



MARICOLTURA DI ROSIGNANO SOLVAY SRL

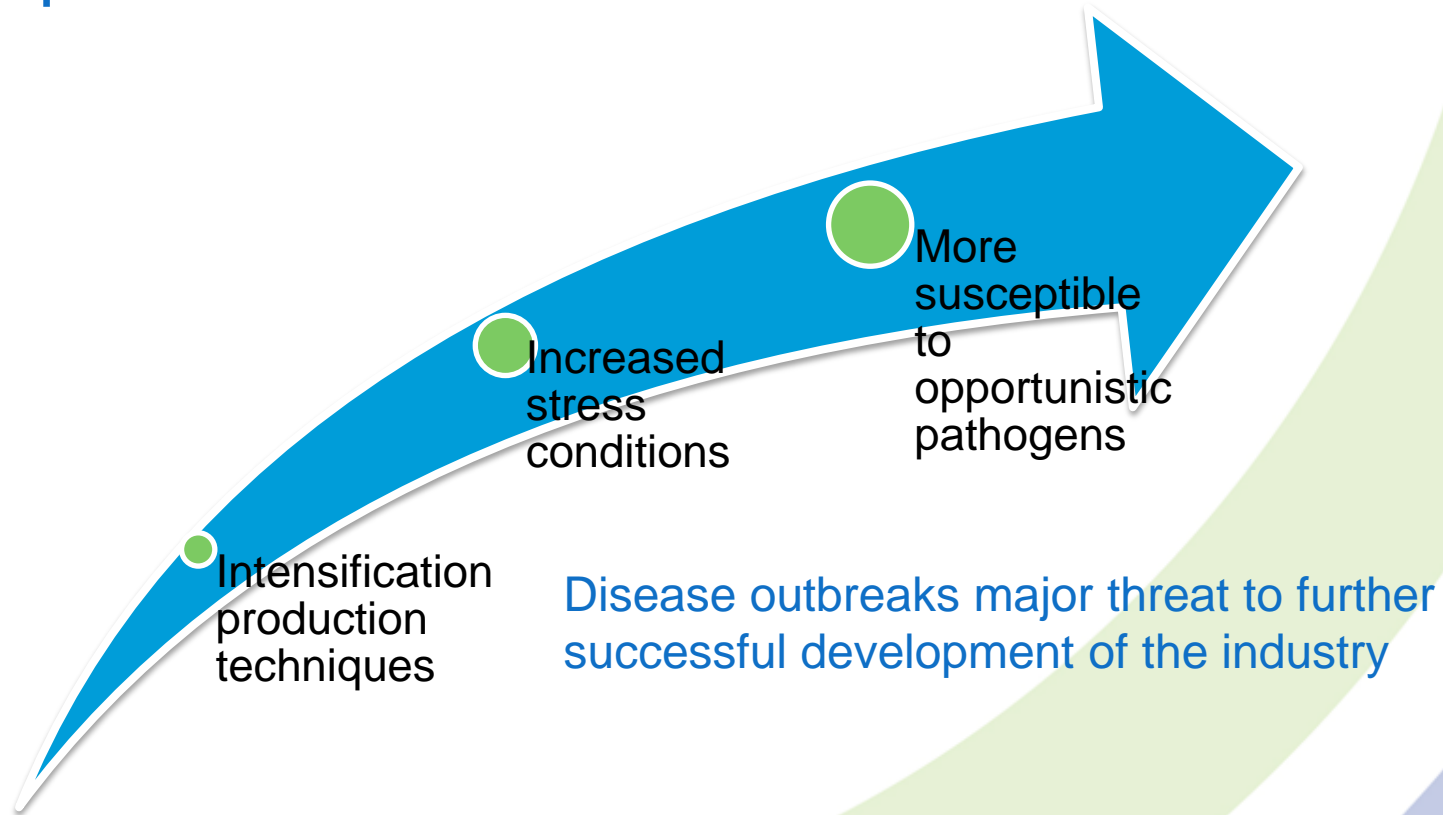


AquaTnet Workshop Microbial Management at Hatchery level

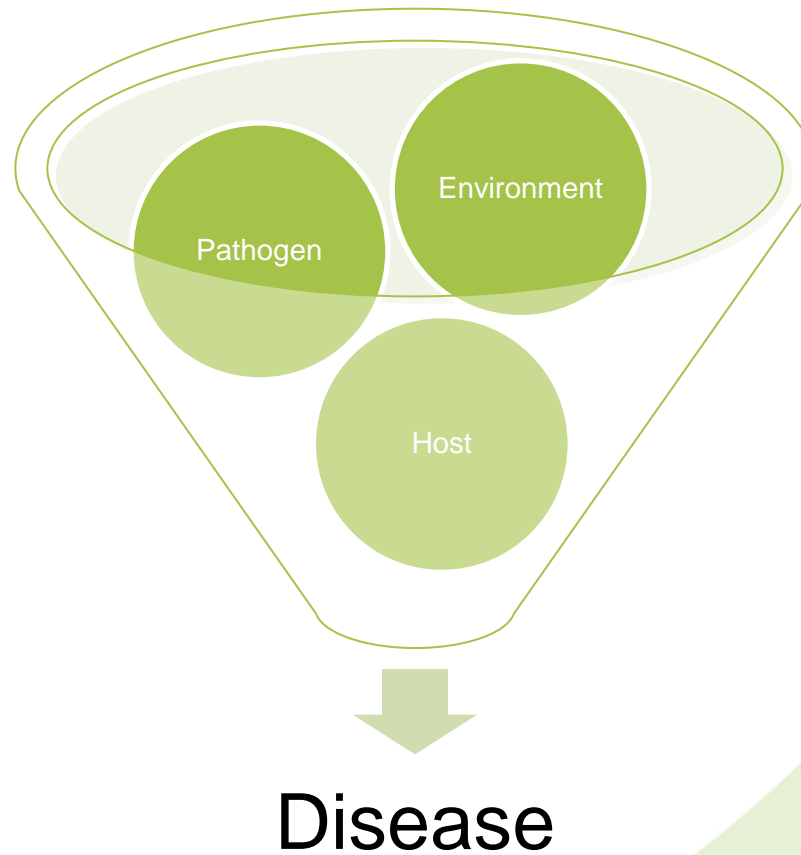
Maricoltura di Rosignano Solvay
T. De Wolf and co-workers

Introduction

Aquaculture



Challenges in disease control



Biosecurity

- “...sets of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another...” (Lotz, 1997).
- “An effective biosecurity program requires an understanding of the aquaculture production systems, the principles of disease transmission, the biology of the reared animals, the advantages and limitations of the products used and management techniques adopted.” (Moriarty & Decamp, 2009)
- It includes prevention, control and contingency planning.

Outline

1. Environment control

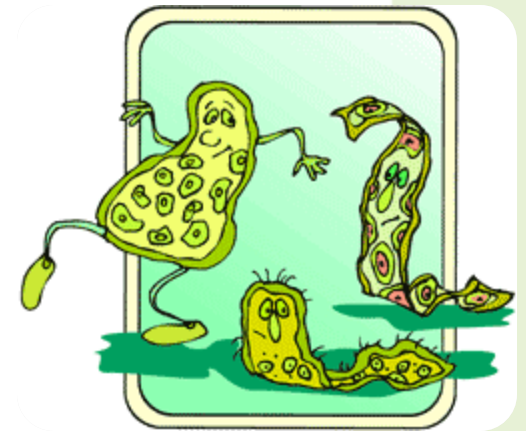
- Water treatment system
- General hygienic measures in the hatchery
- Colonisation of the environment with probionts

2. Control of pathogens

- Hygiene (cleaning procedures and disinfection)
- Treatment of biological material (eggs)
- Treatments on larvae/fish
- Treatments on live food (disinfection, *Vibrio* reduction, SURE, ACE)

3. Host:

- Apply correct rearing techniques
- Support the animal/metabolism/immune system through:
 - Probionts
 - Immunostimulants
 - Nutraceutical enrichments (DHA/asta/ Vit C,.....)





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ENVIRONMENTAL CONTROL



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1. Environment control

General hygienic measures

Cleaning procedures and disinfection

- Water treatment
 - physical filtration
 - filtration up to micrometers
 - UV - ozone
 - chemical disinfection
- Tank preparation and cleaning of devices:
Rinsing – Washing - Disinfecting
- Compartmentalisation
- Disinfecting baths
 - Iodophores
 - Chlorine
 - Chloramine
 - Glutaraldehydes
 - ...



1. Environment control

Correct management of live food production

- Aeration systems / Vorticella traps
 - placement / quantity of airstones
 - “Scotch brite” traps
- Harvesting & rinsing methods
 - concentrator / rinser
 - disinfection baths for filter equipment



1. Environment control

Colonisation of the rearing environment with probionts:

- Rearing live food and fish in a controlled microbiological environment by use of probionts
- Critical: choosing the right probiont for Aquaculture purpose



1. Environmental Control

Characteristics of suitable probionts

- Selection on origin
 - Natural occurrence for the fish
- Selection on safety
 - Not pathogens of animals or humans!
- Selection on functional properties
 - Adapted to production environment (temp; 0-40ppt;...)
 - Increased immunity
 - Active against many fish bacterial pathogens
 - Improving water/pond bottom quality; waste reduction
 - Control over blue-greens and off-flavour
 - Improving growth rate and FCE
- Technological properties
 - Cost-effective manufacture under industrial conditions
 - Good stability and long shelf life

Saarela et. al. Probiotic bacteria: safety, functional and technological properties. J. Biotech. (2000) 84: 197–215



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CONTROL OF PATHOGENS



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2. Control of pathogens

*The physical, chemical and biological methods necessary to protect the hatchery from the consequences of all diseases that represent a high risk will vary with the reared **species**, the animal **stage**, and also with the rearing **conditions***

- Prevention

Many possible sources of entry for a pathogen into an aquaculture facility:

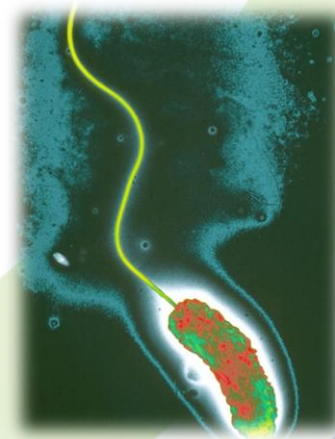
- broodstock, eggs (quarantine areas)
- contaminated water or feed (freeze fresh feeds for broodstock)
- staff
- equipment

- Treatment of biological material (eggs):

- Iodophore based and formalin
- UV or ozone treatment

- Treatments on larvae/fish:

- Formalin bath
- Peracetic acid



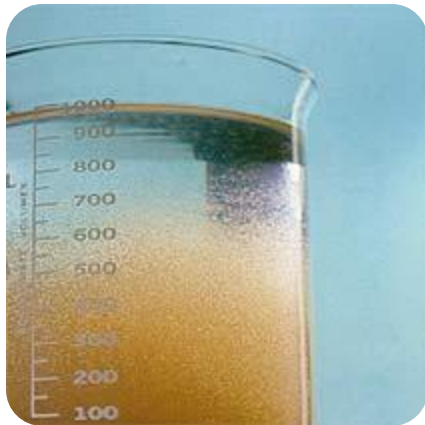
2. Control of pathogens

Live food – problem sketch

- Development of microbial flora in larval gut coincides with commencement of feeding on rotifers. (Munro *et al.* 1995; Reid *et al.* 2009)
- During rotifer feeding stage microbial development has greater influence than lipid nutrition on survival and growth of larvae. (Battaglione *et al.* 2006)
- Despite obvious advantages of improved survival rates, use of antibiotics during production of larval fish is undesirable (Munro *et al.* 1993)
 - development of antibiotic-resistant strains & possible environmental consequences
 - legal constraints in the use of antibiotics

2. Control of pathogens

- Treatments on live food (disinfection, *Vibrio* reduction, SURE, ACE)
 - Adequate culture techniques
 - Importance of harvesting/rinsing techniques
 - Specific *Vibrio* reducers:
 - Example 1: SURE application for *Vibrio* reduction in rotifers
 - Example 2: ACE application for *Vibrio* reduction in Artemia



2. Control of pathogens

- Rotifer culture systems



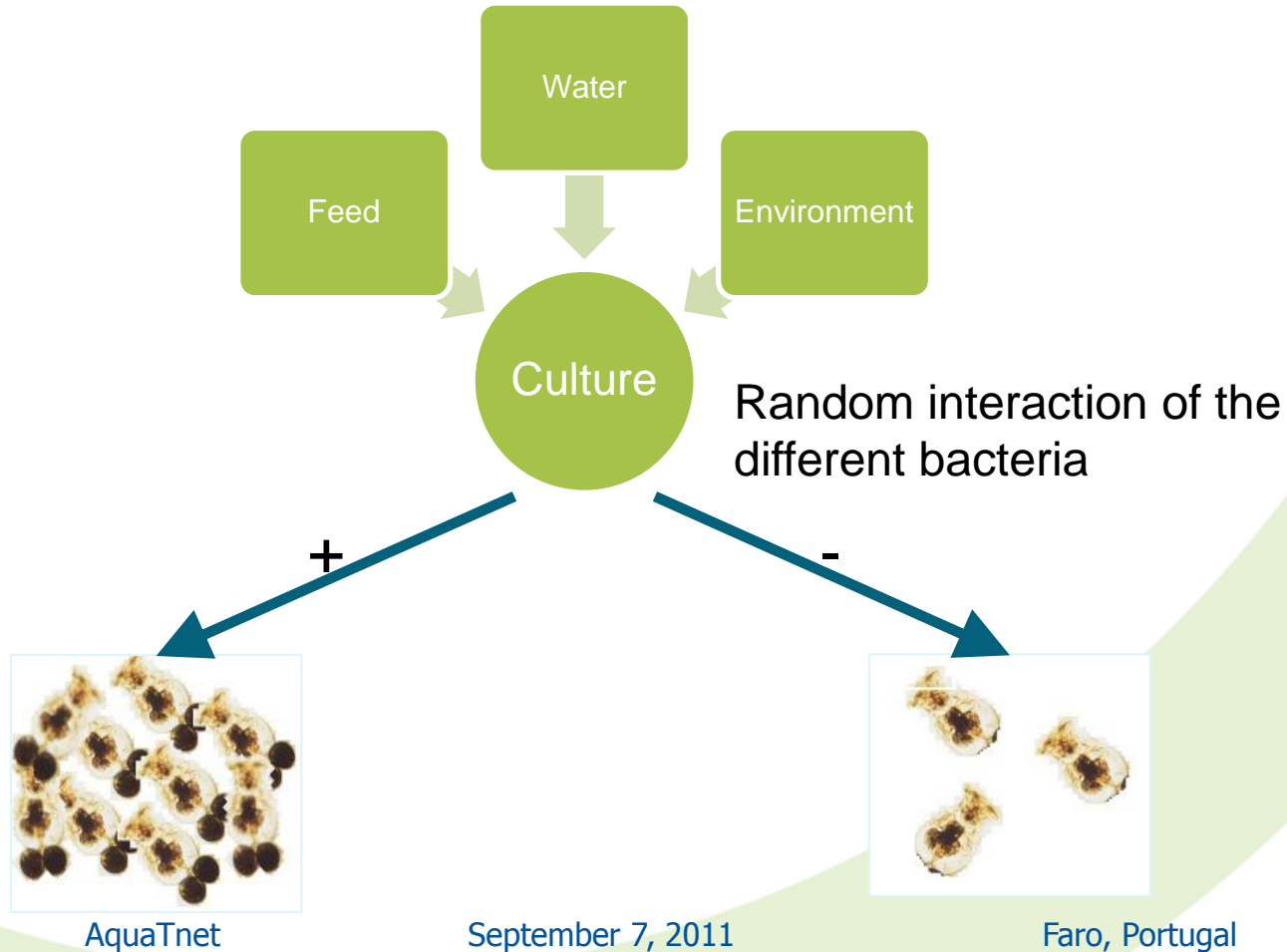
Batch culture



Recirculation
Flow-through

2. Control of pathogens

- Rotifer culture systems

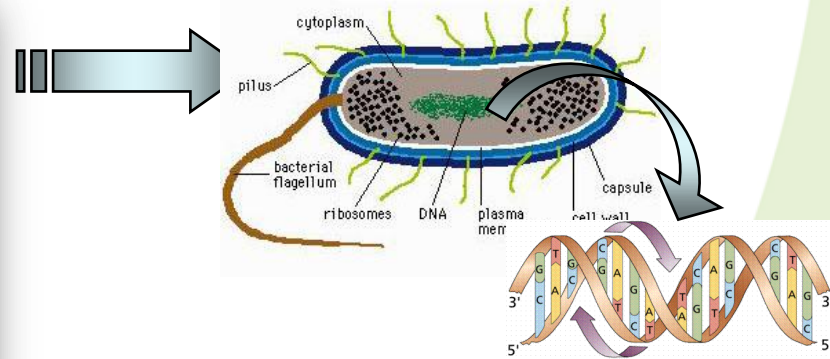


DGGE

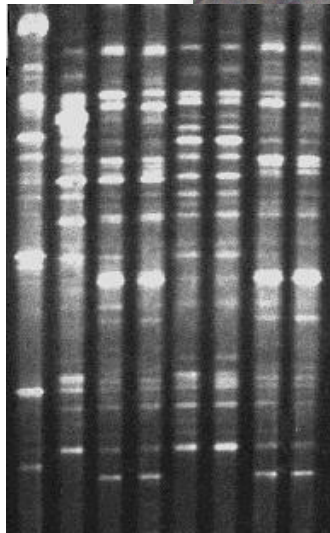
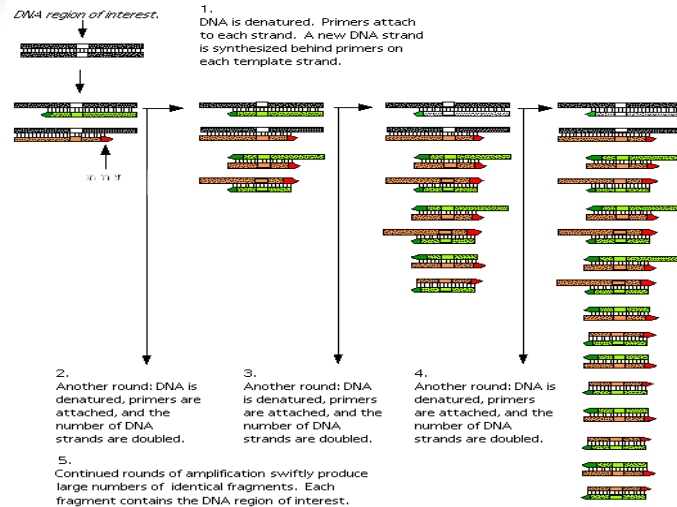
Sampling culture water



DNA extraction

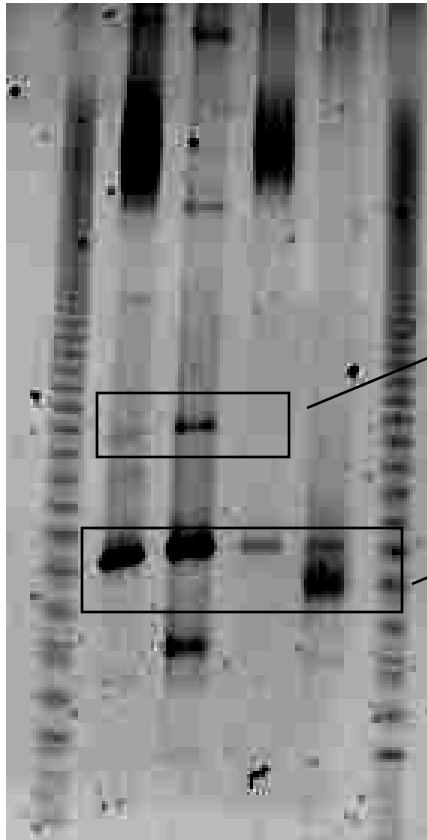


POLYMERASE CHAIN REACTION



DGGE

DGGE profile – Batch culture



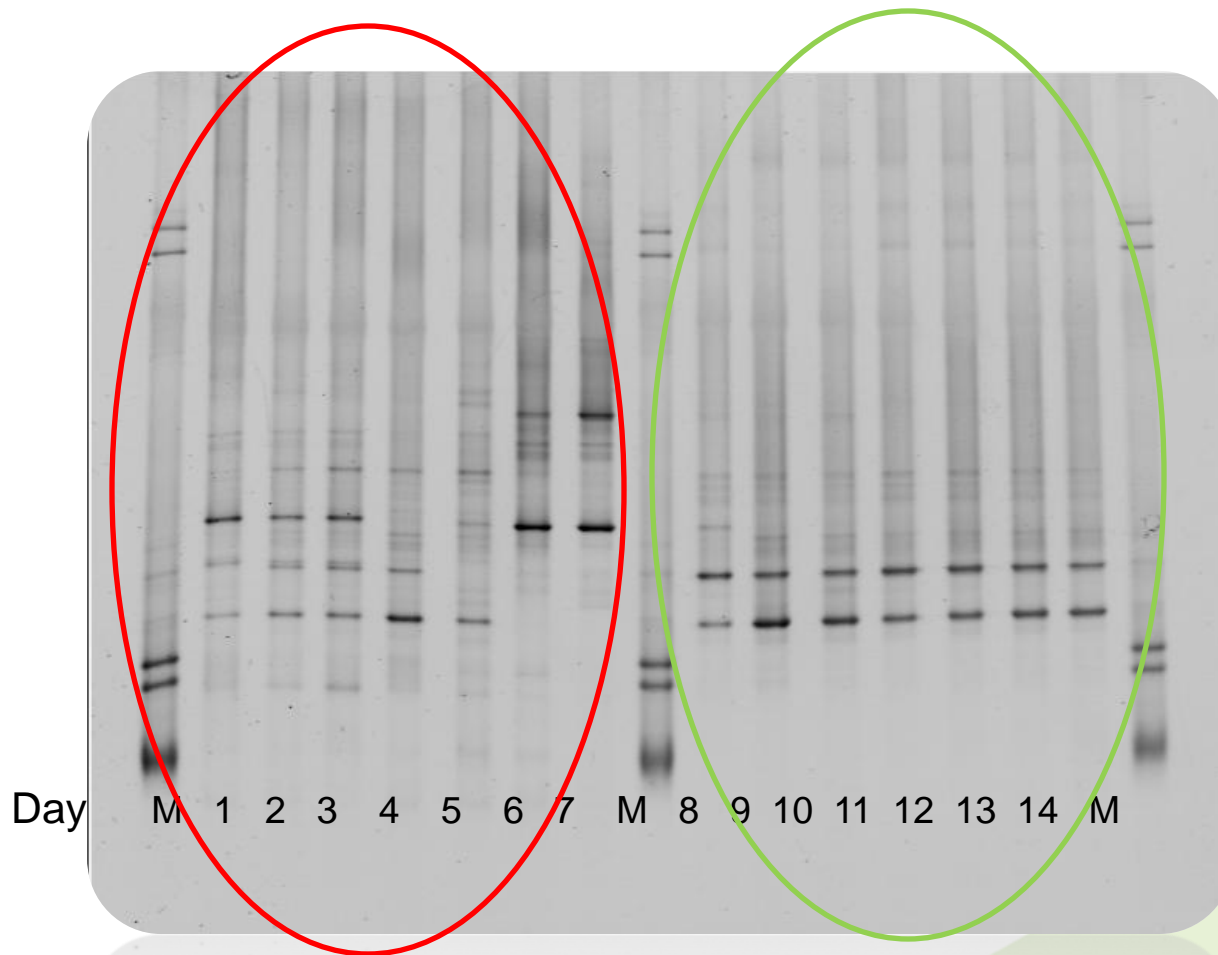
Some dominant bacteria disappear from the culture water

Some dominant bacteria remain for the whole batch culture period

The environment selects!

R d0 d1 d2 d3 R

DGGE profile – recirculation experiment



2. Control of pathogens



Example 1: SURE application for *Vibrio* reduction in rotifers

- Sanocare SURE is a water conditioner, containing herbal ingredients, that can be used at the end of a standard rotifer culture or enrichment
- Application: directly in the rotifer tank or in the concentrator/rinser



2. Control of pathogens

Example 1: SURE application for *Vibrio* reduction in rotifers

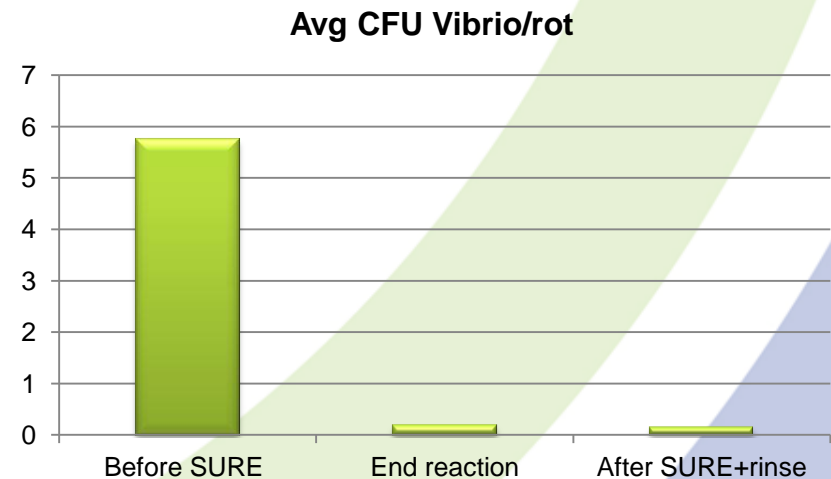


- Benefits:
 - Reduced bacterial load in rotifers and surrounding water, strong *Vibrio* suppression



Resulting in:

- Improved rearing environment for larval fish
- More reliable production of fish larvae



2. Control of pathogens

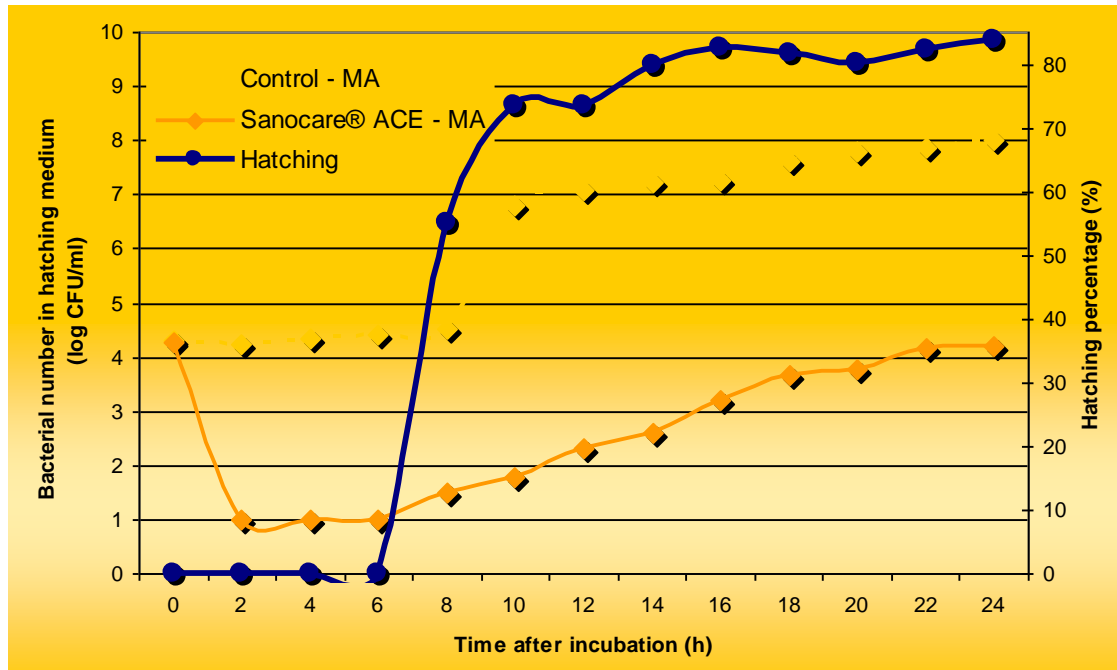


Example 2: ACE application for *Vibrio* reduction in Artemia

- Sanocare ACE is a water conditioner that can be used during hatching and enrichment of *Artemia* in order to decrease the bacterial load
- Application: directly in the *Artemia* tank
- Benefits:
 - **Reduced bacterial load**
 - Increased viability of the nauplii
 - Improved (artemia) tank conditions

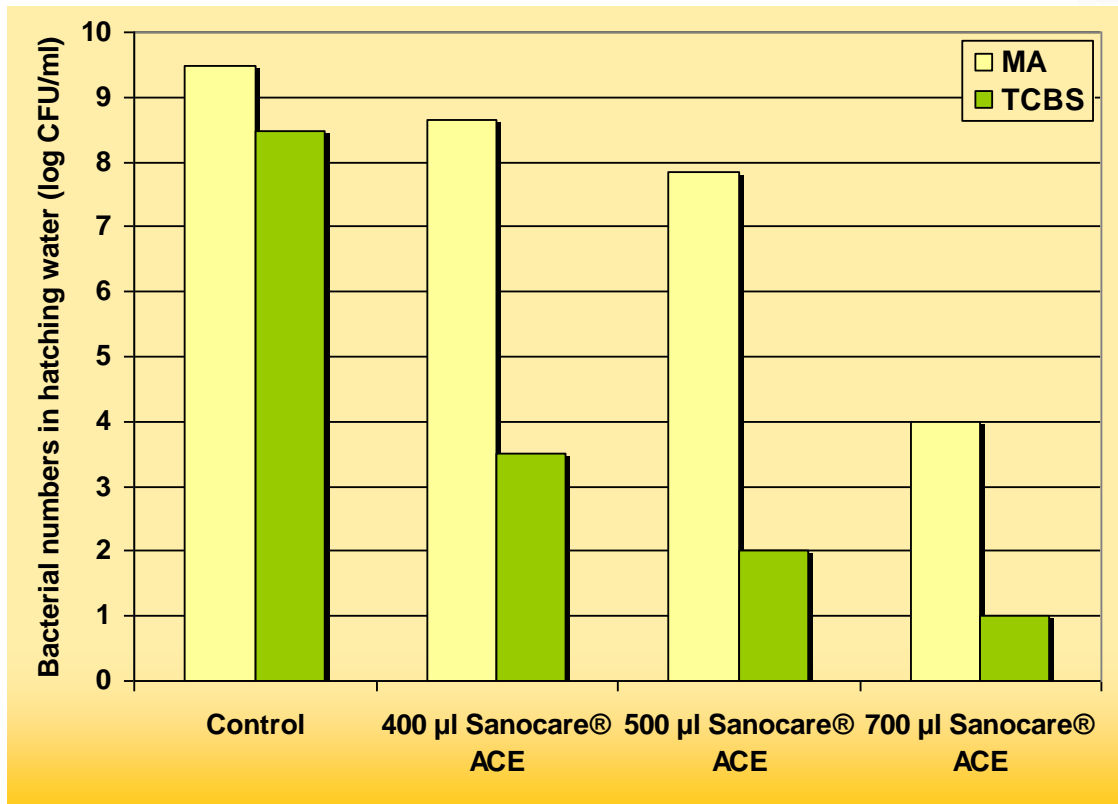
2. Control of pathogens

The bacterial development of heterotrophic marine bacteria during the hatching of *Artemia* nauplii in the presence and absence of Sanocare® ACE



2. Control of pathogens

Use of ACE during hatching of Artemia





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HOST ORGANISM



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3. Host

Applying a correct tank management and correct feeding strategies are of crucial importance



3. Host



Support the host organism through the use of nutraceuticals in order to improve the metabolism and boost the immune system

Category of nutraceuticals used in aquaculture:

- HUFAs
- Vitamins (C, E, D, A,.....)
- Antioxidants (astaxanthine, Se,.....)
- Immunostimulants (beta-glucanes, trace elements,.....)
- **Probiotics**
- ...

3. Host

Probiotics in Aquaculture to support the host organism



- **Disease control**
- **Gut microflora colonization**
- **Production of exo-enzymes**
- **water quality improvement**

Mix of selected *Bacillus* species:
Bacillus subtilis, *B. licheniformis*,
B. pumilus

Use: directly in the water or through the live food chain

3. Host

Probiotics in Aquaculture to support the host organism

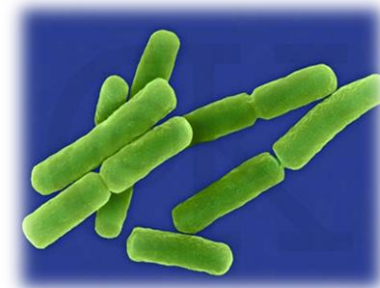
Active against many pathogens

- Directly by growth inhibition



3. Host

Fish pathogens directly inhibited by Sanolife
Bacillus strains



Fish species	Pathogen
Japanese flounder (<i>Paralichthys olivaceus</i>)	<i>Vibrio</i> spp.
Gizzard shad (<i>Konosirus punctatus</i>)	<i>Vibrio</i> spp.
Gilthead seabream (<i>Sparus aurata</i>)	<i>Vibrio harveyi</i>
	<i>Vibrio proteolyticus</i>
	<i>Vibrio</i> spp.
Rainbow trout (<i>Salmo gairdneri</i>)	<i>Listonella anguillarum</i>
Herring (<i>Clupea harengus</i>)	<i>Photobacterium illopiscarum</i>
European seabass (<i>Discentrarchus labrax</i>)	<i>Vibrio</i> spp.
Turbot (<i>Scophthalmus maximus</i>)	<i>Vibrio</i> spp.
Pacific cod (<i>Gadus macrocephalus</i>)	<i>Vibrio</i> spp.
Atlantic salmon (<i>Salmo salar</i>)	<i>Vibrio</i> spp.
Coho salmon (<i>Oncorhynchus kisutch</i>)	<i>Vibrio ordali</i>
Croaker (<i>Micropogon opercularis</i>)	<i>Vibrio</i> spp.
Tilapia (<i>Oreochromis niloticus</i>)	<i>Streptococcus</i> spp.
Channel catfish (<i>Ictalurus punctatus</i>)	<i>Edwardsiella</i> sp.

3. Host

Probiotics in Aquaculture to support the host organism – gut microflora colonisation

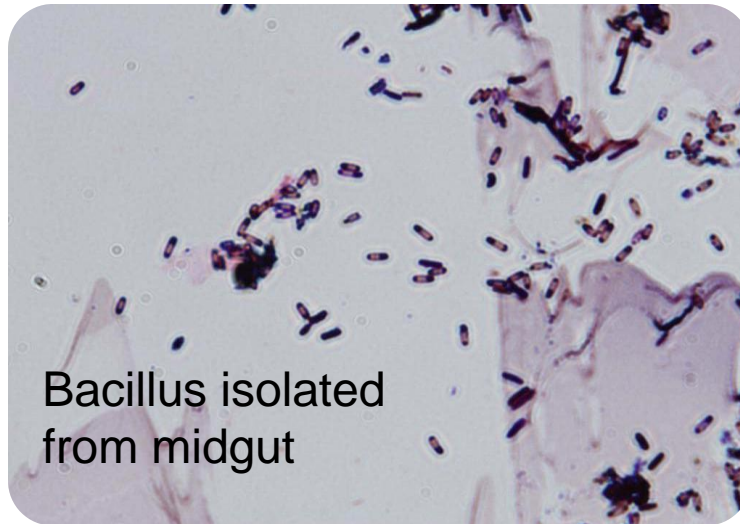
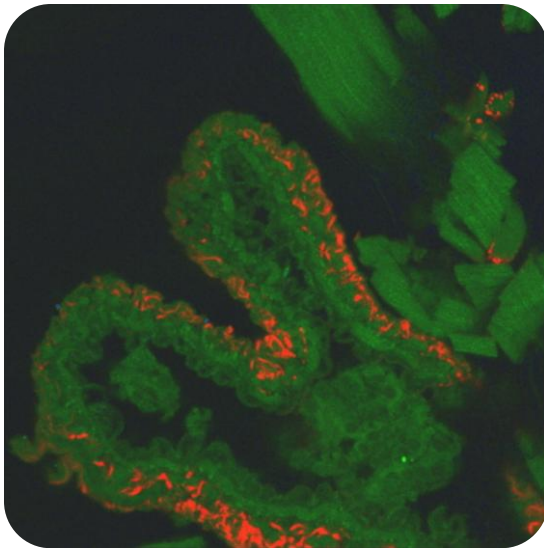


Active against many pathogens

- Indirectly by competitive exclusion

Nutrients

Attachment sites





3. Host

Probiotics in Aquaculture to support the host organism – production of exo-enzymes



Ghent, November 2006

REPORT ON ENZYMATIC ACTIVITY OF SANOLIFE BACILLUS STRAINS

Trials carried out at the Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, under contract research for INVE Technologies SA, Dendermonde, Belgium and coordinated by Dr Olivier Decamp.

OBJECTIVE:
To confirm the production of enzymes by Bacillus strains provided by INVE Technologies.

Benefit on food assimilation



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Conclusions

- Biosecurity and hygiene remain the most important tools to avoid pathogen introduction
- Assure adequate nutritional condition of the live food and fry through correct feeding strategies
- Apply best management practices in the hatchery (tank management, fish and live food handling)
- Use of nutraceuticals can improve the long term health status of your fish



Thank You!

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