

EFFECTS OF ENVIRONMENTAL FACTORS ON BIOFILM FORMATION BY *Vibrio fischeri* ISOLATES

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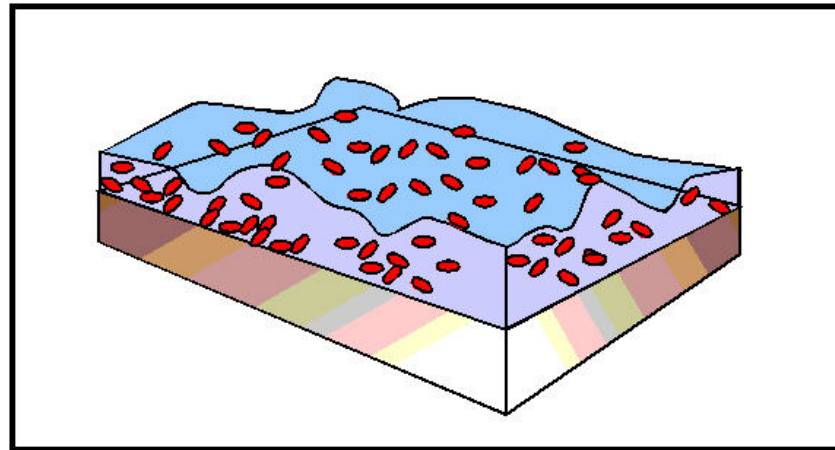


Outline

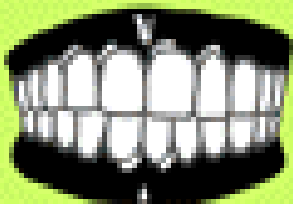
- Introduction.
- Research questions. Aims.
- Experimental design. Methods.
- Results.
- Future directions.
- Acknowledgements.

Biofilms?

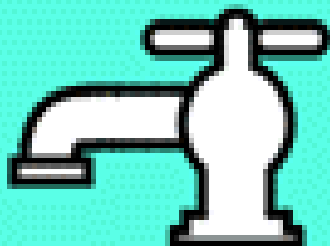
- Community of microbes attached to: surface, each other.
- Matrix: Polysaccharides.



Biofilms Impact . . .

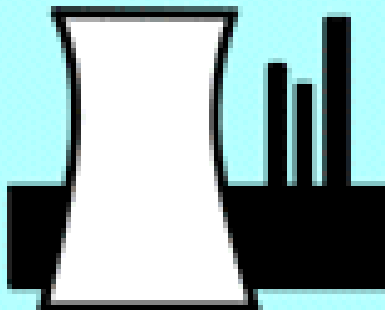


Teeth

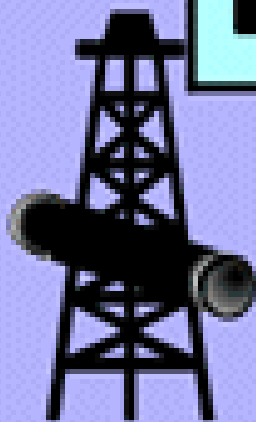


Drinking Water

Cooling Water



Food Processing

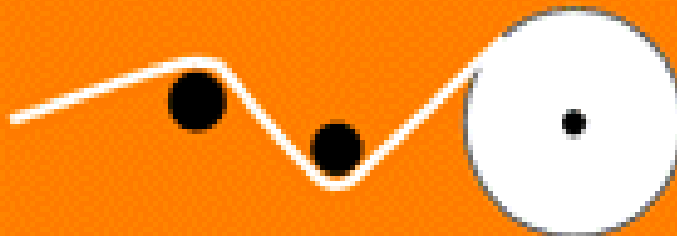


Oil Recovery

Ship Hulls

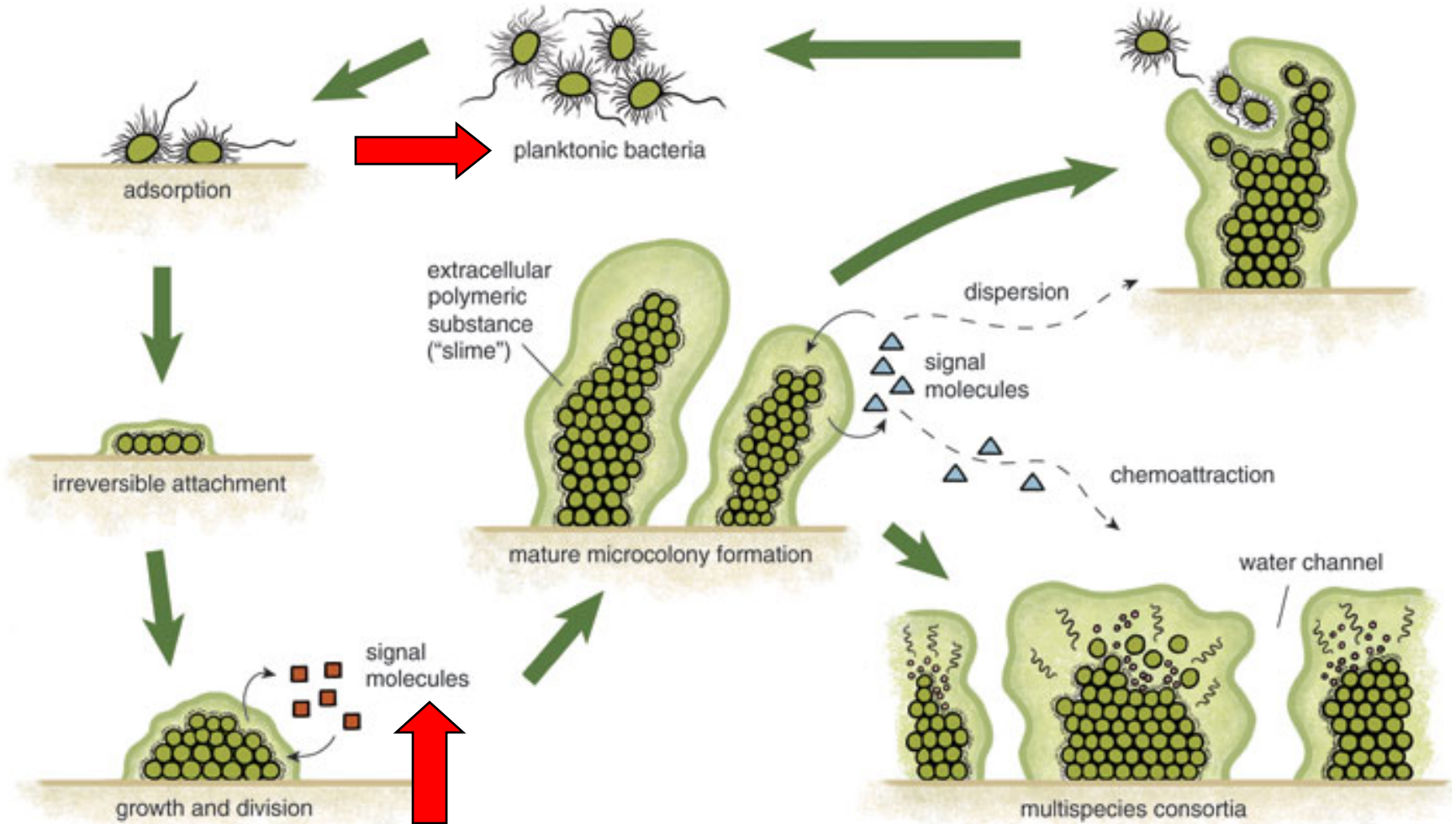


Paper Manufacturing

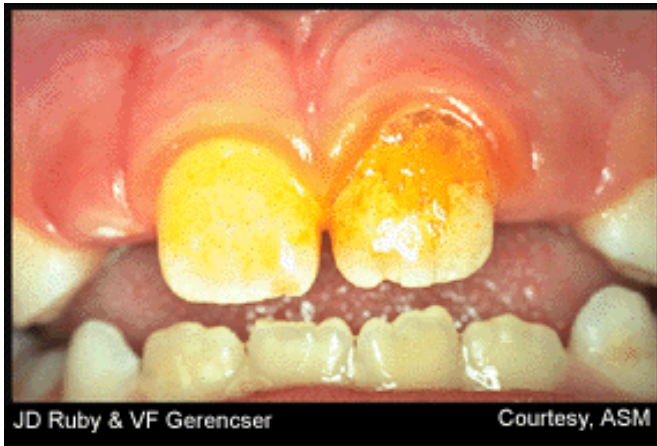


Medical Implants

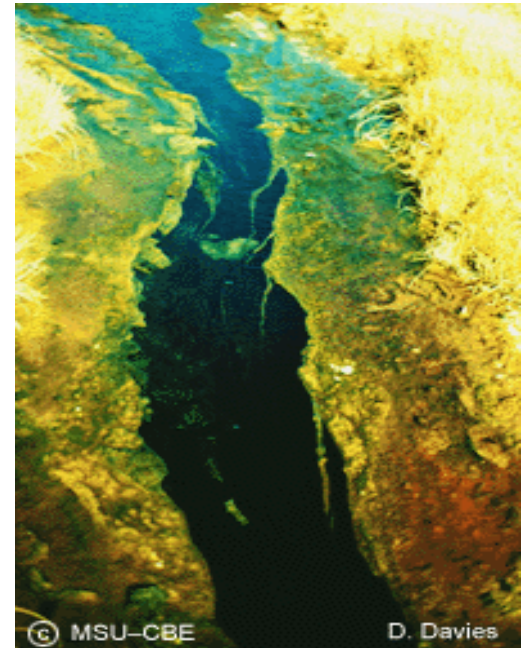
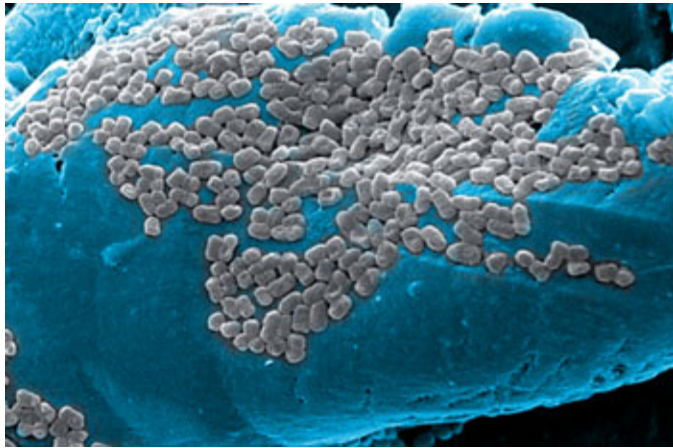
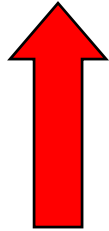
Biofilms: Cycle.



"United we stand"



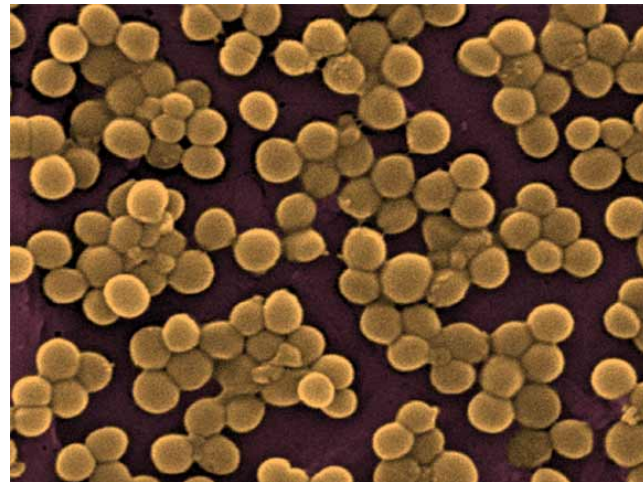
Impact of Biofilms



- A number of organisms have been intensively studied for their biofilm-forming properties:



Pseudomonas aeruginosa



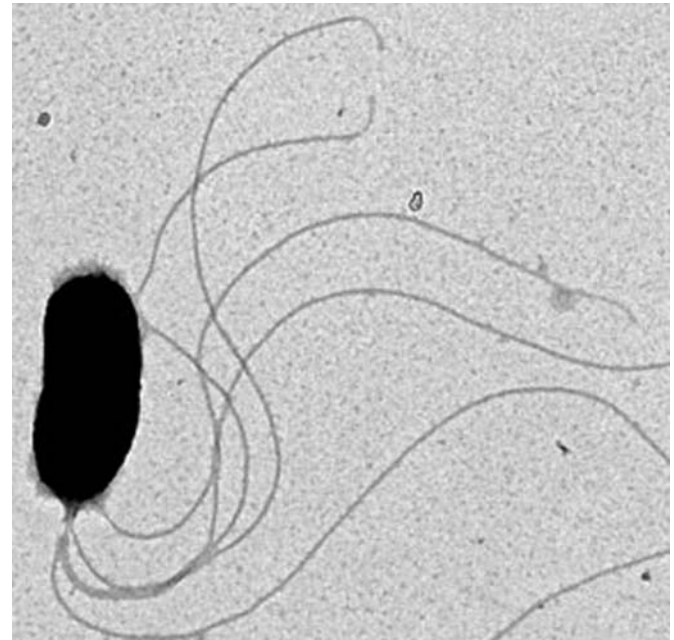
Staphylococcus spp.



Vibrio cholerae

Model organism: *Vibrio fischeri*

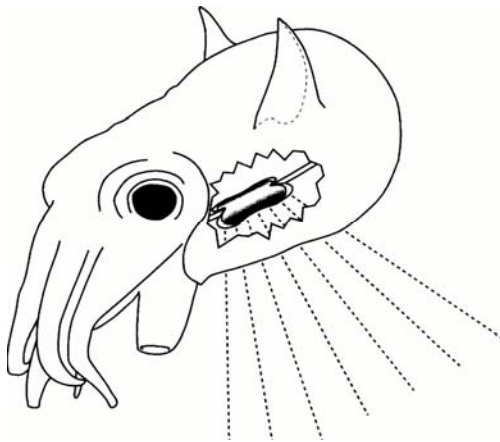
- Marine heterotrophic, facultative Gram (-).
- Novel locus: Polysaccharide production.
- **Symbiotic** bacteria.



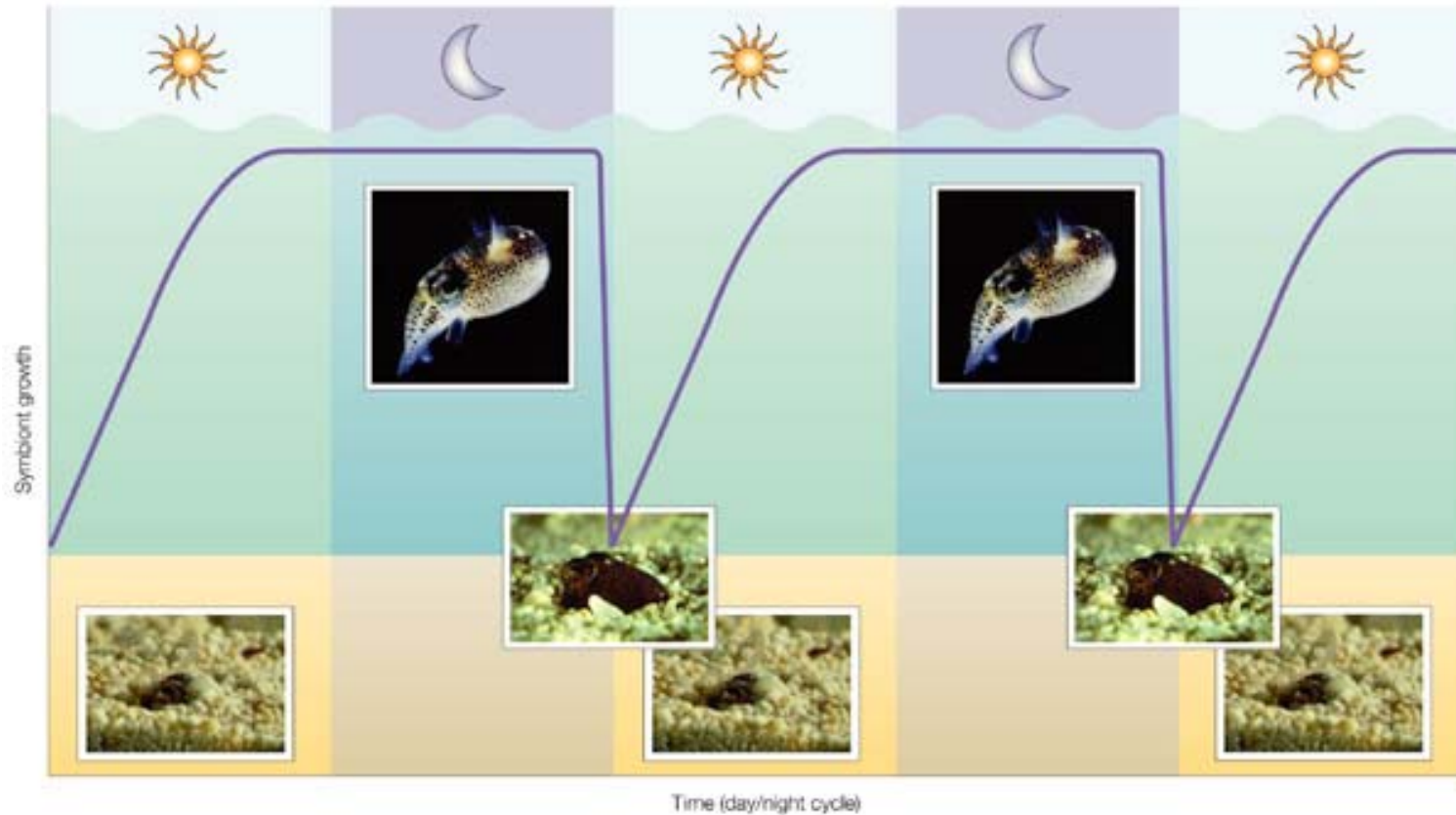
- Free-living planktonic state or as a symbiont.
- Light organ: Biofilm \longrightarrow Bioluminescent.
- Seawater: biofilmlike aggregate.



Photography by William Boyle



The host behavior and the associated symbiont density in the light organ

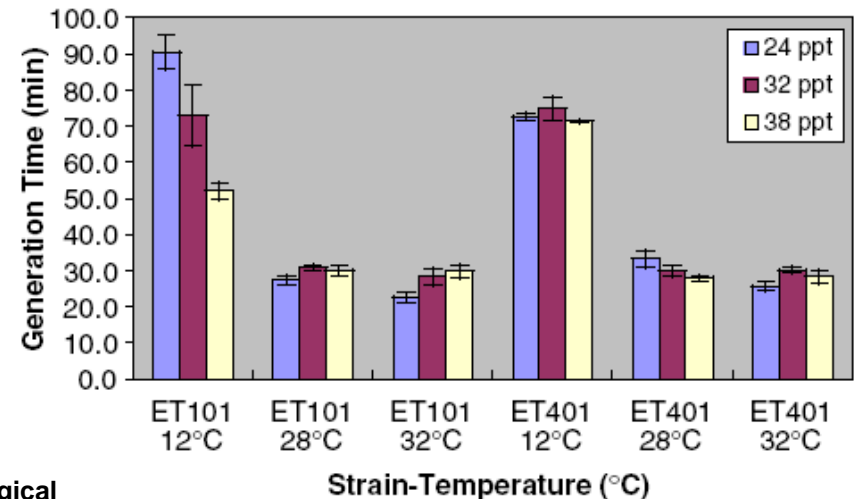
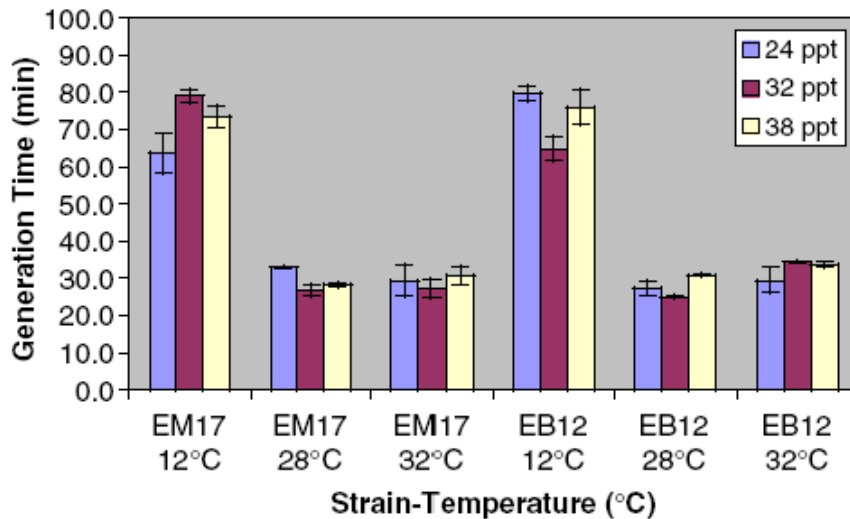


Nature Reviews | Microbiology

Nyholm, S.V., and McFall-Ngai, M. (2004). *Nat Rev Microbiol* 2: 632–642.

Effect of the Environment

- Regulation of gene expression in response to environmental signals.



Soto W. *et al.*, 2009. Salinity and temperature effects on physiological responses of *Vibrio fischeri* from diverse ecological niches.

- Growth rates are influenced by temperature.

TABLE 4. Infection of *Sepioloa* juveniles with *V. fischeri* strains

Temp (°C) ^a	<i>V. fischeri</i> strain	Concn in squid host species (10 ⁵ CFU/squid) after 48 h	
		<i>S. affinis</i>	<i>S. ligulata</i>
26	SA1	4.1	4.5
	SA8	4.0	4.0
	SL2	4.1	3.9
	SL8	3.7	3.9
	SI2	4.4	4.0
	SI4	4.2	4.1
	SR5	3.8	4.2
	SR10	3.9	3.9
18	SA1	0.24	0.20
	SA8	0.31	0.35
	SL2	0.30	0.34
	SL8	0.31	0.34
	SI2	0.28	0.25
	SI4	0.31	0.33
	SR5	0.19	0.22
	SR10	0.24	0.23

^a Preparations were incubated at 18 or 26°C.

Nishiguchi M.K., 2000. Temperature affects species distribution in symbiotic populations of *Vibrio* spp.

- 
- A grayscale micrograph showing a dense, complex network of filamentous structures, likely a biofilm. The structures are interconnected and form a mesh-like pattern. The background is dark, and the filaments are lighter, creating a high-contrast image. The overall appearance is that of a highly organized, interconnected community of microorganisms.
- Alterations to aquatic environments affect biofilm formation.
 - Features: Remain elusive.



NASA Science mission:

QuickTime™ and a
decompressor
are needed to see this picture.

How microbial
associations establish and
survive under extreme environments?



Aim:

Temperature, salinity, atmosphere,
starvation and static-dynamic conditions:
measure their influence on biofilm formation
of 15 isolates.

- **Experiment 1.** Environmental factors that affect biofilm formation (15 free-living and symbiotic strains).
- **Experiment 2.** Role of *lux* and *NaMP* operons on biofilm formation.

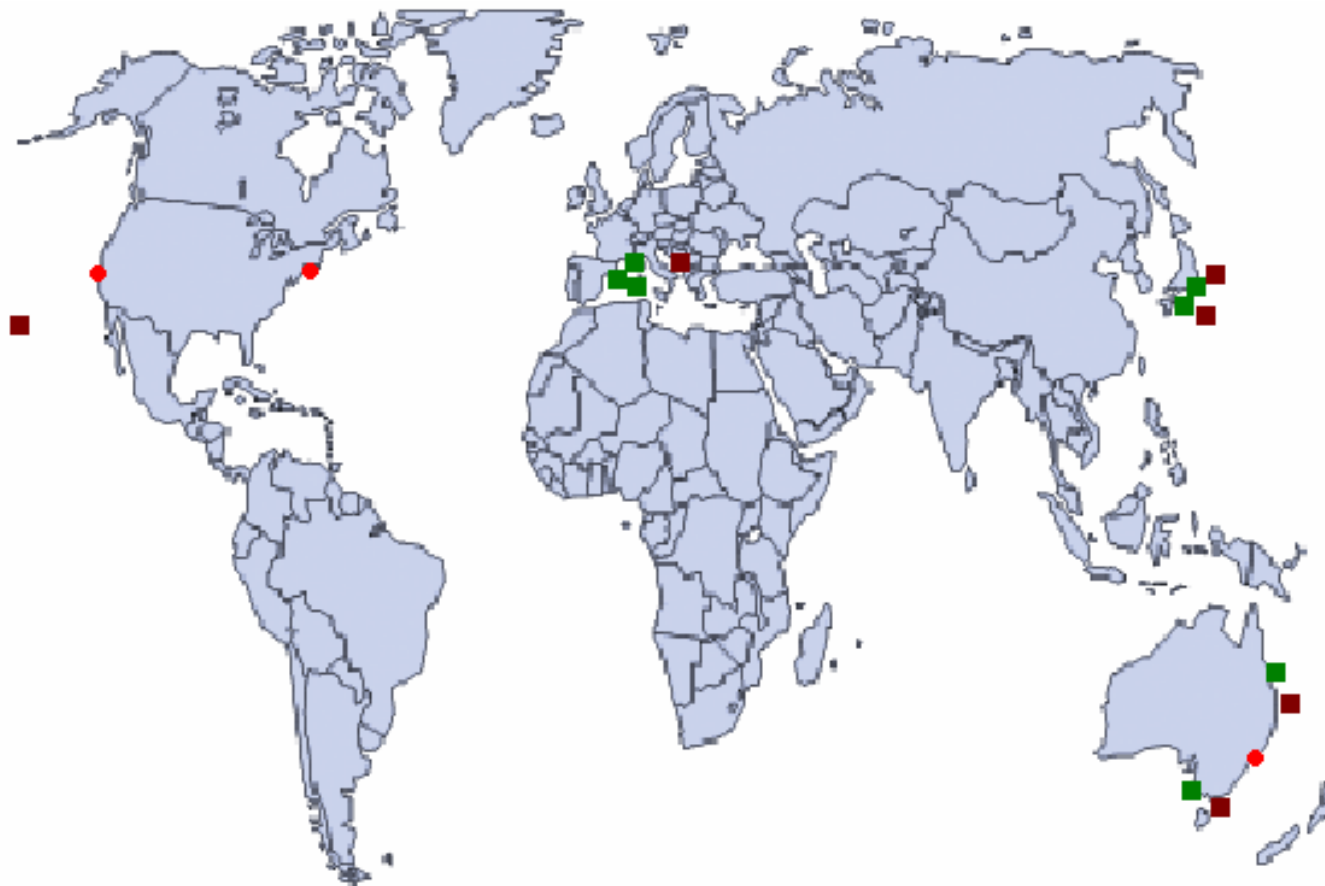


Experiment 1 :
Effect of the Environment

Strains of *Vibrio fischeri* used in this study.

Strain	Host	Location
WH1	Free-living	USA (Massachusetts, Woods Hole)
MDR7	Free-living	USA (California, Marina del Rey)
CB37	Free-living	Australia (Coogee Bay, New South Wales)
ATCC 7744	Free-living	American Type Culture Collection
MJ101	<i>Monocentris japonicus</i>	Japan (Tokyo Bay)
CG101	<i>Cleidopus gloriamaris</i>	Australia (Townsville, Queensland)
SR5	<i>Sepiola robusta</i>	France (Banyuls sur Mer)
SL518	<i>Sepiola ligulata</i>	France (Banyuls sur Mer)
SA1G	<i>Sepiola affinis</i>	France (Banyuls sur Mer)
SI66	<i>Sepiola intermedia</i>	Italy (Bari)
EM17	<i>Euprymna morsei</i>	Japan (Tokyo Bay)
ET101	<i>Euprymna tasmanica</i>	Australia (Melbourne, Victoria)
ET401	<i>Euprymna tasmanica</i>	Australia (Townsville, Queensland)
EB12	<i>Euprymna berryi</i>	Japan (Tosa Bay)
ES114	<i>Euprymna scolopes</i>	USA (Kaneohe Bay, Hawaii)

Soto W. *et al.*, 2009. Salinity and temperature effects on physiological responses of *Vibrio fischeri* from diverse ecological niches.



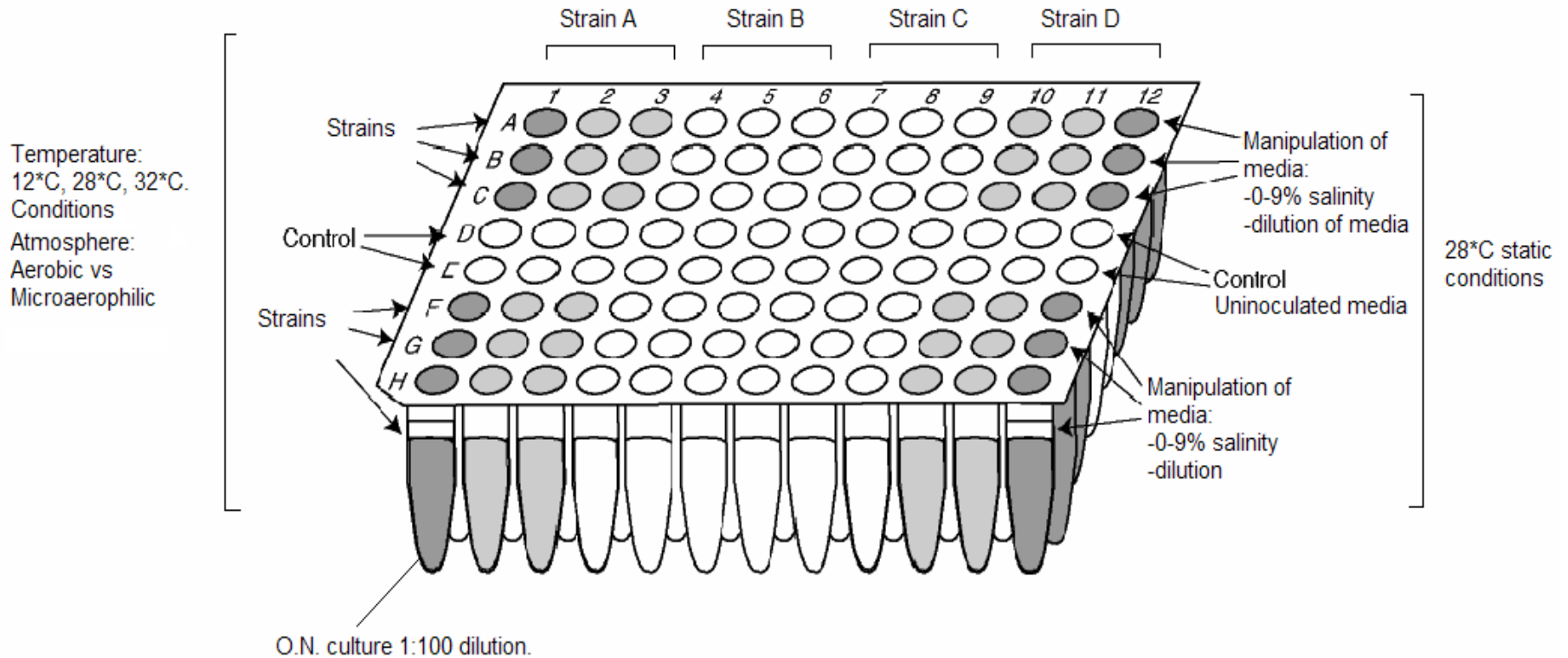
● Free-living

■ *Sepioloidea*

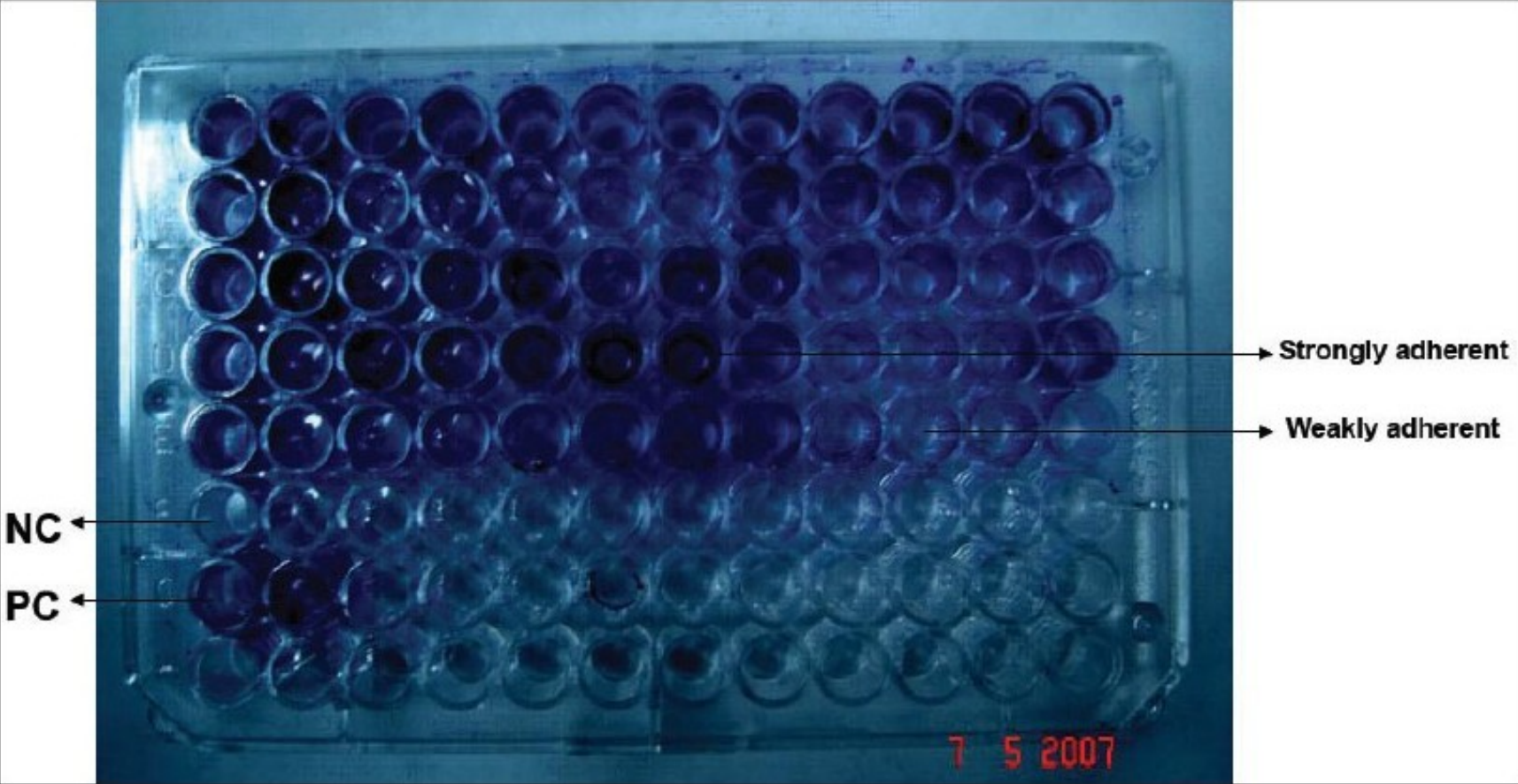
■ *Euprymna*

Methods

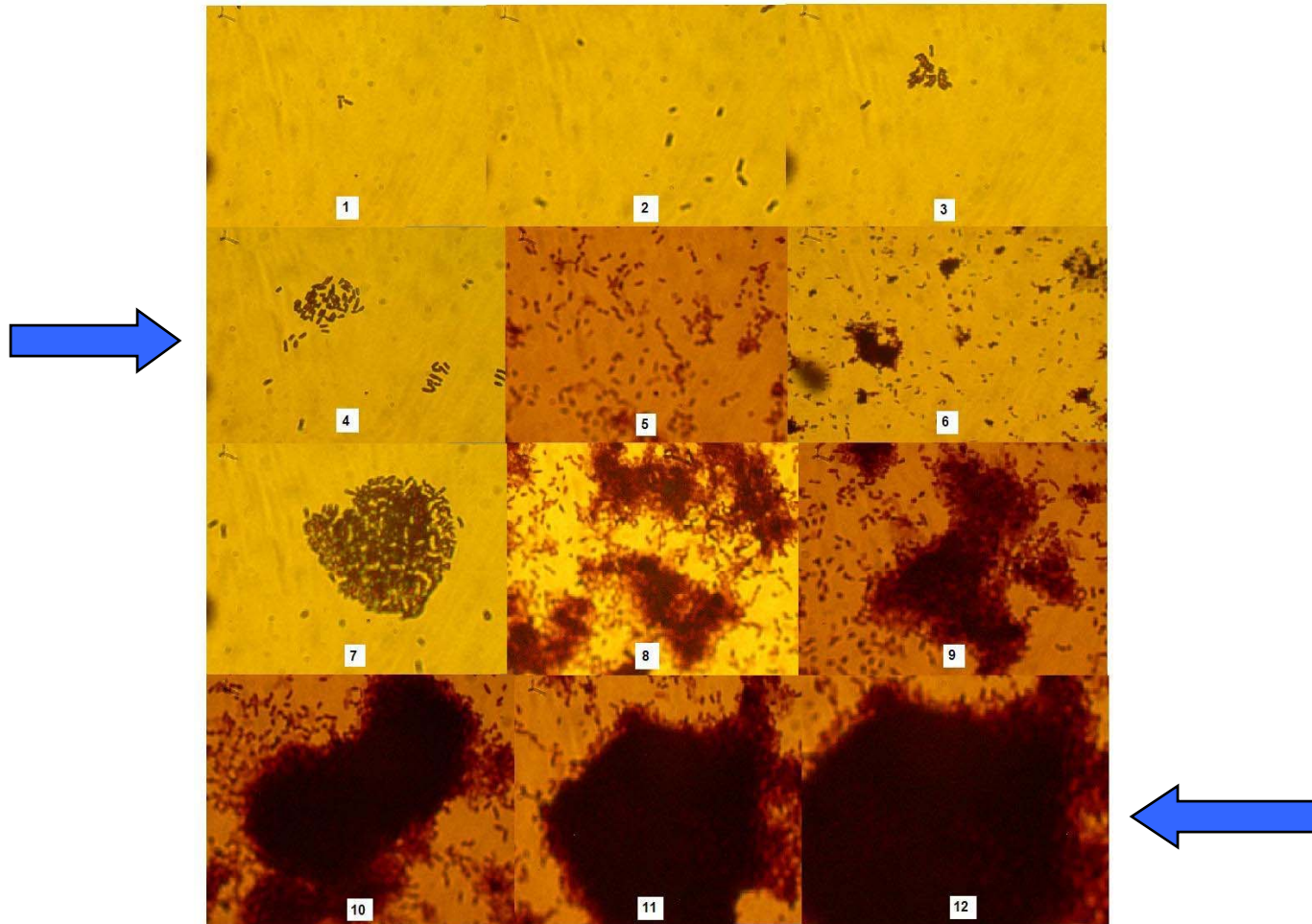
Microtiter plate assay



- Incubation for 24 h.
- Wells washed 3 times.
- Crystal violet 0.1% v/v for 30 min.
- Wells washed 3 times.
- Solubilization with 70% ethanol.
- Measurement of O.D. (562nm) on plate reader.

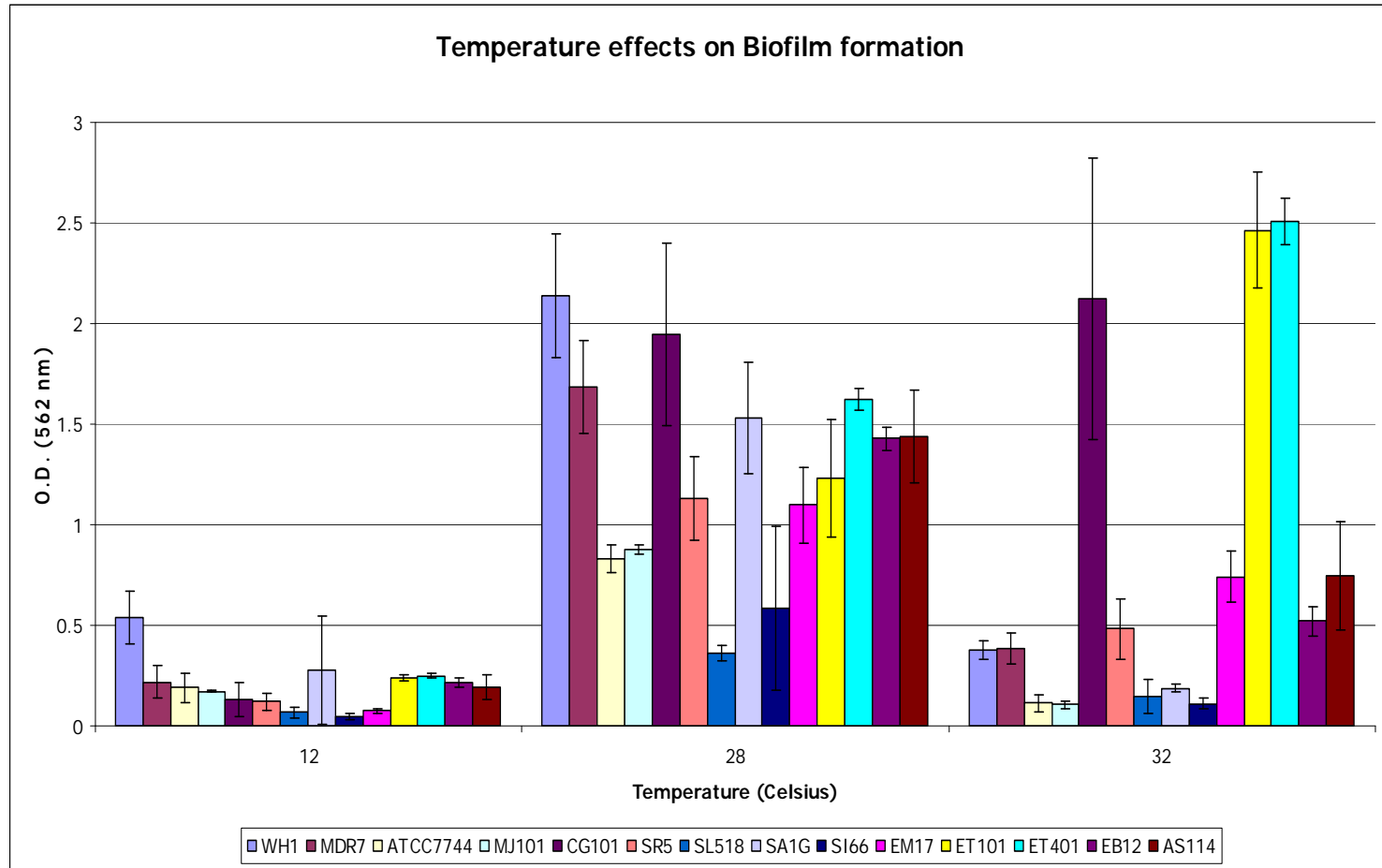


Results



Biofilm formation dynamics

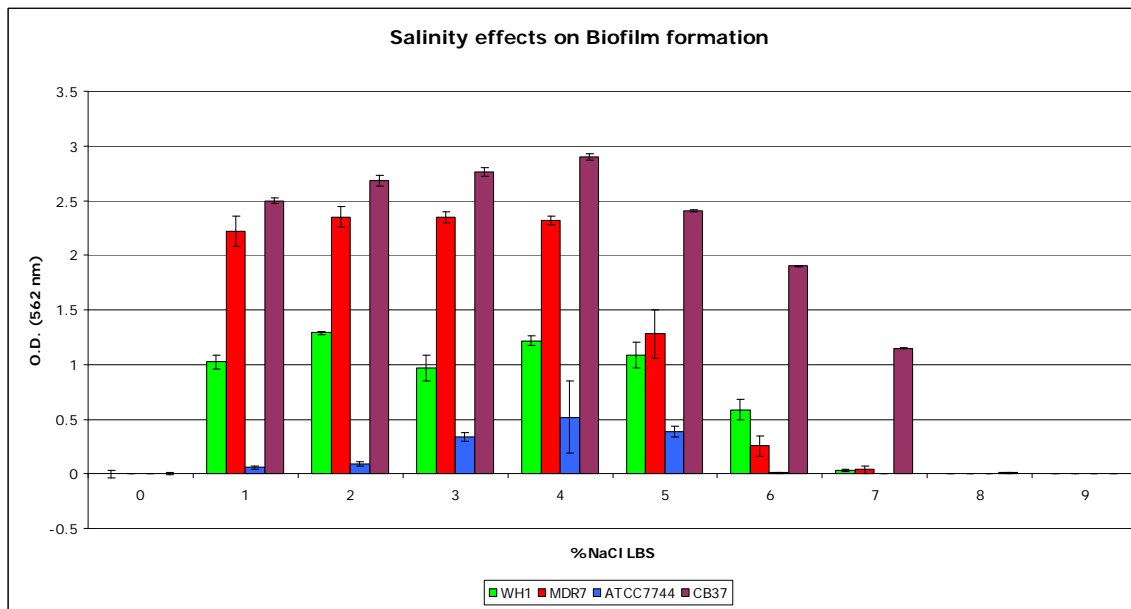
Temperature



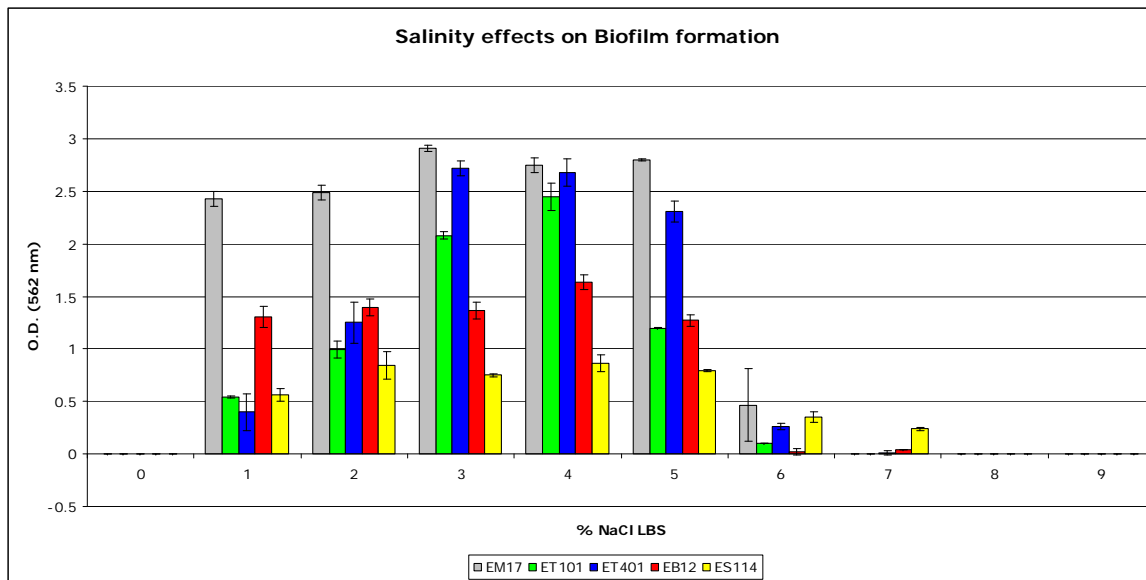
Most strains (>78%) classed as strong biofilm producers.

Salinity

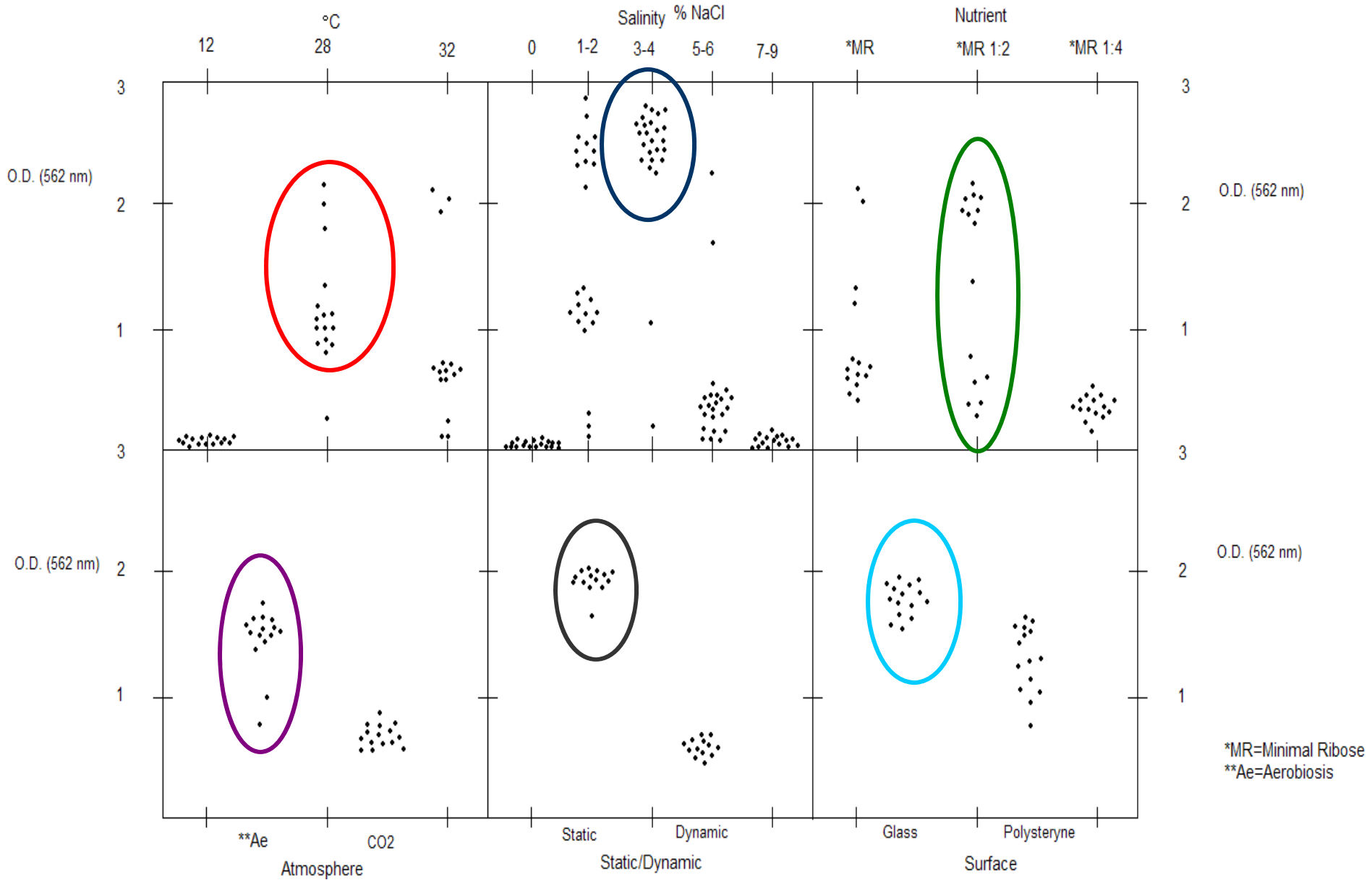
Free-living



Symbiotic



Most of the strains (>80%; $p < 0.01$) form strong biofilms: 1-5% NaCl.



Conclusions

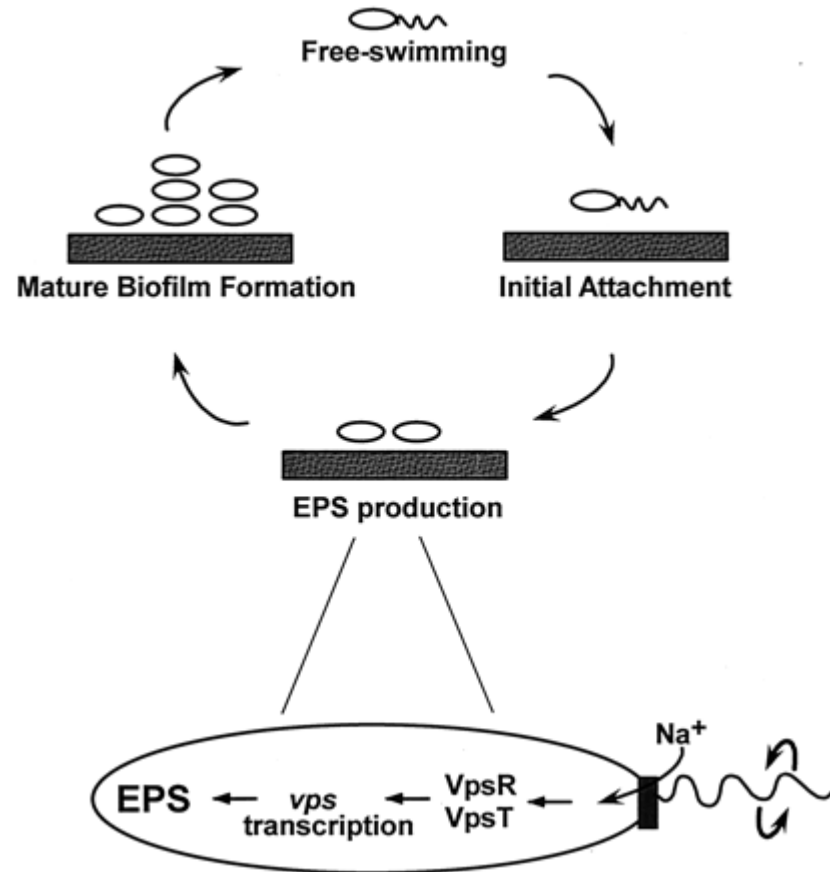
- These factors tested resemble how the bacterium response to fluctuations in its natural environment.
- Biofilms: influenced by host.

- Outside the host: Salinity, temperature changes and starvation.
- Inside the host: Oxygen/CO₂, temperature, nutrient availability, dynamic conditions.

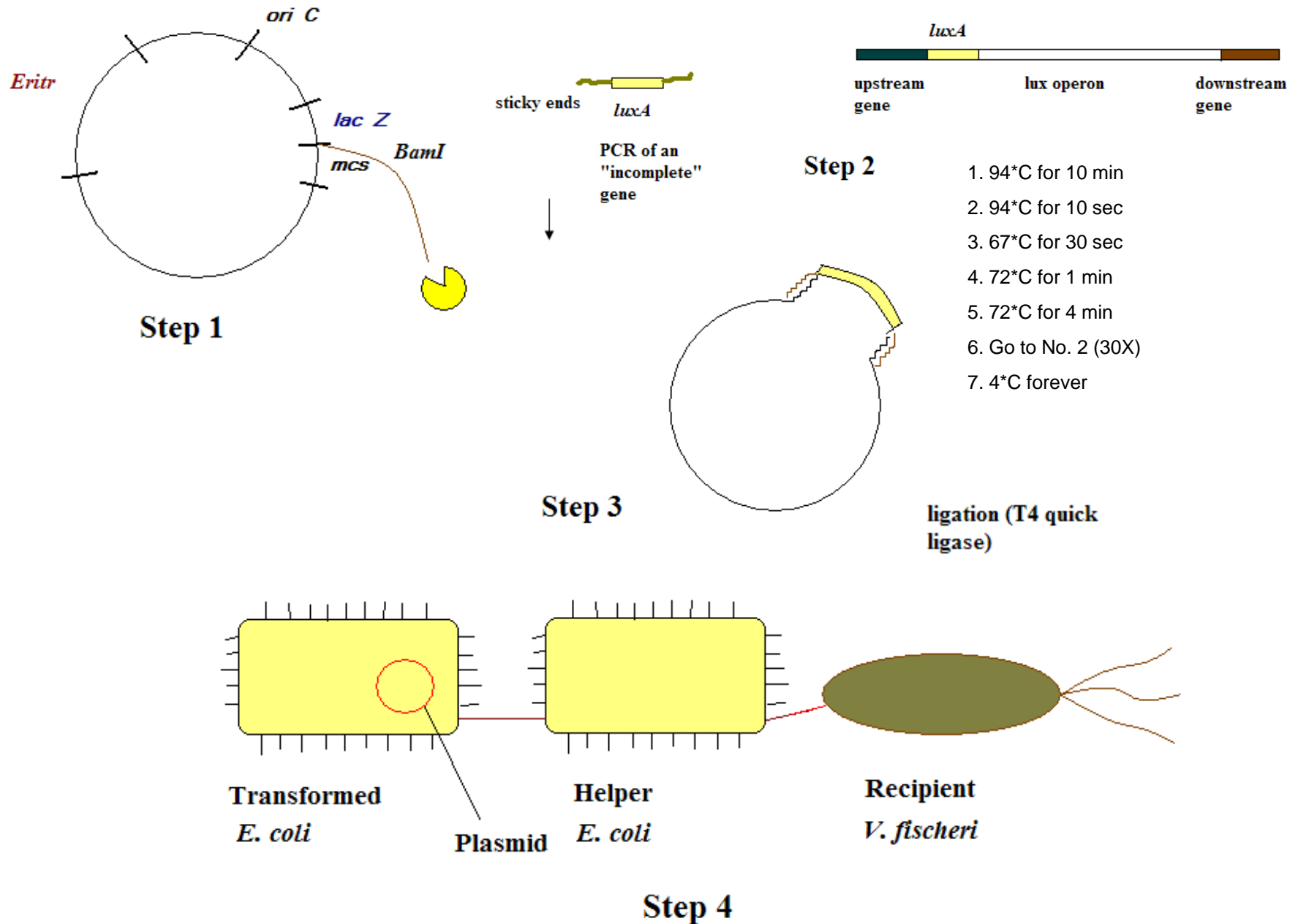


Experiment 2 :
Role of operons *lux* and *NaMP*

Why *NaMP*?

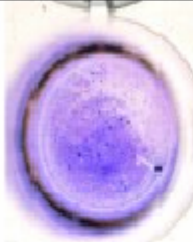





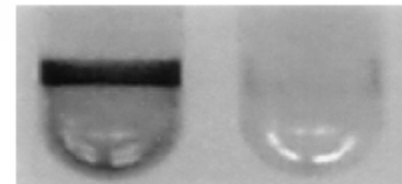
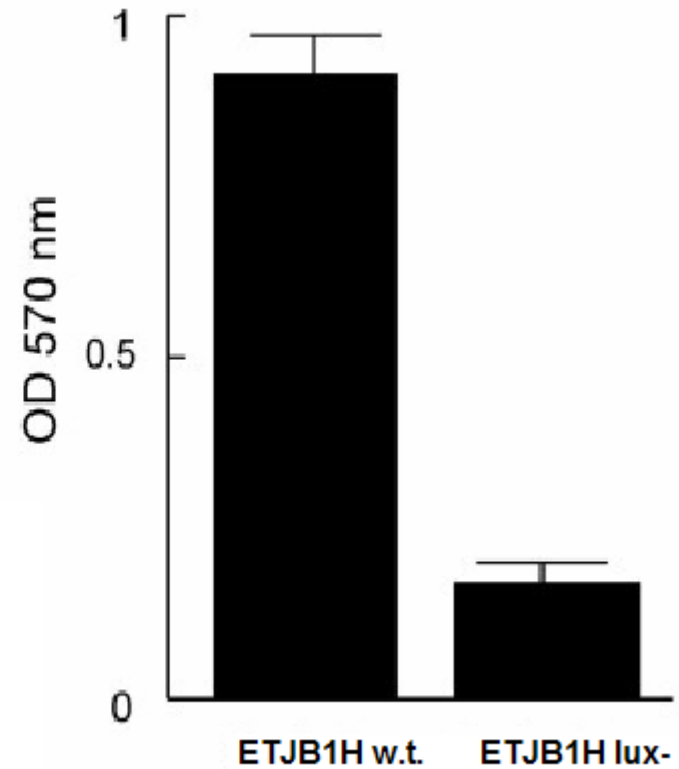
Methods

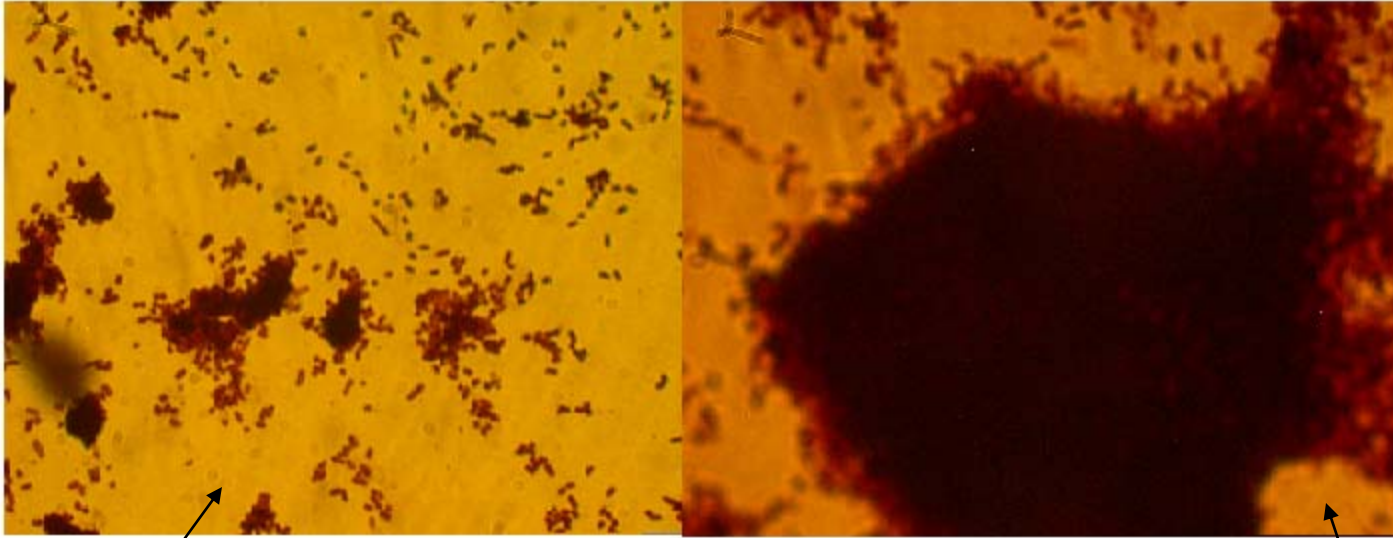


Results:

Colorimetric method for Biofilm quantification

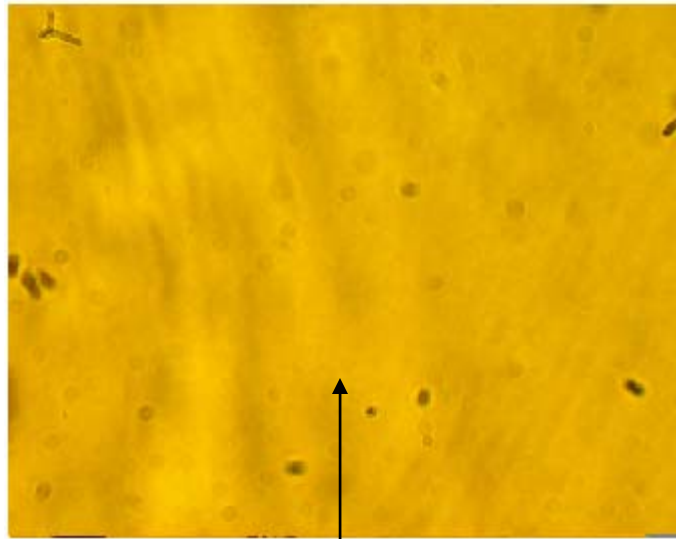
Operon/Strain	W.T.	mutant
Lux		
NaMP		





V. fischeri
ETJB1H NaMP-

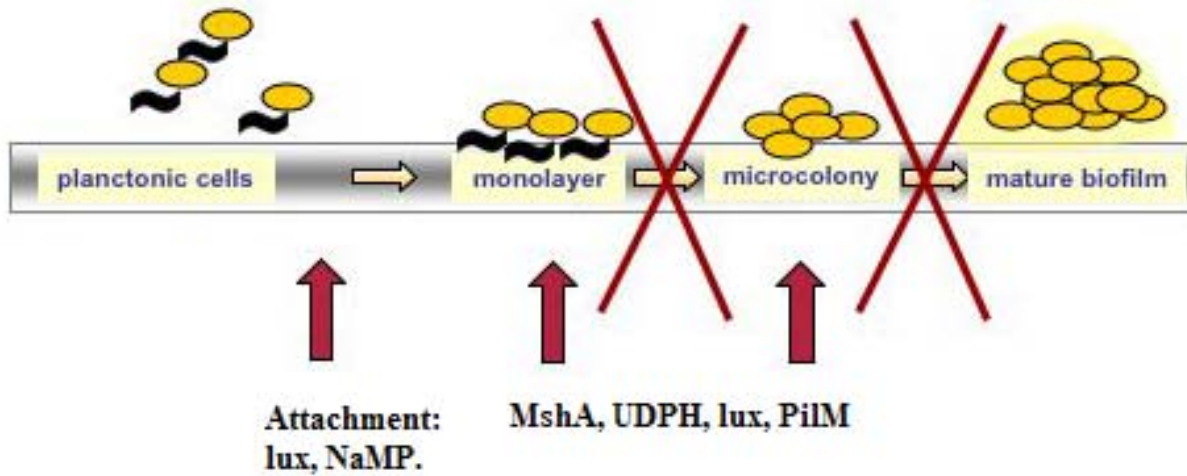
V. fischeri ETJB1H W.T.



V. fischeri
ETJB1H lux-

Conclusion

Mutation= No Attachment, no Quorum sensing, No Biofilm.



Future studies

- Genes responsible of biofilm formation in *Vibrio fischeri*.
 - What genes are activated and/or suppressed under a specific environmental stress?

Acknowledgements

- NMSU Department of Biology
- Michele Nishiguchi
- Members of the Nish lab
- NMSGC



RISE-doctorate



New Mexico Space
Grant Consortium.

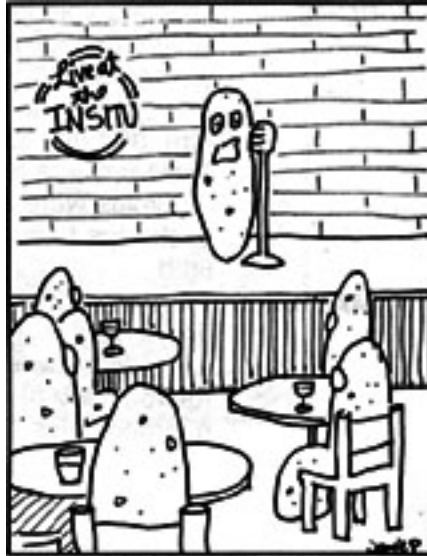


NSF IOS 0744498



NIH-NIAID 1SC1AI081659
NIH-NIAID 3SC1AI081659-02S1

Questions?



*I just got back from a lab grown culture,
and, boy, are they homogeneous...*