Studying host microbial interaction in larviculture: the way forward

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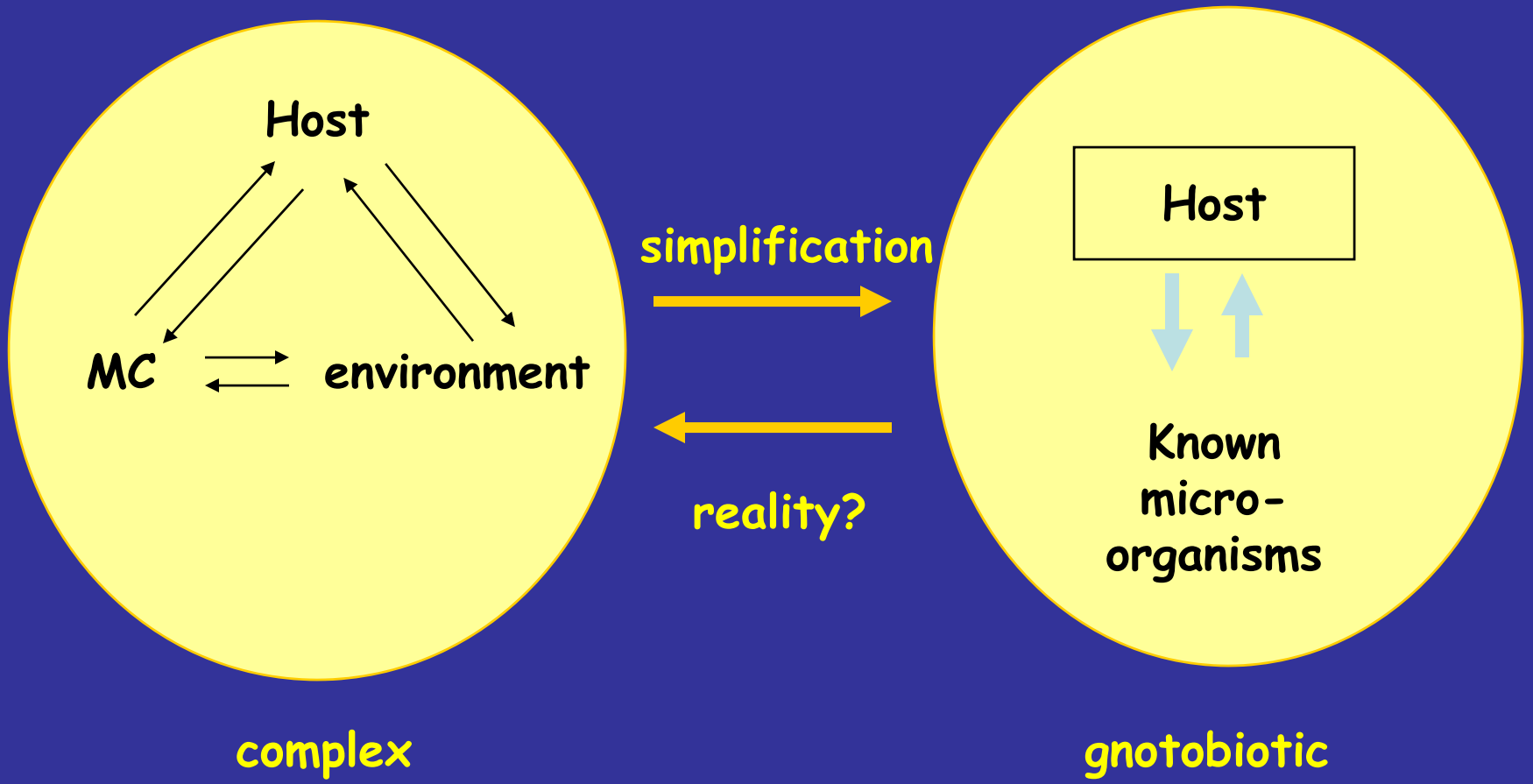
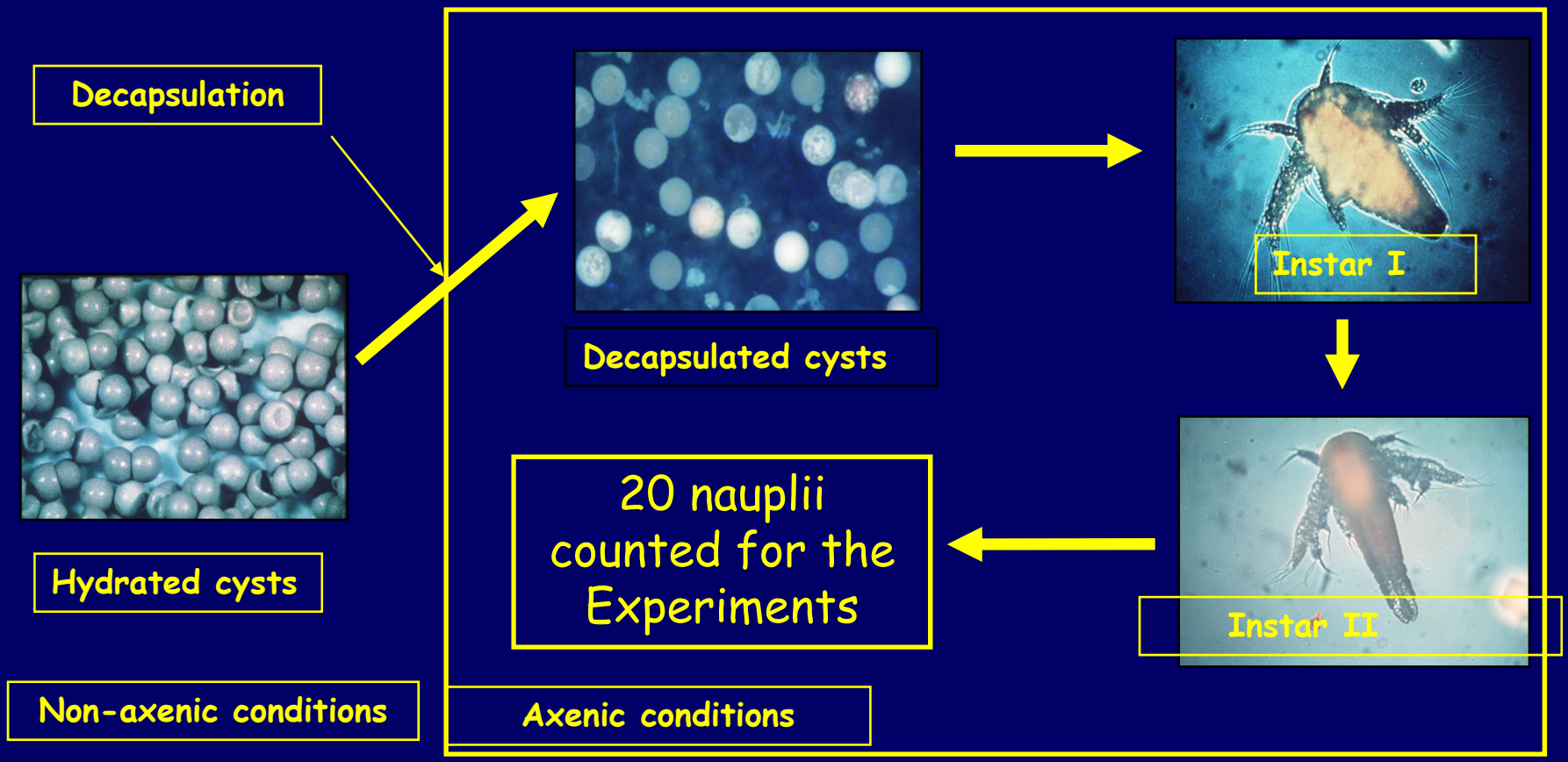
How to study host-microbial interactions?



Gnotobiotic systems

- Mammals
- Zebrafish
- *Artemia*
- *Brachionus*
- *Seabass and Cod (under development)*

Gnotobiotic Artemia: GART

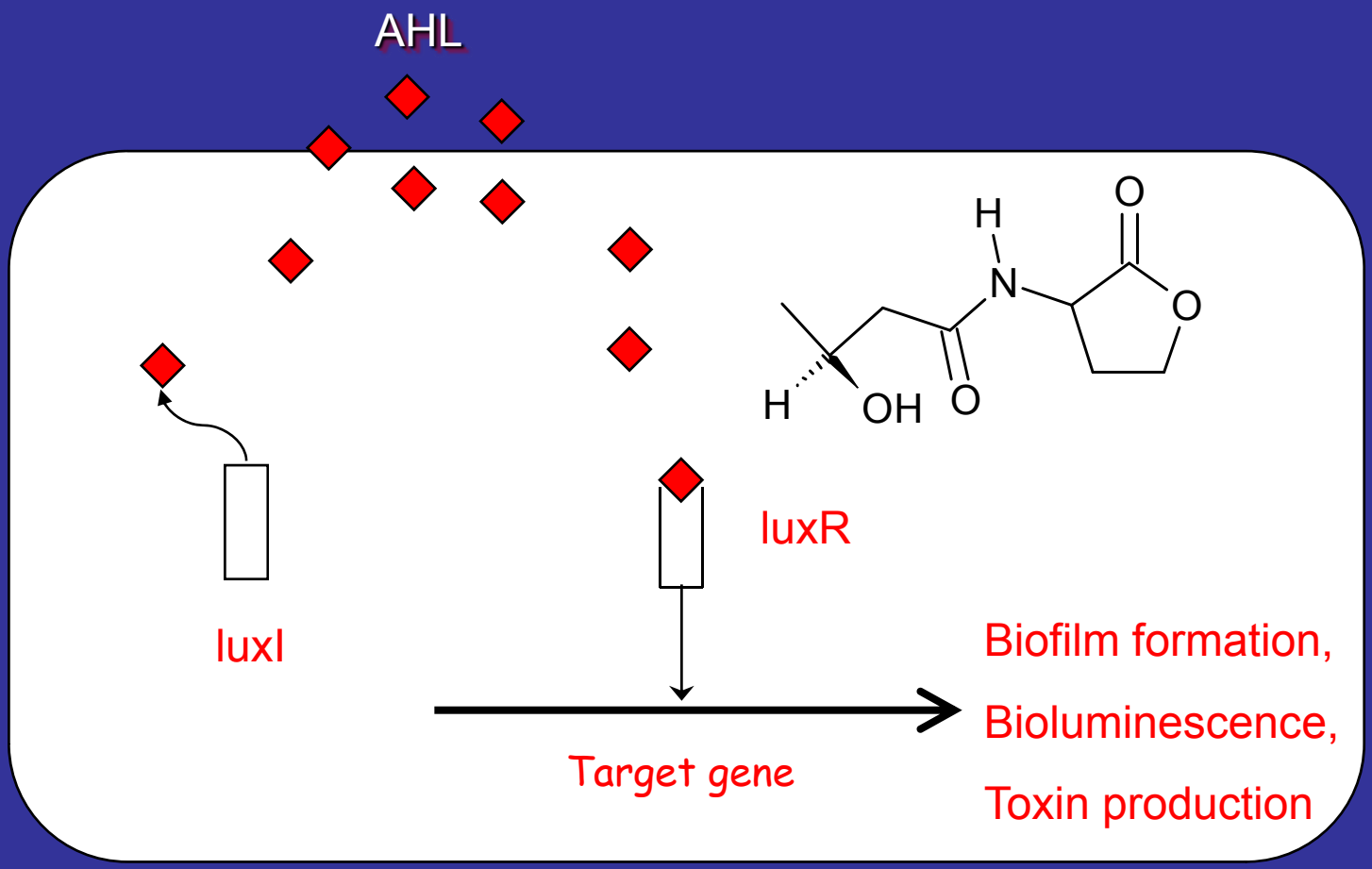


Processes

- Quorum sensing
- Polyhydroxybutyric acid
- Yeast-bound glucan
- Heat shock proteins

Quorum sensing

What is quorum sensing?



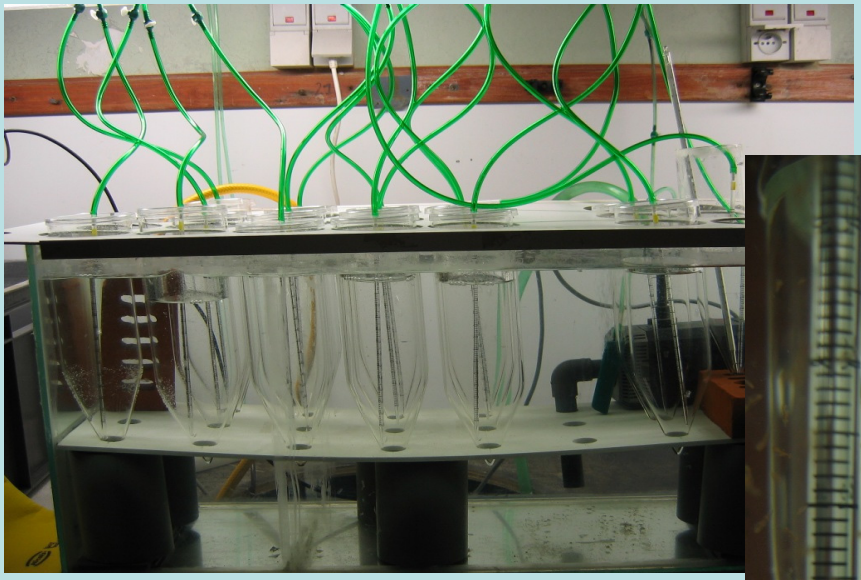
AHL-mediated pathway in Gram-negative bacteria

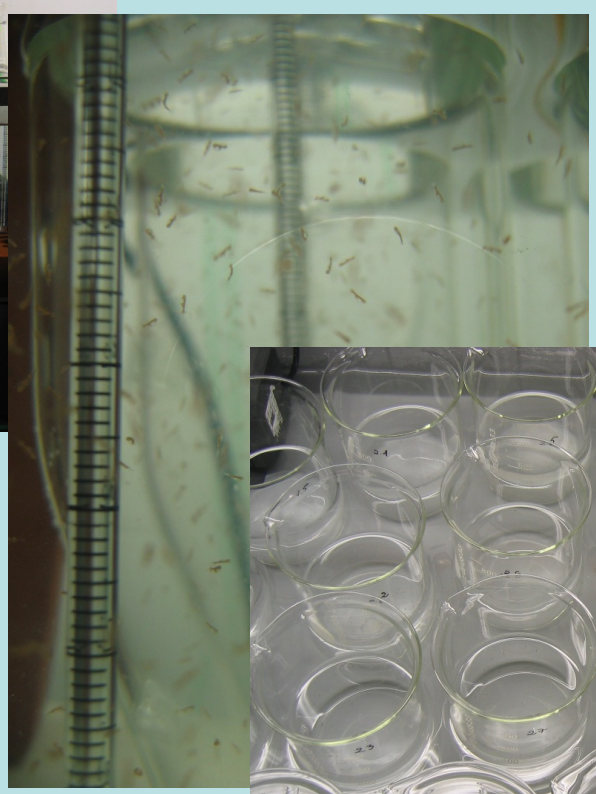
Quorum sensing and quenching in the *Brachionus - Turbot* food chain

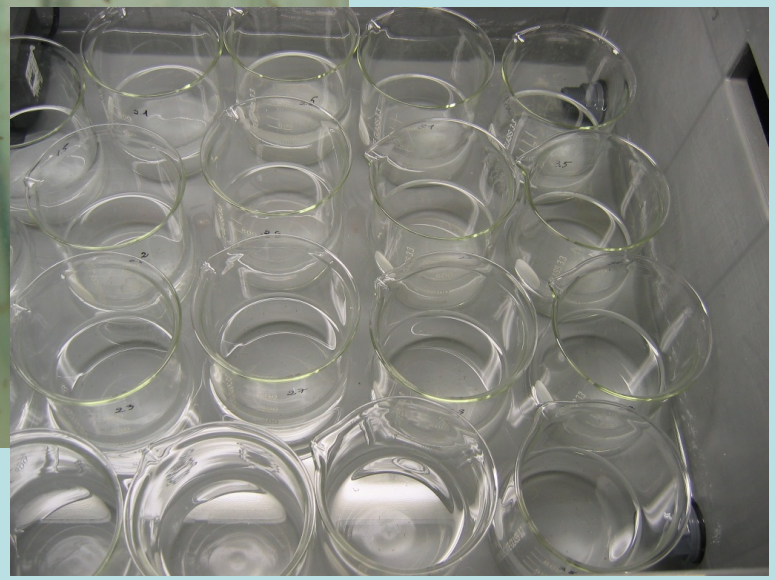
Objectives

- Study the effect of AHL on turbot larval survival
- Study the effect of an N-acyl homoserine lactone (AHL) degrading bacterial mixtures (EC5) in turbot larviculture.

Experiment setup







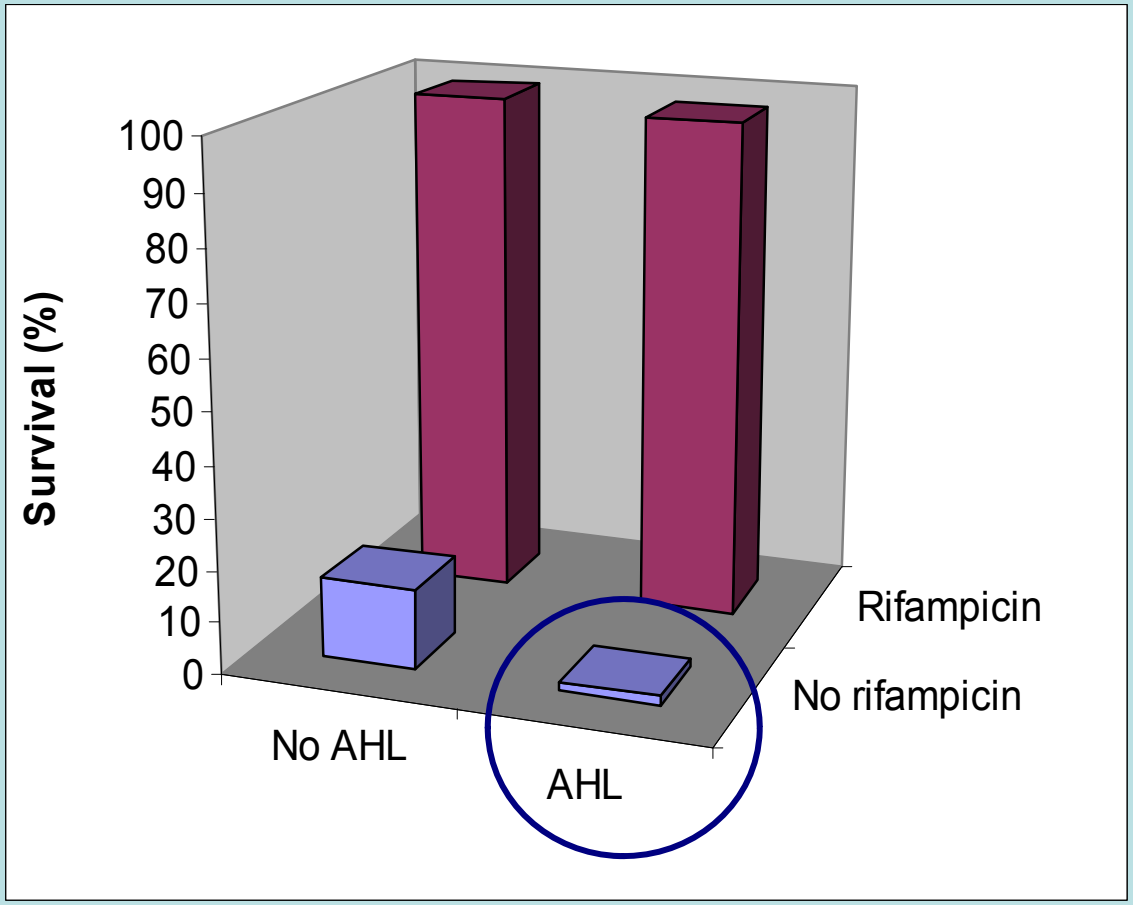
- **Egg disinfection: 0.005% glutaraldehyde + 50 mg l^{-1} rifampicin**
- **Temperature: 16°C**
- **Light intensity: 14 lux**
- **Mild aeration for cones, no aeration for beakers**

Experiment outline

Aim: To investigate the mode of action of AHL mixture (1 mg l^{-1})

Exp	Treatment					
	1	2	3	4	5	6
1	**Control**	**AHL addition**	**Rifampicin**	**Rifampicin + AHLs**		
2						
3						

Survival of turbot larvae at 5 dph (Exp 1)



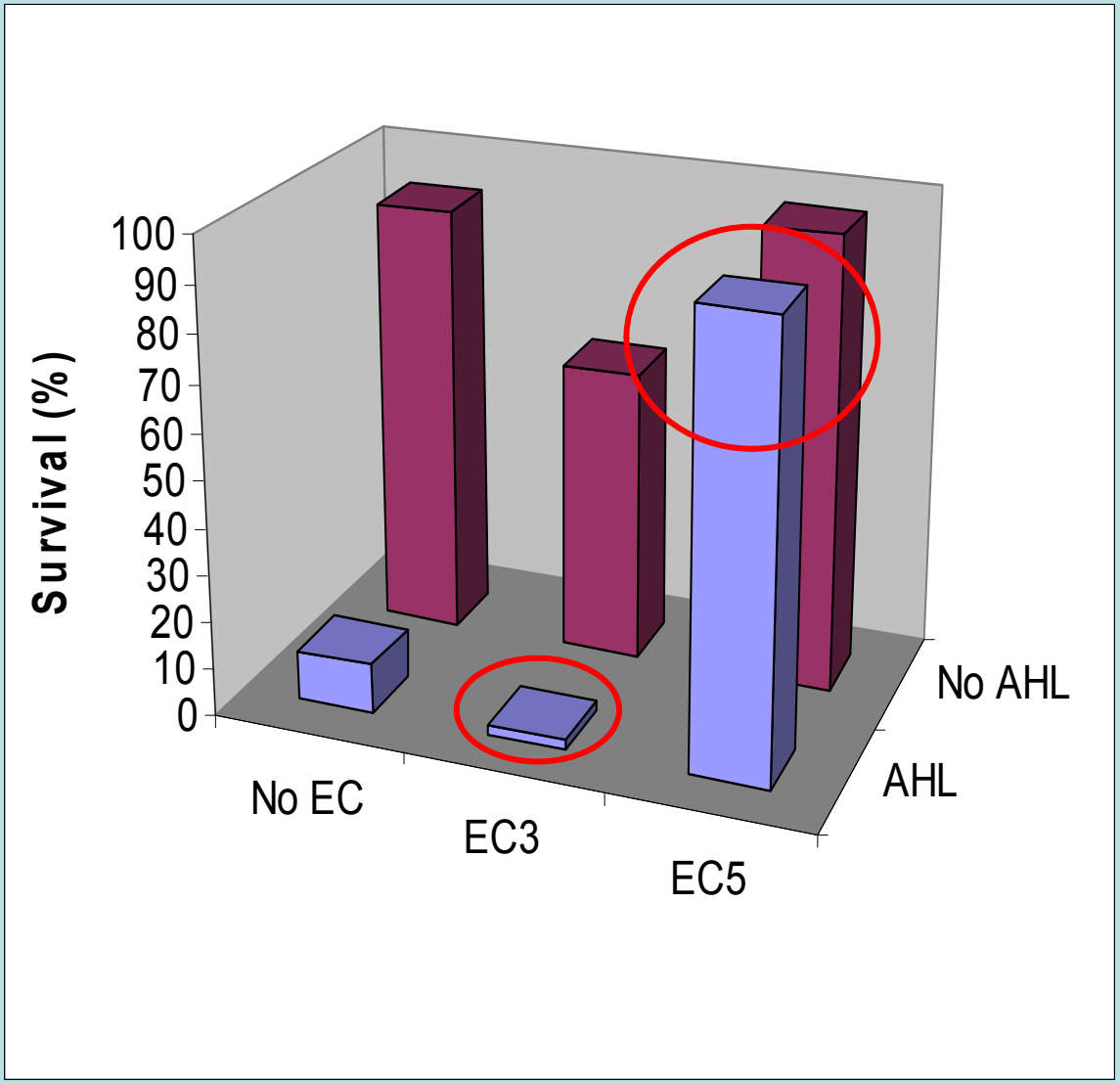
- No negative effect of AHLs in the presence of antibiotic
- Effect of AHL probably through the stimulation of the virulence of opportunistic bacteria

Experiment outline

Aim: To compare the effect of EC3 and EC5

Exp	Treatment					
	1	2	3	4	5	6
1	Control	AHL addition	Rifampicin	Rifampicin + AHLs		
2	Control	AHL addition	EC5 (added to water)	EC5 (added to water) + AHLs	EC5 (added to water + via rotifers)	EC5 (added to water + via rotifers) + AHLs
3	**Control**	**AHL addition**	**EC3 (added to water + via rotifers)**	**EC3 (added to water + via rotifers) + AHLs**	**EC5 (added to water + via rotifers)**	**EC5 (added to water + via rotifers) + AHLs**

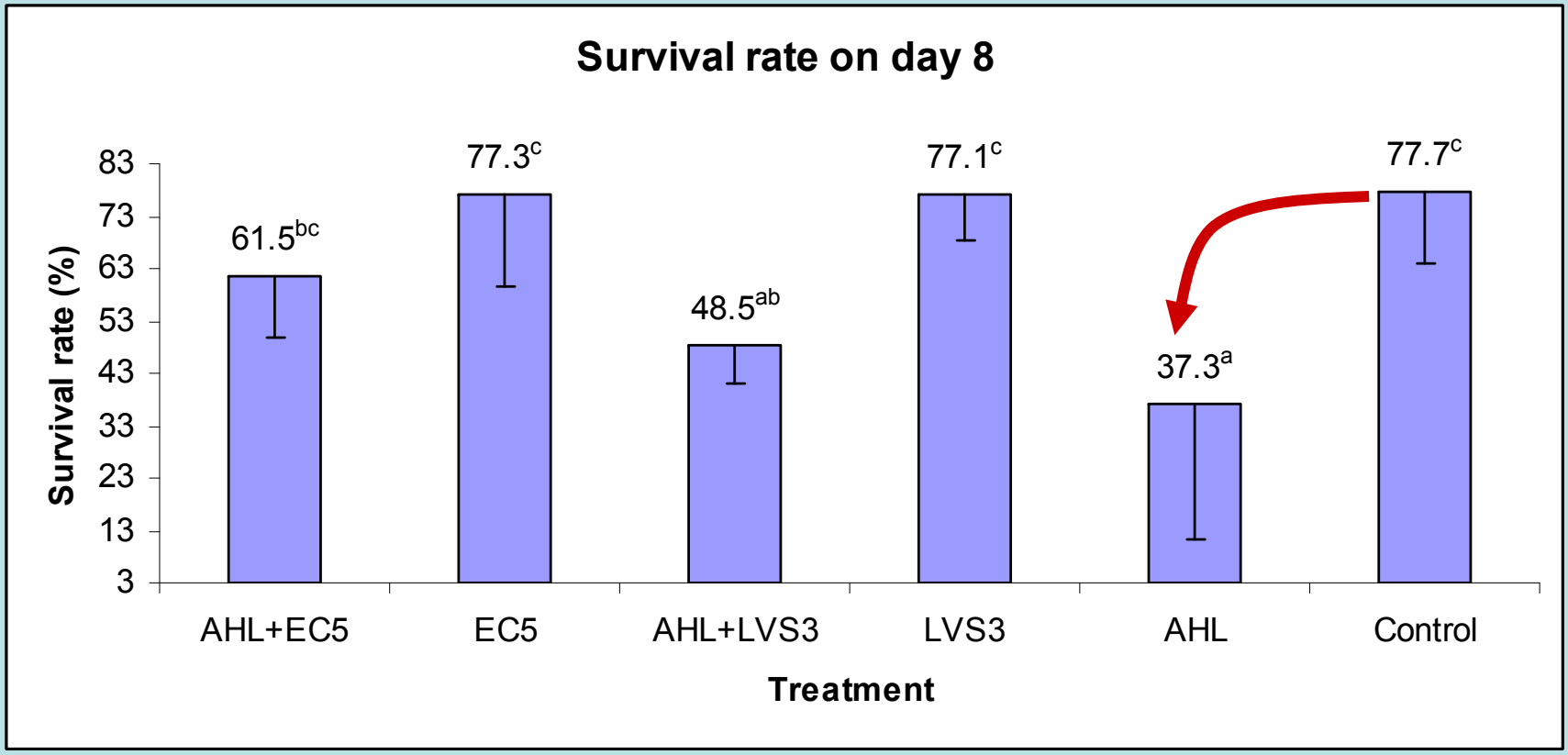
Survival of turbot larvae at 7 dph (Exp 3)



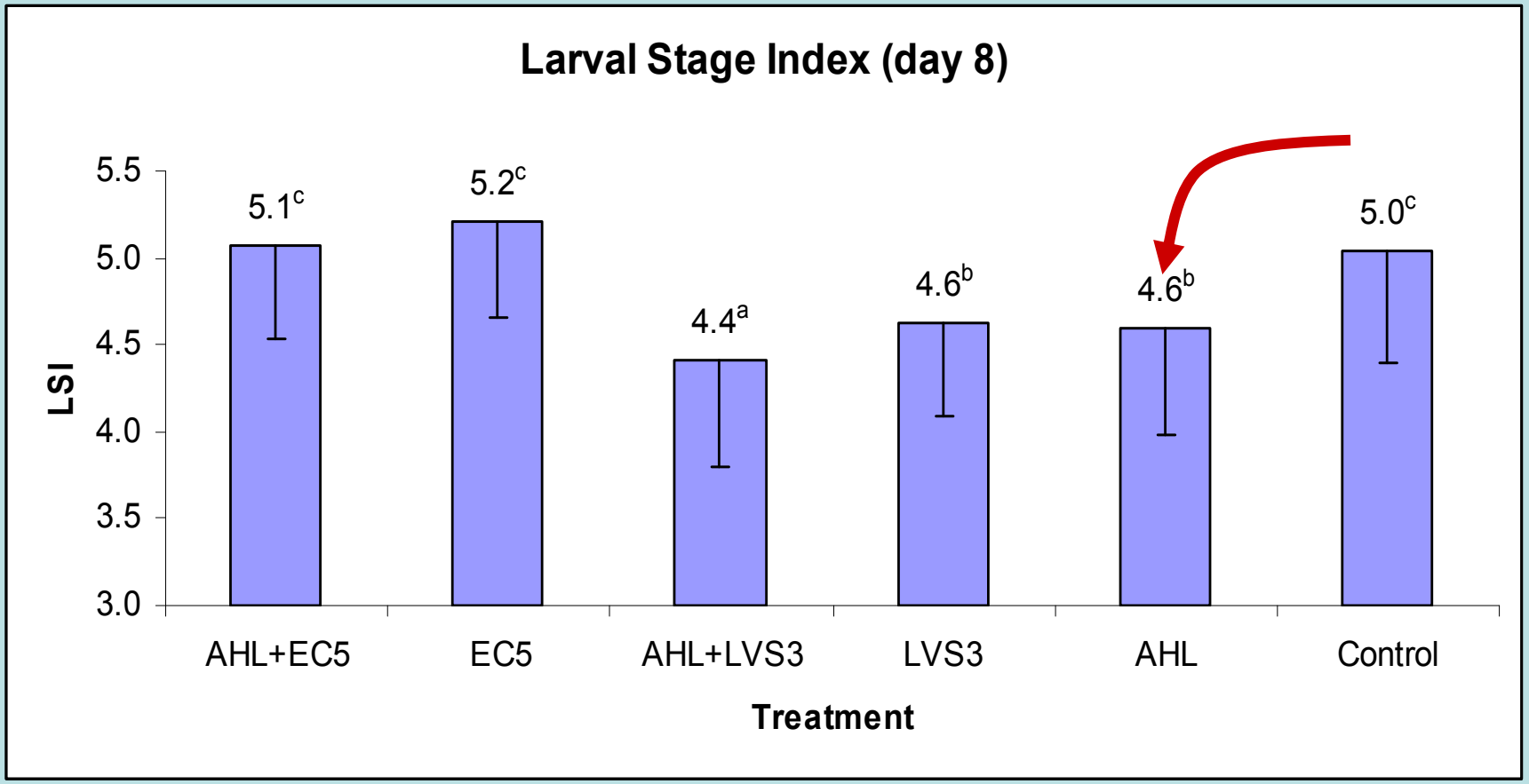
EC5 can neutralize the negative effect of AHLs, while EC3 cannot

Quorum sensing in the *Artemia - Macrobrachium* food chain

Effect of AHL on *Macrobrachium* larviculture



Effect of AHL on Macrobrachium larviculture: LSI



Conclusions

- QS determines virulence *in vivo* as demonstrated by using qs mutant
- In *Artemia* disruption of AI2 is sufficient to abolish virulence
- In *Brachionus* disruption of AI1 and AI2 is needed
- In larviculture of turbot and macrobrachium AHL molecules have a strong negative effect on survival
- Added QS molecules can be quenched by AHL degrading MC
- Is AHL mediated virulence by prevalent opportunistic pathogenic bacteria a problem in larviculture?

Heat shock proteins

as

immunostimulants?

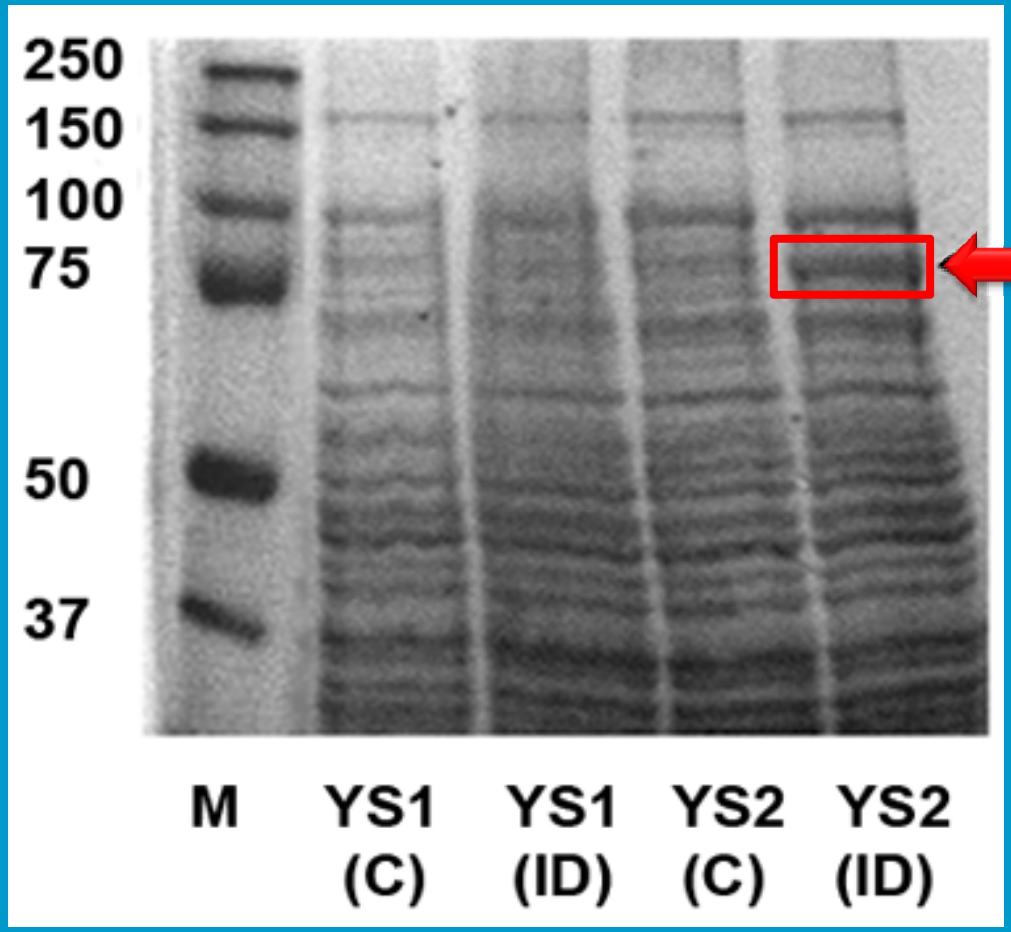
Experimental design

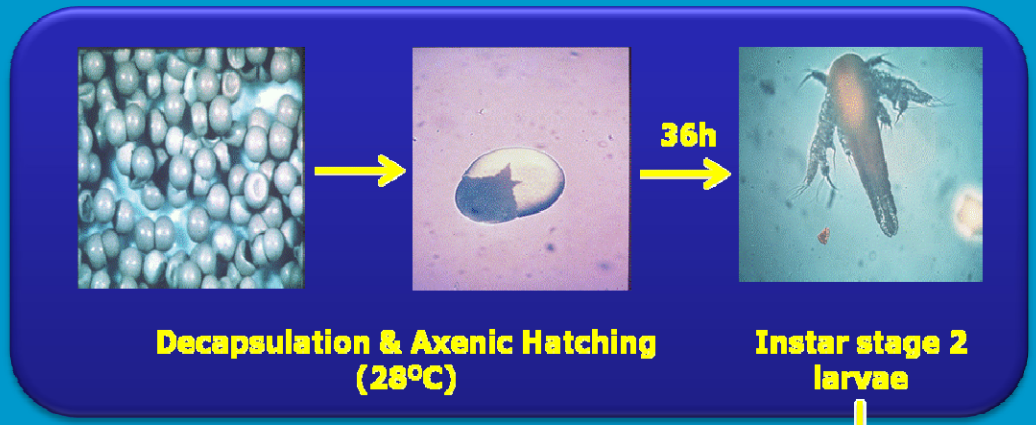
To test the ability of *E. coli* overproducing DnaK to protect *Artemia* larvae against Vibrio infection

Construction of bacteria over-producing DnaK

E. coli strains	Plasmids	Induction	Hsps encoded by plasmids
YS1	pblDnaK (constructed using pBAD TOPO® vectors, Invitrogen™, USA)	L-arabinose (0.5 mg/ml for 1 h)	-
YS2	pDnaK (constructed using pBAD TOPO® vectors, Invitrogen™ , USA)	L-arabinose (0.5 mg/ml for 4 h)	DnaK

DnaK expression of YS1 and YS2 on SDS-PAGE





Counting of 50 larvae/tubes

Feeding bacteria (10^7 cells/ml)

Vibrio challenge (10^7 cells/ml)

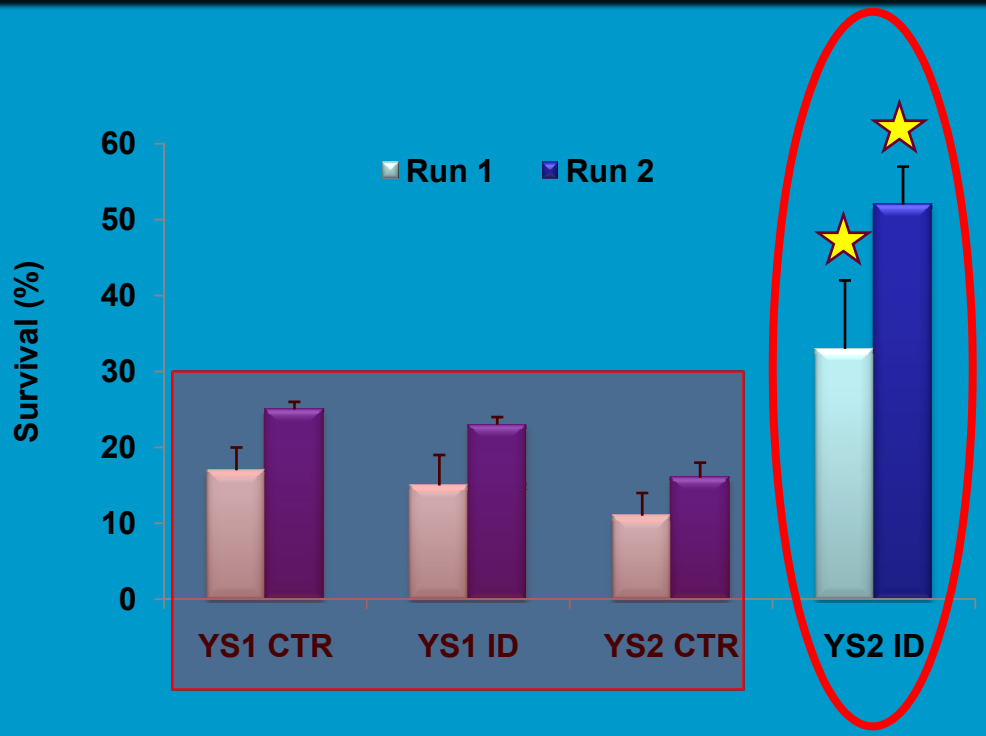
Artemia survival (24h)

Ingestion period 6h

Hsp expression by SDS-PAGE & Western Blot

Correlation

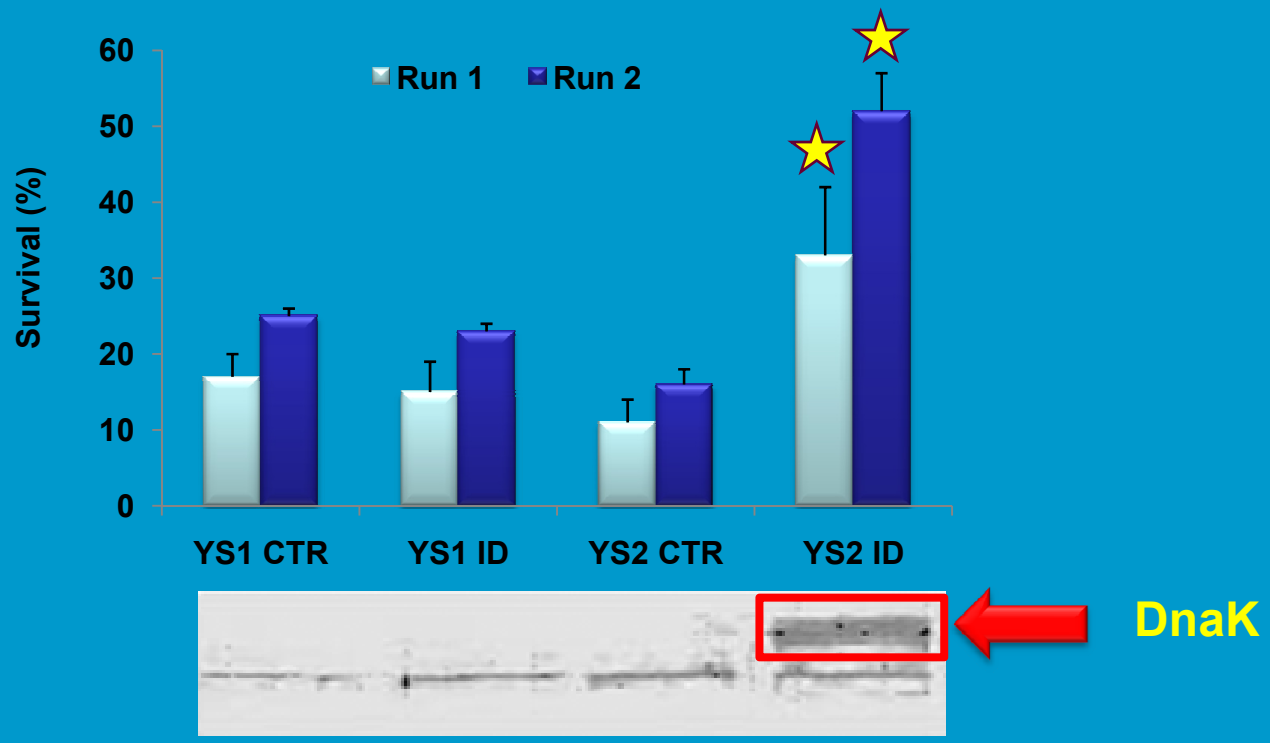
Survival after Vibrio challenge



- Survival of *Artemia* larvae fed either induced or non-induced strain YS1 was low, results similar to those obtained with non-induced YS2

- A significant 2 to 3-fold increase in survival occurred when larvae fed with arabinose-induced YS2 were exposed to *V. campbellii*

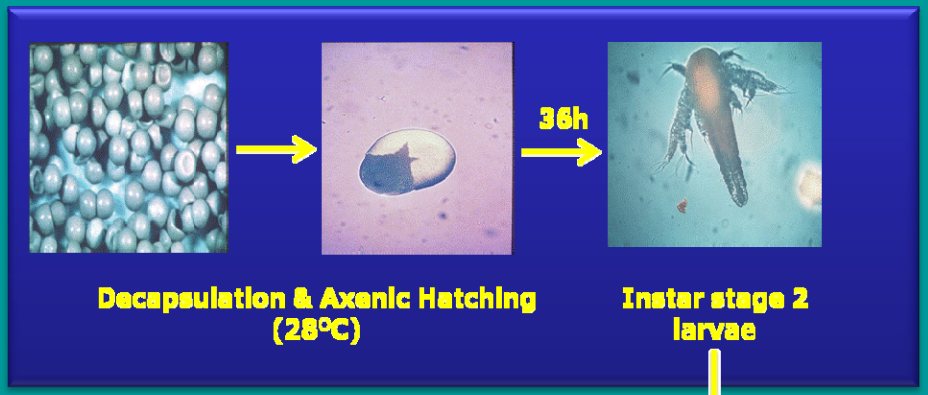
Correlation of enhanced resistance vs. DnaK accumulation in bacteria

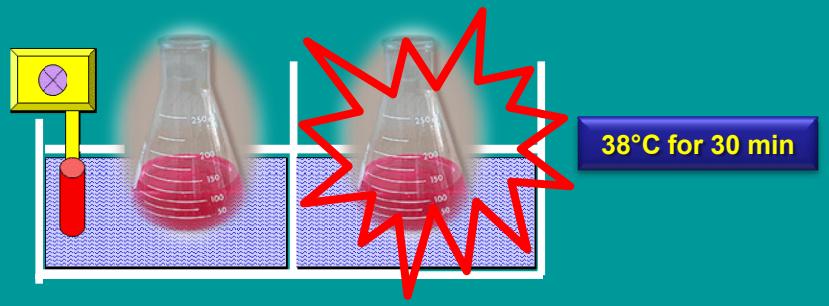


- DnaK accumulation correlated with enhanced survival of *Artemia*

- Clearly, protection against vibrio challenge is improved by DnaK, although the possibility that other Hsps have this capability is not discounted

Experimental design





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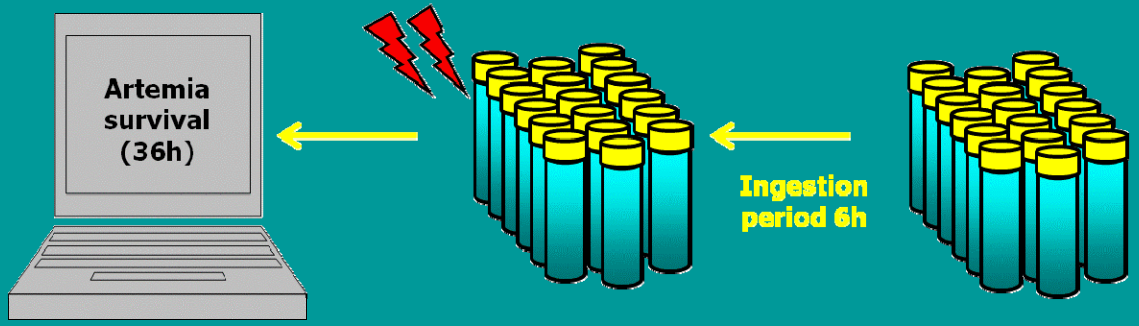
Vibrio challenge (10^7 cells/ml)



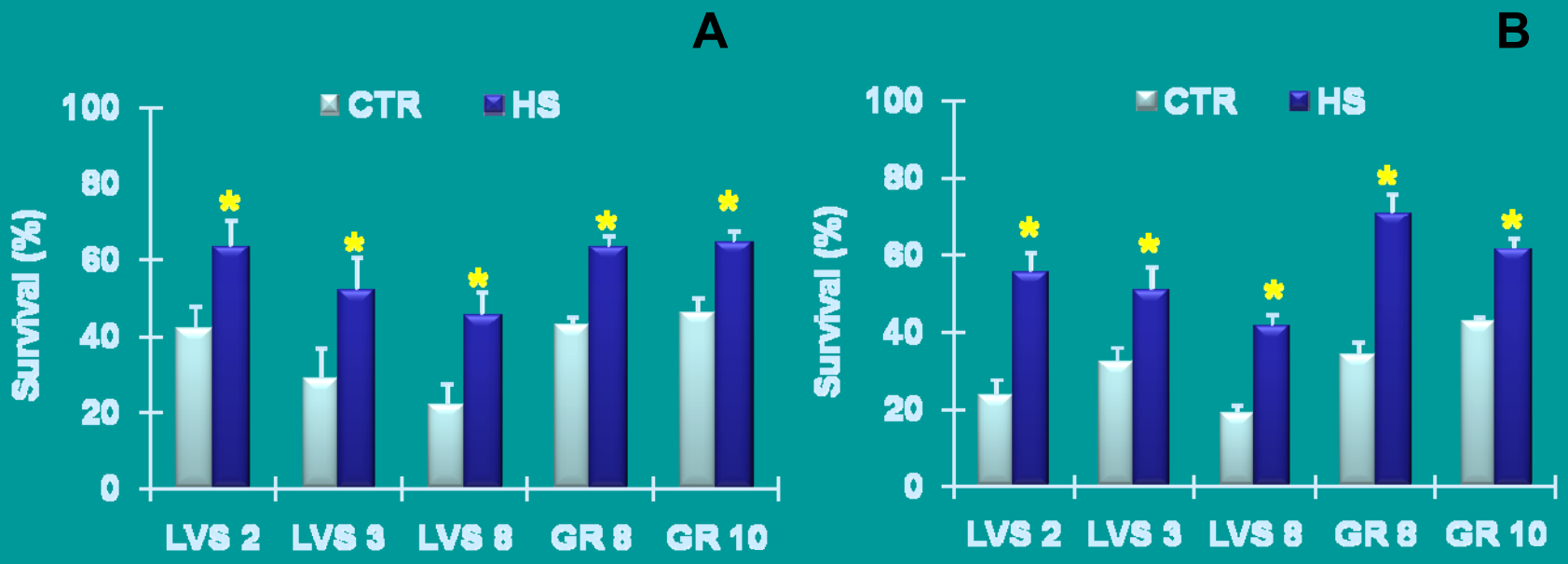
Bacteria	Strain
Bacillus sp.	LVS 2
Aeromonas hydrophila	LVS 3
Vibrio sp.	LVS 8
Cythophaga sp.	GR 8
Roseobacter sp.	GR 10

DnaK expression by SDS-PAGE & Western Blot

DnaK quantification by ELISA

Results

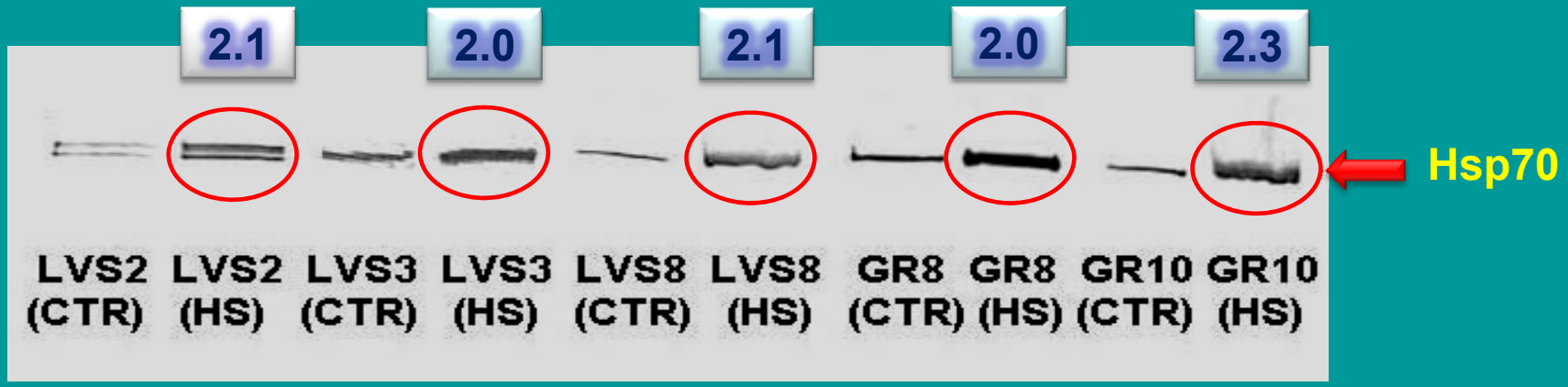
1. Survival after Vibrio challenge



- Feeding HS as opposed to non-HS bacteria significantly increased survival upon exposure to *V. campbellii*

- Protection occurred in all HS bacterial strains employed

3. DnaK accumulation in bacteria



- Western blot revealed that HS increased DnaK production by bacteria

- Quantification by ELISA demonstrated that DnaK increased from 2.0 - 2.3 fold in heated bacteria

- Higher amounts of DnaK in HS bacteria correlated with enhanced ability to promote survival of *Artemia* larvae

Summary

- Feeding gnotobiotic *Artemia* with *E. coli* over-producing different prokaryotic Hsps (DnaK-DnaJ-GrpE) increased larval resistance to *V. campbellii*

- A definitive role for DnaK was demonstrated by feeding *Artemia* larvae with transformed bacteria over-producing only this protein (YS2), with survival augmented approximately 3-fold after pathogenic vibrio exposure

- Immunoprobing of western blots showed that enhanced resistance to *V. campbellii* correlated with DnaK production in *E. coli*

- Exogenous Hsps possibly trigger the *Artemia* innate immune response, producing anti-inflammatory substances which suppress infection

GENERAL CONCLUSIONS

- Gnotobiotic systems allow to test novel strategies for disease abatement in larviculture
- Potential treatment that deserve to be tested in non-gnotobiotic environments are
  - Quorum sensing interference
  - polyhyroxybutyric acid
  - Yeast cell wall bound glucan
  - Heat shock proteins