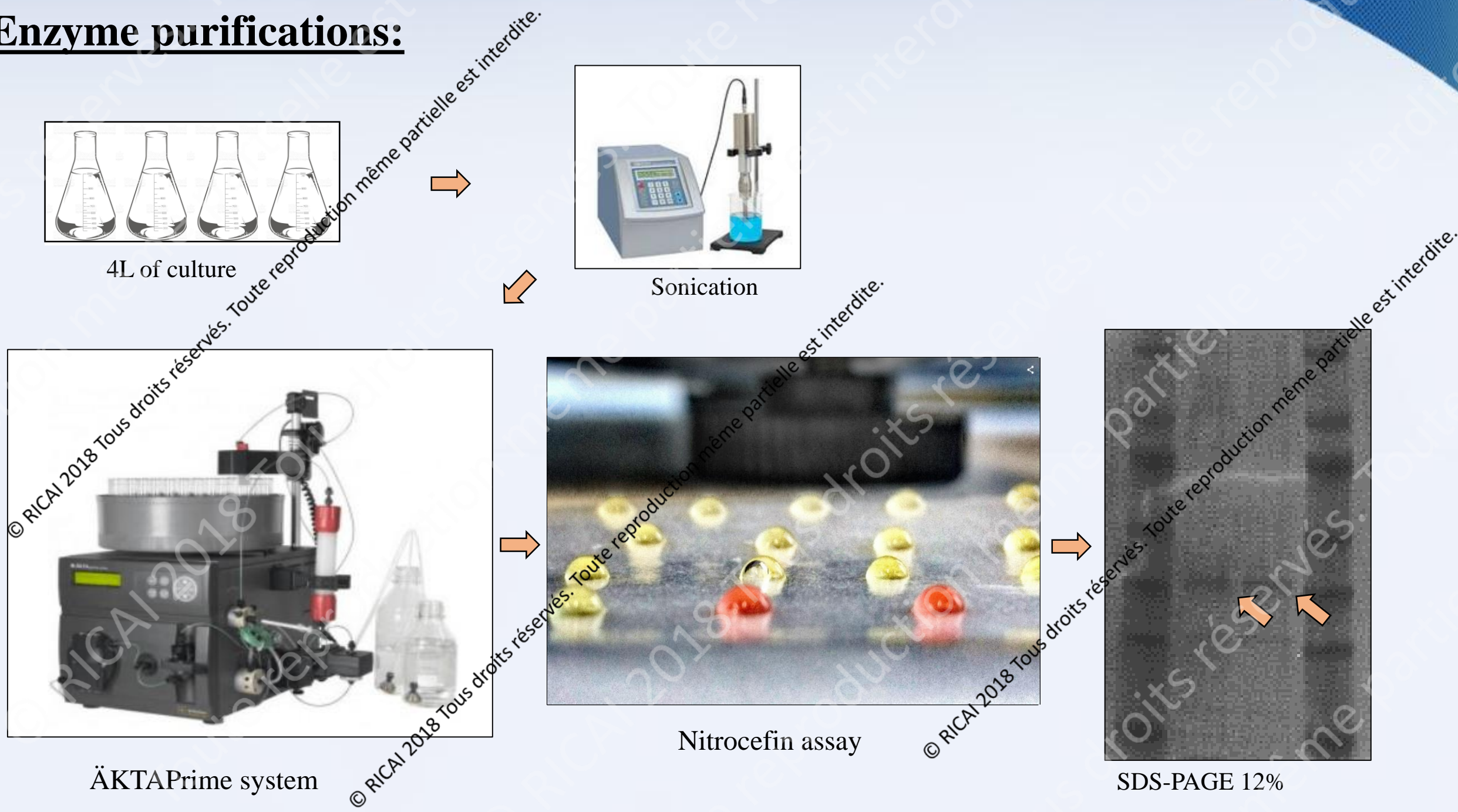
ÄKTAPrime system



Nitrocefin assay



SDS-PAGE 12%



Enzymatic activity of ZHO-1

Enzymatic activity using HEPES buffer supplemented with ZnSO_4 (50 μM)

Antibiotics	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\text{mM}^{-1} \cdot \text{s}^{-1}$)
Penicillin G	83.6	146.6	570.3
Piperacillin	43	1358	31.6
Cephalotin	4411	19	10000
Cefoxitin	1.6	147	143
Ceftazidime	0.62	150	4.1
Cefotaxime	25.4	33.9	749.3
Cefepime	0.26	843	0.3
Aztreonam	<0.01	>1000	ND
Imipenem	39.3	121	324.7
Meropenem	8.1	15.5	519
Ertapenem	ND	ND	ND

Summary:

- We identified 1 new class B carbapenemases from environmental isolates from the sea
- This carbapenemase confers resistance to many β -lactams including carbapenems
- Remaining questions:
 - Why this metallo-beta-lactamase genes are present in environmental isolates ?
 - Metallo- β -lactamases in marine environment ?
- Other species carries putative B1-M β L :
 - Cellvibrio* sp.
 - Gilvimarinus agarilyticus*

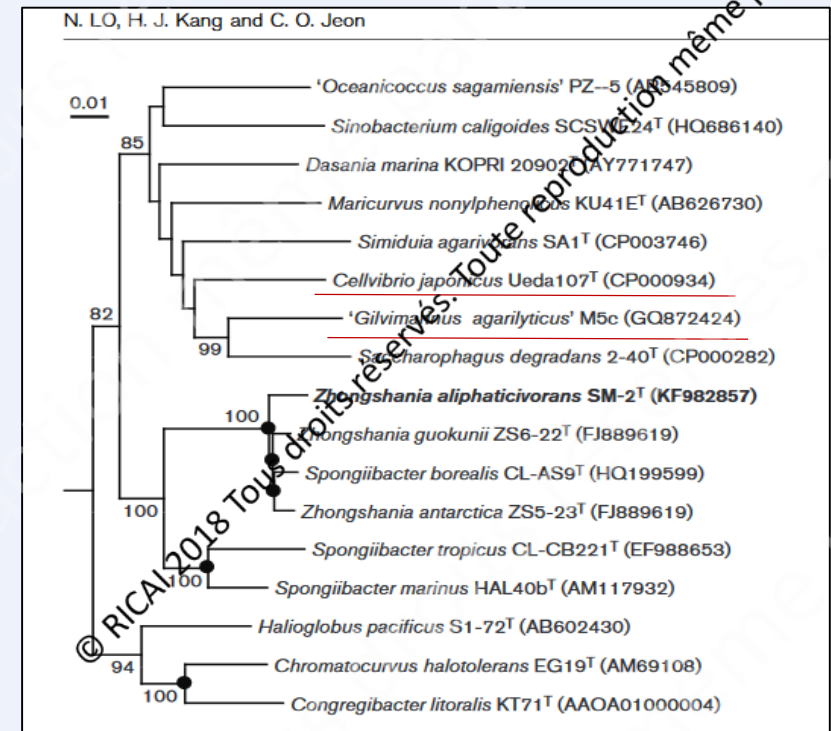


Table 1. MICs of β -lactams for *E. coli* porin proficient and deficient strains with and without the ZHO-1 β -lactamase gene

MICs ($\mu\text{g/ml}$)	<i>E. coli</i> TOP10 (pZHO-1)	<i>E. coli</i> HB4* (pZHO-1)	<i>E. coli</i> TOP10 empty vector	<i>E. coli</i> HB4 empty vector	<i>Z. aliphacitivorans</i>
Antibiotics					
Amoxicillin	128	> 128	2	16	0.5
Amoxicillin+ clavulanic acid ^a	128	> 128	1	16	0.5
Piperacillin	32	64	1	8	0.5
Cefalotin	128	> 128	4	128	0.5
Cefoxitin	128	> 128	4	64	0.5
Ceftazidime	4	128	0.12	1	0.06
Cefotaxime	64	64	0.06	0.5	0.06
Cefepime	0.5	4	0.12	0.5	0.06
Aztreonam	0.06	0.5	0.06	0.5	0.06
Imipenem	0.5	0.5	0.06	0.06	0.06
Meropenem	1	4	0.06	0.25	0.06
Ertapenem	1	4	0.06	1	0.06

Grey shaded MICs values correspond to intermediate susceptibility; boldened MIC values correspond to resistance.

^aClavulanic acid was added at a fixed concentration of 2 $\mu\text{g/ml}$. **E. coli* HB4 is an OmpC/OmpF porin-deficient strain.