

Effect of zinc accumulation on imipenem resistance of *Stenotrophomonas maltophilia*

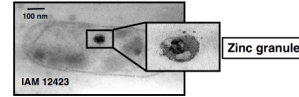
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Stenotrophomonas maltophilia is a gamma-proteobacterium, and habitats clinical and natural environment. Some strains of the species are notorious for their resistance to most beta-lactam antimicrobial agents by L1 and L2 type beta-lactamases. The former is a binuclear zinc enzyme (so-called metallo-beta-lactamase), and decomposes even imipenem having a broad antibacterial spectrum.

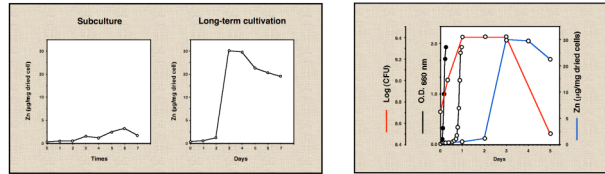
We have researched zinc accumulation of *S. maltophilia* with zinc-resistant strains isolated from clinical specimens (two strains) and environments (two strains). It was observed that these strains accumulated zinc in intracellular granules at a stationary phase. Additionally, they were tested the resistance to imipenem and ceftazidime by the disk diffusion method. Clinical strains were resistant to two antibiotics, and an L2 type lactamase were detected by PCR and sequencing. For the meantime, environmental strains were sensitive or weakly resistant to them. Interestingly, they increased imipenem resistance by the zinc accumulation, and were detected the gene of an L1 type lactamase but not an L2 type one by the same method. Therefore, zinc accumulation in granules is effective for imipenem resistance of *S. maltophilia* strains having L1 type metallo-beta-lactamase.

Background

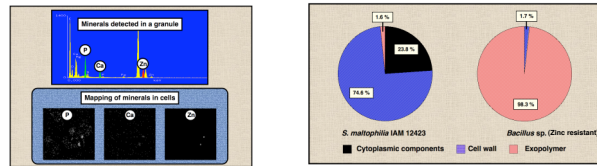
Zinc accumulation of *S. maltophilia* IAM 12423



1. *S. maltophilia* IAM 12423 accumulated zinc at a stationary phase by long-term cultivation



2. *S. maltophilia* IAM 12423 accumulated zinc in granules with other components



Strains and media

Strains used in this study and their zinc resistance and accumulation

Strain	Zinc	
	Resistance	Accumulation (mg dried cells)
<i>S. maltophilia</i>		
Clinical strains		
IAM 12423	10 mM	5 μ g
GN 12873	10 mM	12 μ g
NCB 0306-284	10 mM	12 μ g
Environmental strains (Source)		
IAM 1566 (Rice paddy)	5 mM	10 μ g
JCM 1976 (Rice paddy)	10 mM	5 μ g
IAM 12672 (Fish)	3 mM	0.1 μ g
<i>P. putida</i>		
NCB 0308-456 (IMP type metallo- β -lactamase)	10 mM	1 μ g

Media used in this study

Media	Normal growth	Zinc-accumulation growth
	YP medium	YPZn medium
YP medium	1% Polypeptone	YP medium
YPZn medium	1% Yeast extract (Difco)	ZnCl ₂ requirements
	0.5% NaCl	pH 6.0
	pH 6.0	
Antibiotic resistance test	Muehler-Hinton Sugar (Eiken)	

Antibiotic resistance test

Antibiotics	Kirby-Bauer (KB) disk (Eiken)	Experiment
Carbapenem		Pre-cultured cells
Imipenem (IPM)	10 μ g (titer)	Washed with 0.2mM Maleate-50mM EDTA buffer (pH7.0)
Meropenem (MEPM)	10 μ g (titer)	Suspended with buffer (OD ₆₀₀ =0.5)
Paipenem (PAPM)	10 μ g (titer)	Spread suspension with a cotton-tipped swap on Mueller-Hinton S agar
Cephalosporin		Put KB disks on a plate
Ceftazidime (CAZ)	30 μ g (titer)	Cultivated at 30 °C for 24 hours
Cephalexidine (CER)	30 μ g (titer)	
Monobactam		
Aztreonam (AZT)	30 μ g (titer)	

Results and Discussion

Zinc-accumulated cells increased imipenem and ceftazidime resistance in *S. maltophilia*

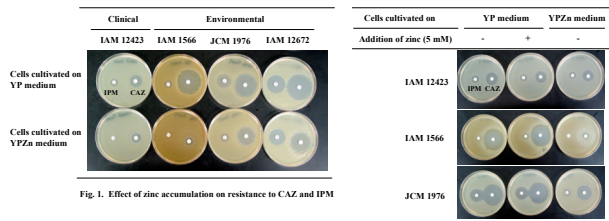


Fig. 1. Effect of zinc accumulation on resistance to CAZ and IPM

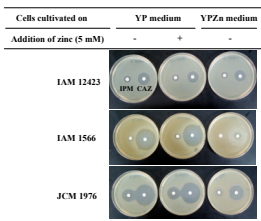


Fig. 2. Effect of zinc on resistance to CAZ and IPM

Why does zinc accumulation affect antibiotic resistance of *S. maltophilia*? (Especially environmental strains)

1. Activation of β -lactamase \rightarrow Correlation with metallo- β -lactamase?
 2. Activation of efflux pumps for antibiotics \rightarrow Correlation with multidrug efflux pumps?
 3. Inhibition of incursion of antibiotics into cells \rightarrow (Some strains were sensitive to antibiotics after cultivation on YPZn medium.)
- *Correlation with metallo- β -lactamase
S. maltophilia strains are known to produce L1 and L2 β -lactamases. L1 metallo- β -lactamase is a binuclear zinc enzyme and decomposes imipenem.
 \rightarrow Effect of a metallo- β -lactamase inhibitor (sodium 2-mercaptoacetate (SMA) disk (Eiken)) on resistance to antibiotics
- *Correlation with multidrug efflux pumps
S. maltophilia strains are also known as resistant to antibiotics of tetracycline by multidrug efflux pumps. (Especially clinical strains)
 \rightarrow Effect of zinc accumulation on resistance to Carbapenem, Cephalosporin, and Monobactam antibiotics

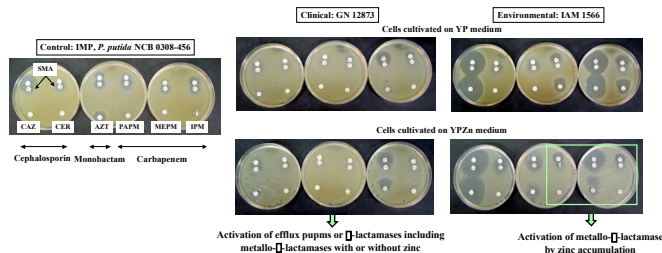


Fig. 3. Effect of SMA on resistance to Carbapenem, Cephalosporin, and Monobactam antibiotics of cells cultivated YP and YPZn media

L1 and L2 β -lactamase sequences of *S. maltophilia* strains

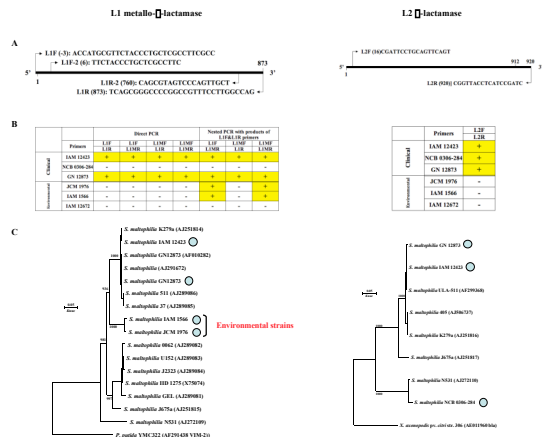


Fig. 4. PCR and sequencing of L1 and L2 lactamase of *S. maltophilia* strains
 A. Primers designed with InforBIO. Numbers were referred from data of J675a strain (AJ2518) to L1 and J675a strain (AJ25187) to L2.
 B. Amplification by PCR. +: amplified; -: not amplified. PCR condition is the following: pre-heat, 96 °C, 2 min.; amplification, 30 cycles of 96 °C, 1 min., 60 °C, 1 min., 72 °C, 1 min.; final 72 °C, 5 min.; 4 °C.
 C. Phylogenetic tree based on L1 and L2 gene sequences of *S. maltophilia* strains. \odot : Detected in this study

Conclusion

The result of environmental strains revealed that zinc accumulation in granules is effective for carbapenem including imipenem resistance of *S. maltophilia* strains having L1 type metallo- β -lactamase. Additionally, two environmental strains formed a cluster on a phylogenetic tree based on the gene of the β -lactamase. (Environmental strain of IAM 12672 was not detected the gene of β -lactamases by PCR and did not accumulate zinc after cultivation on YPZn medium.) Clinical strains did not show increasing antibiotic resistance by the zinc accumulation because they may have other antibiotic-resistance system.