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Background

Many scientist believe that increasing *Vibrio* illnesses may be associated with global warming and rise in sea surface temperature (1). The seasonal detection of *Vibrio* spp. in marine systems depends on temperature. In fact, low temperature has been demonstrated to affect the entry of some vibrios species into the viable but nonculturable (VBNC) state (2). This phenotype hinders their detection during winter period. Moreover, this population can suppose a risk for public health due to their possible resuscitation under appropriate conditions.

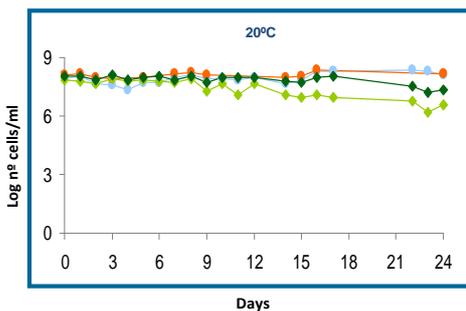
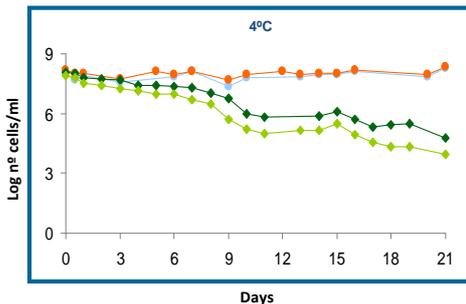
Aim of the work

- ✓ To study the phenotypical/physiological changes occurring in *Vibrio harveyi* during the exposure to 4°C and 20°C in seawater
- ✓ To analyze the potential resuscitation of the VBNC population of *V. harveyi*

Materials and Methods

Microorganisms		Bacterial populations enumerated				
Strain	Characteristics	Survival experiment	Total cells (●)	Active cells (●)	Culturable cells	Resuscitation
<i>Vibrio harveyi</i> ATCC 141126	Psychrophile oligotroph marine	Sterile natural seawater	Acridine orange stained samples and epifluorescence microscopy (3)	Dead Cell Discriminator Kit (Invitrogen™)	Marine Agar (◆) Tryptone Soya Agar (◆)	Culturable cells in Marine Agar after VBNC cells temperature upshift • Rifampicin (protein synthesis inhibitor) • Nalidixic Acid (DNA replication inhibitor)

Results and Discussion



Days	Non-antibiotics (CFU/ml)				Rifampicin (CFU/ml)				Nalidixic Acid (CFU/ml)			
	20°C		26°C		20°C		26°C		20°C		26°C	
	SW	ASW	SW	ASW	SW	ASW	SW	ASW	SW	ASW	SW	ASW
1	↑↑↑	↑↑	↑↑↑	↑↑↑	→	→	↓	→				
2	↑	↑	↑	→	↓	→	→	→	↑↑	↑↑	↑↑	↑↑
3	→	↑	→	↑	→	↓	↓↓					
4	→	→	→	→	→	→	→	→				
5					→	↑↑	↑	↑↑↑	↑	→	→	→
6	→	→	→	→					→	↑	↑	→
7	→	↓	↑	↓								

Quantifying expressed as increases in the order of magnitude compared to previous time (↑ or ↓ = 1 order; → no modification)
SW: natural sea water ASW: artificial sea water

- ✧ *V. harveyi* exposed to 4°C in sea water loses the culturability accompanied by maintenance of metabolic activity → entry of the cells into the VBNC state
- ✧ The recovery of the culturability after temperature upshift in presence of nalidixic acid, along with maintenance of CFUs in the experiments carried with rifampicin suggest that a subpopulation of VBNC cells resuscitate even if the process require specific protein synthesis

Conclusion

Ongoing warming and reduced salinity of coastal regions could support the spread of vibrios at a global scale, especially in northern latitudes. In these regions, even though vibrios entry into the VBNC state, with punctual temperature upshift these subpopulation of VBNC cells could resuscitate.

References

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