



University di Modena e Reggio Emilia, Modena, Italy

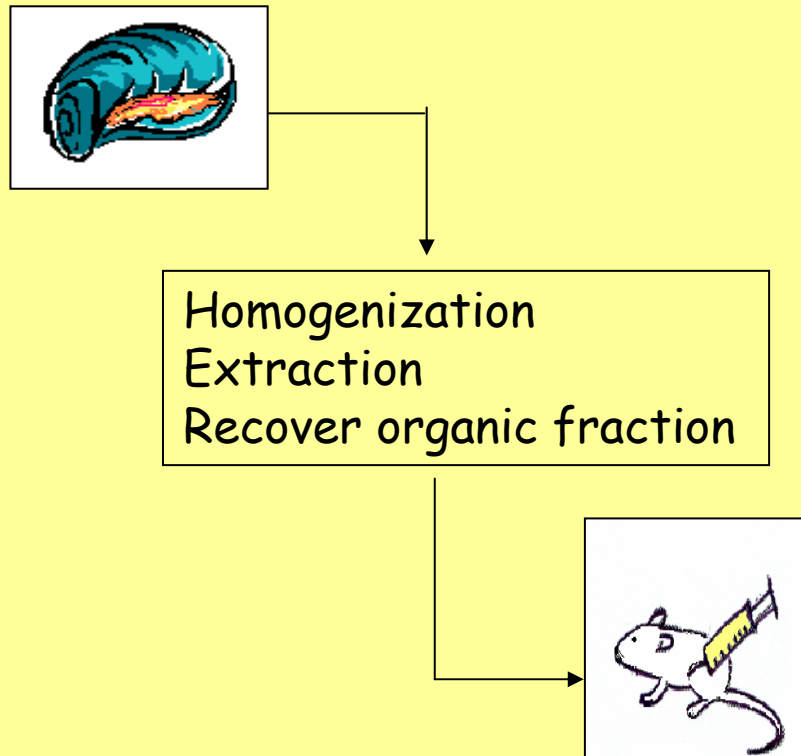
Dr.ssa Mirella Bellocci -PhD student-

Un metodo citolitico
per il dosaggio delle palitossine
basato sull'uso di cellule coltivate in monostrato

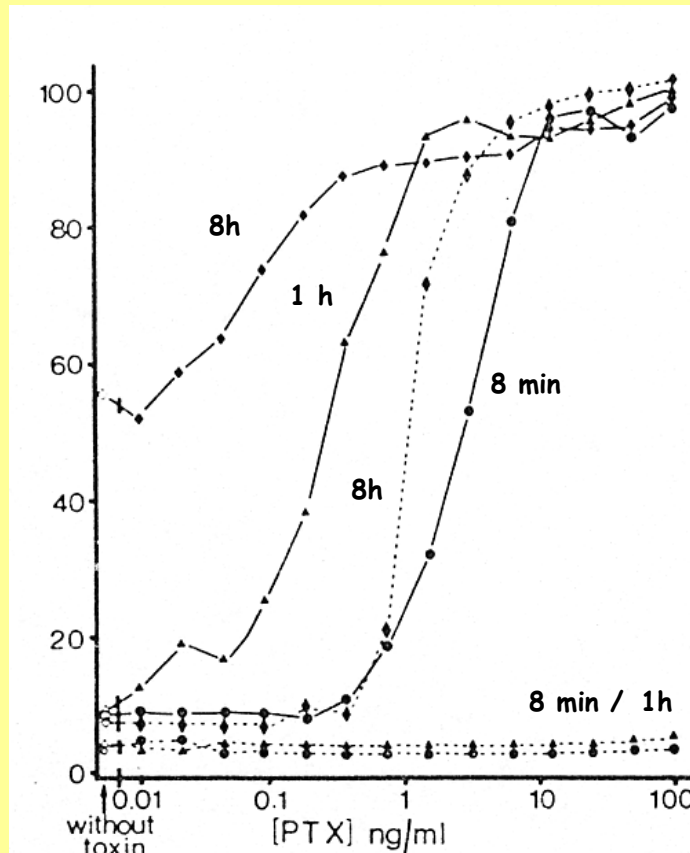
*"Alternative alla sperimentazione animale nello studio e rilevazione delle biotossine marine",
IZSTeramo 9 marzo 2009*

To protect public health

- ✓ Routine monitoring programmes of algal populations at sea and ...seafood toxicity



Eritrociti di ratto



fuoriuscita di K⁺ (——)
emolisi (-----)

Habermann et al., 1981

Palytoxin



Na⁺ K⁺ ATPase

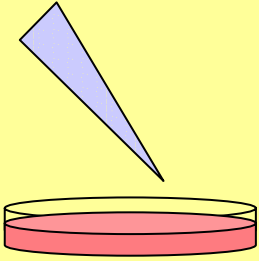


Ion imbalance

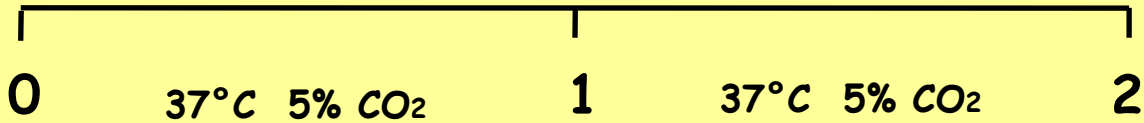
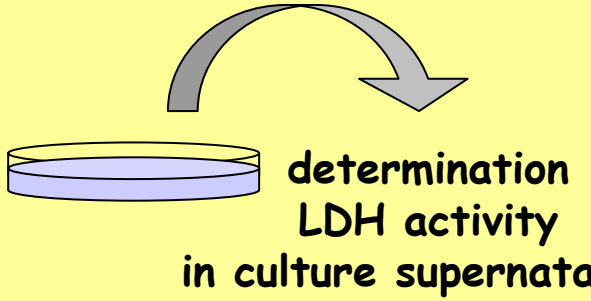
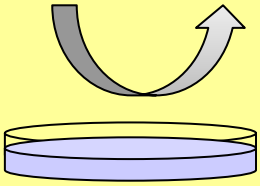


Cell lysis

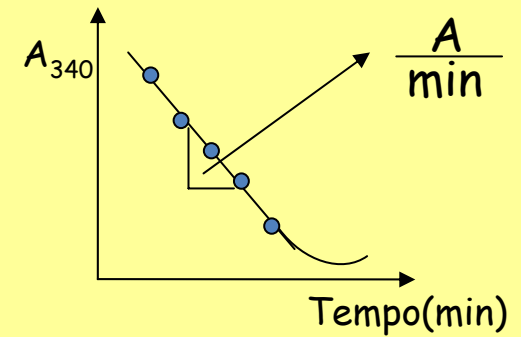
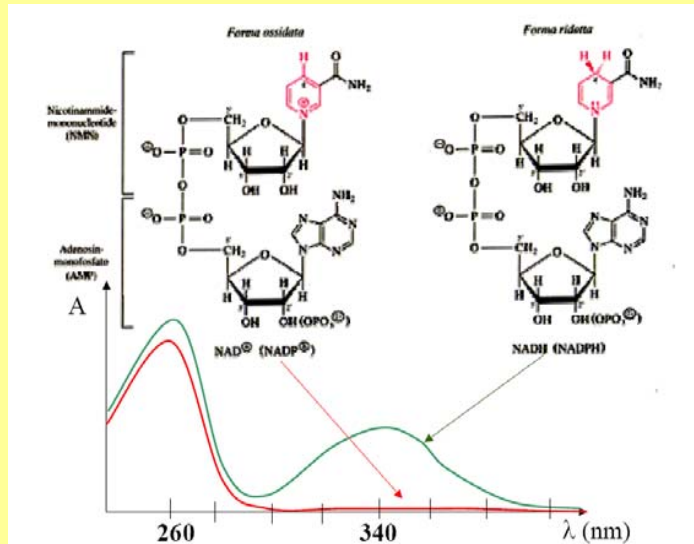
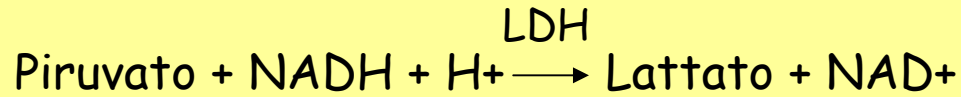
Palytoxin/vehicle



PBS **culture medium**

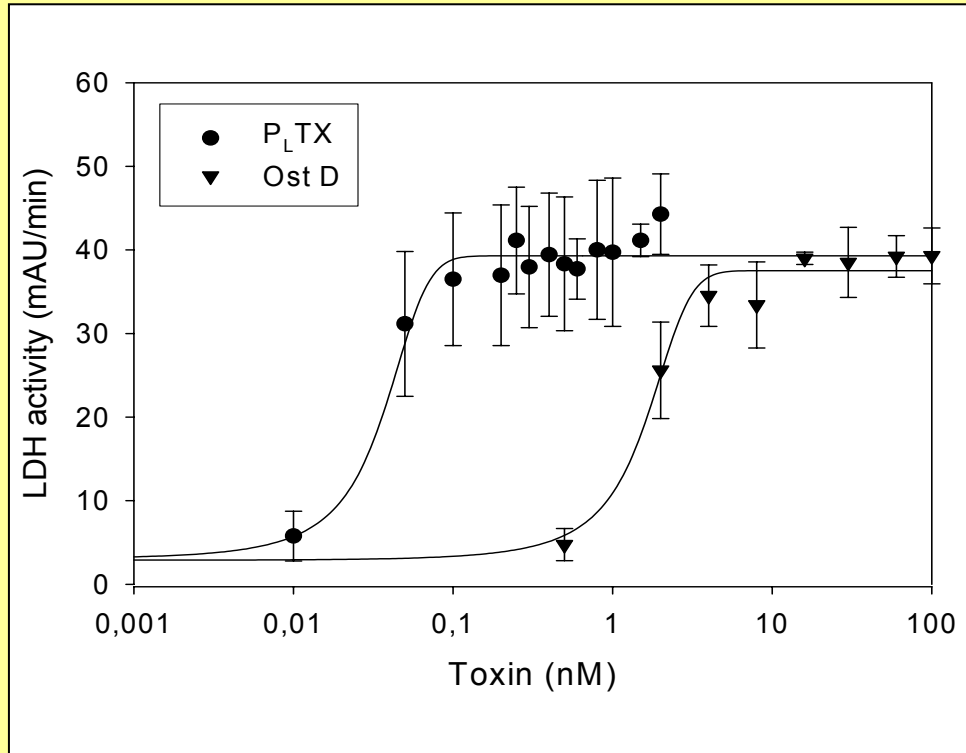


time (h)



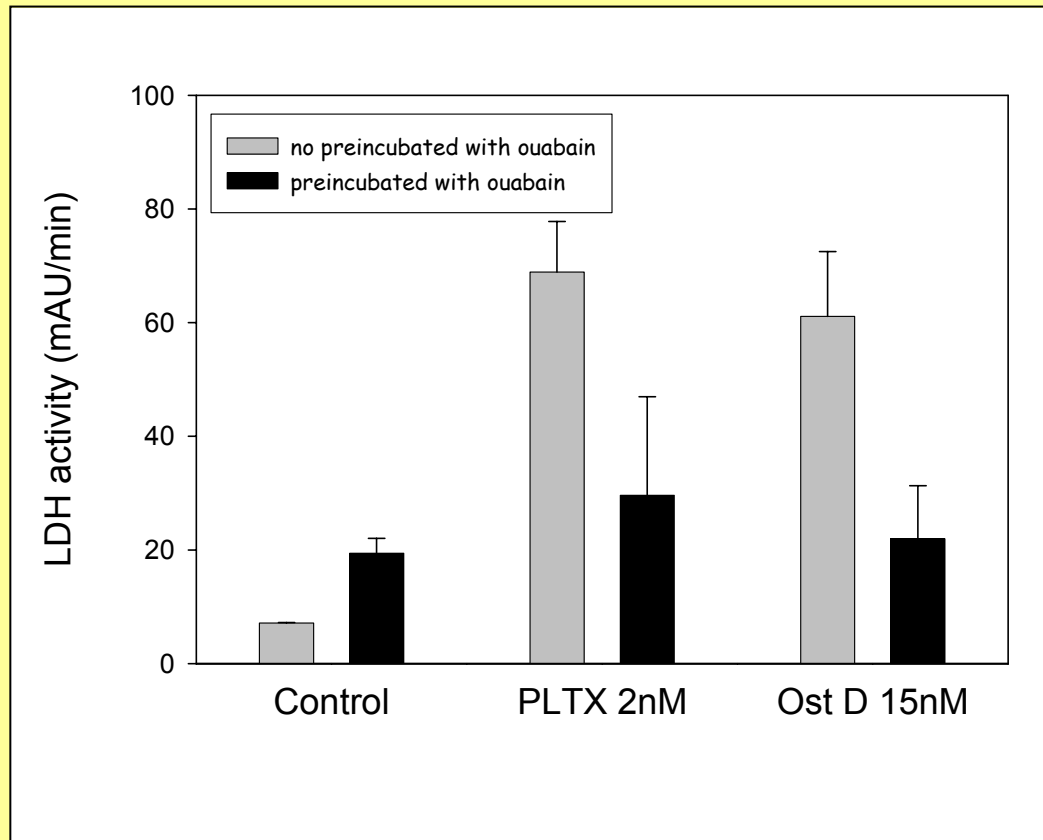
Decrement of $A_{340\text{nm}}$
due to oxidation
of NADH to NAD⁺

Sensitive



LOD 50 pg P_LTX/ ml culture medium

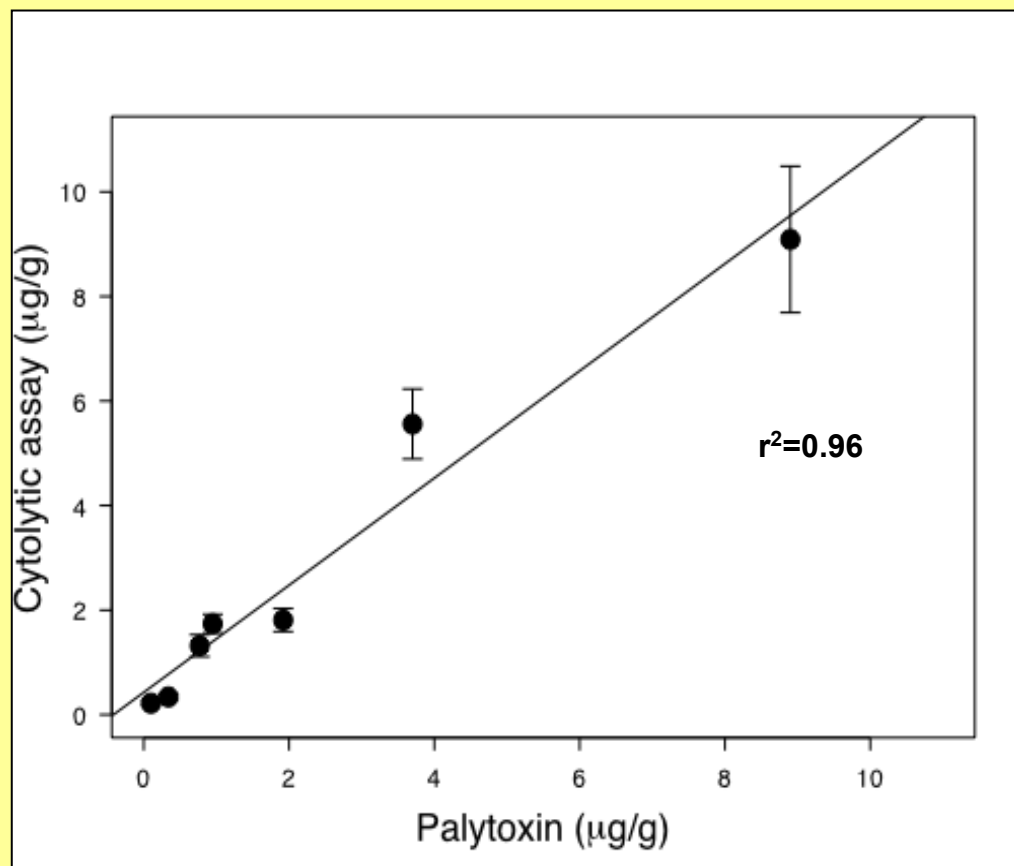
Specific



Selective

Treatment	LDH activity (mAU/min)
Control	7.9 \pm 2.0
Palytoxin (2 nM)	88.2 \pm 14.0
Ostreocin D (15 nM)	79.1 \pm 14.6
Maitotoxin (10 nM)	2.7 \pm 1.5
Tetrodotoxin (100 nM)	5.7 \pm 2.6
Yessotoxin (1 μ M)	6.2 \pm 3.9

Spiked mussels extract



Naturally contaminated materials

Material	Palytoxin content	
<i>Algal samples</i>	pg/cell	
	Cytolytic assay	LC-MS
6440/2	0.32	0.44
6448/2	0.08	n.d.
6450/2	0.06	0.37
6452/2	0.31	0.57
6942/2	0.11	0.54
<i>Mussels</i>	µg/g	
	Cytolytic assay	LC-MS
2162/07 (digestive gland)	0.16	0.12
2162/07 (whole shellfish)	0.01	n.d.
<i>Sea Urchin</i>	µg/g	
	Cytolytic assay	LC-MS
1641/06/N	0.44	n.d.

Assay Performance

LIMIT OF DETECTION -LOD-(PLTX standard)	50 pg/ml
LIMIT OF QUANTIFICATION -LOQ-(Shellfish)	8 ngPLTX/Kgfood

Conclusions

- ✓ Solid alternative to existing methods used to quantify Palytoxins
- ✓ Interlaboratory validation to extend the use of cytolytic assay