

# AQUA TECHNA

*Role of essential oils to replace antibiotics in European feed industry and Effect of Aquaviance on the pathogenic flora*



# Plan

- ⇒ Antibiotic Growth Promoters( AGP) ban in European context
- ⇒ Essential Oils : Generalities and background
- ⇒ Study of Aquaviance efficiency against fish pathogenic bacteria strains
- ⇒ Aquaviance : an innovative technology
- ⇒ Analytical control



# European context

⇒ 1997

Avoparcin ban

⇒ 1999

Virginiamycin and  
Bacitracin ban

⇒ 2006

CGPs free

⇒ OBJECTIVE : To find alternatives to AGP



# Essential Oils Generalities

## ⇒ DEFINITION

- ✓ any concentrated, hydrophobic liquid containing volatile aroma compounds from plants

## ⇒ MODE OF EXTRACTION

- ✓ Steam distillation, extraction with solvent

## ⇒ COMPOSITION

- ✓ Very variable
- ✓ Terpens, phenols, aldehydes, coumarins, *etc*



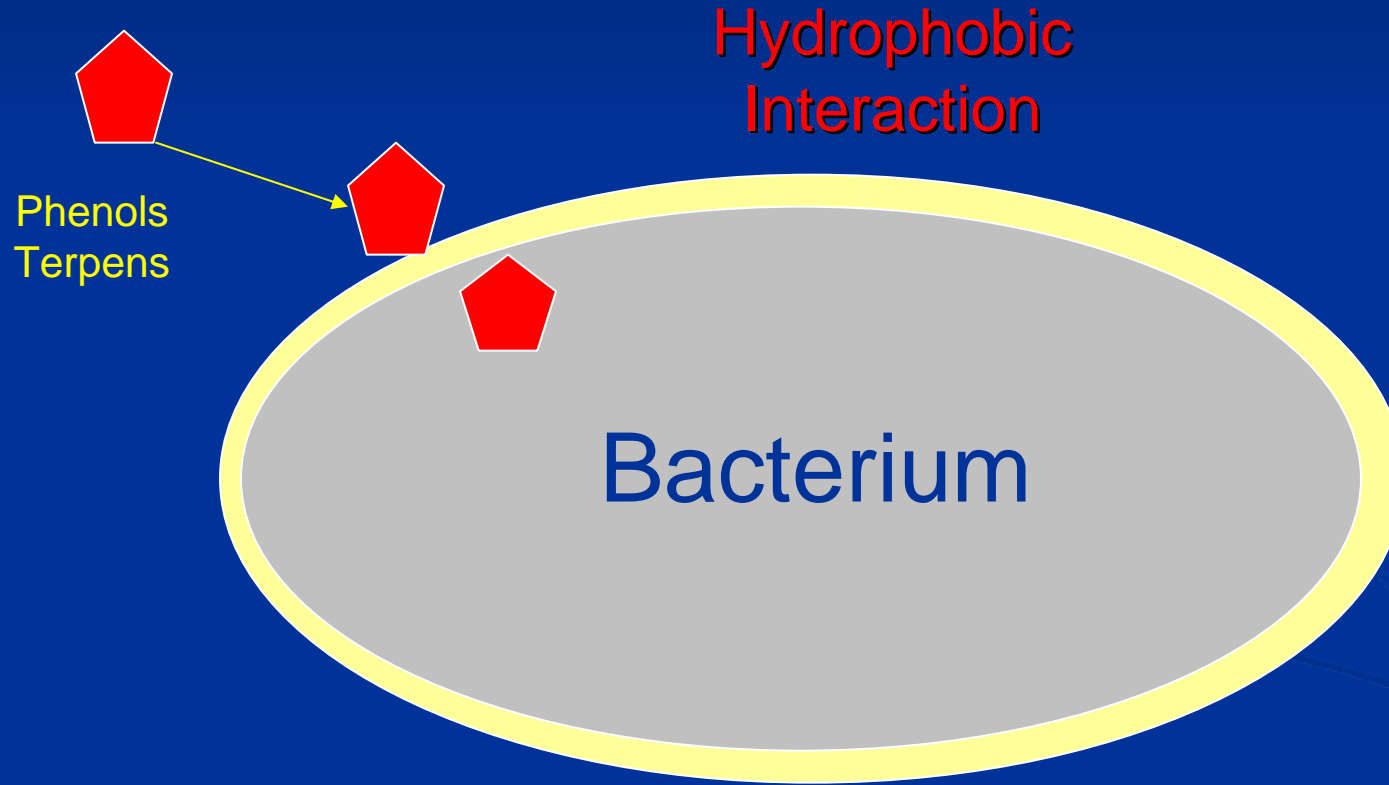
# Essential Oils Properties

- ⇒ Antibacterial
- ⇒ Antifungal activity
  - ⇒ Antiviral
- ⇒ Antiparasitic agent
  - ⇒ Antioxidant
- ⇒ Role on hepatic activities
- ⇒ Stimulation of digestive enzymes
- ⇒ Stimulation of immun system
  - ⇒ Aromatic properties
  - ⇒ *etc*



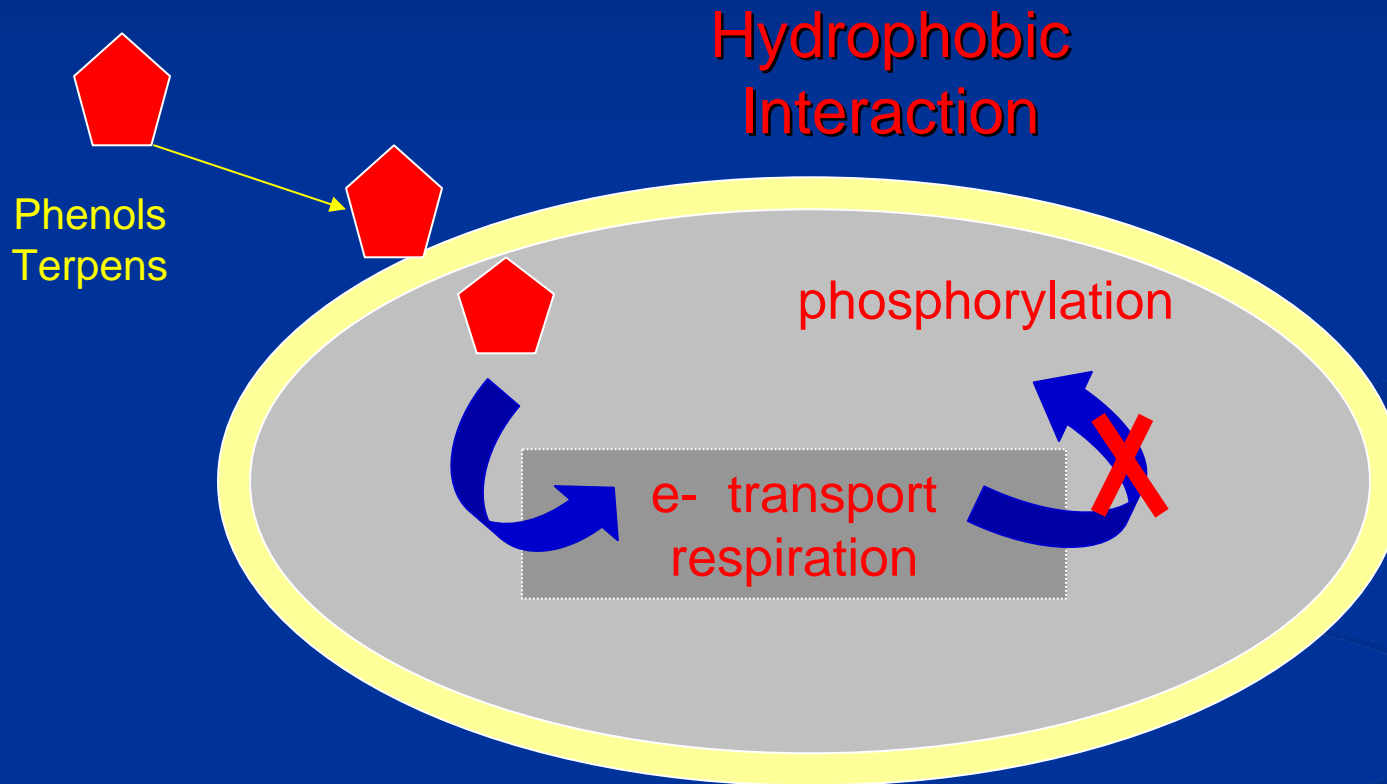
# Essential Oils

## Antibacterial mode of action (1)



# Essential Oils

## Antibacterial mode of action (2)



# Antibacterial properties against fish pathogenic bacteria

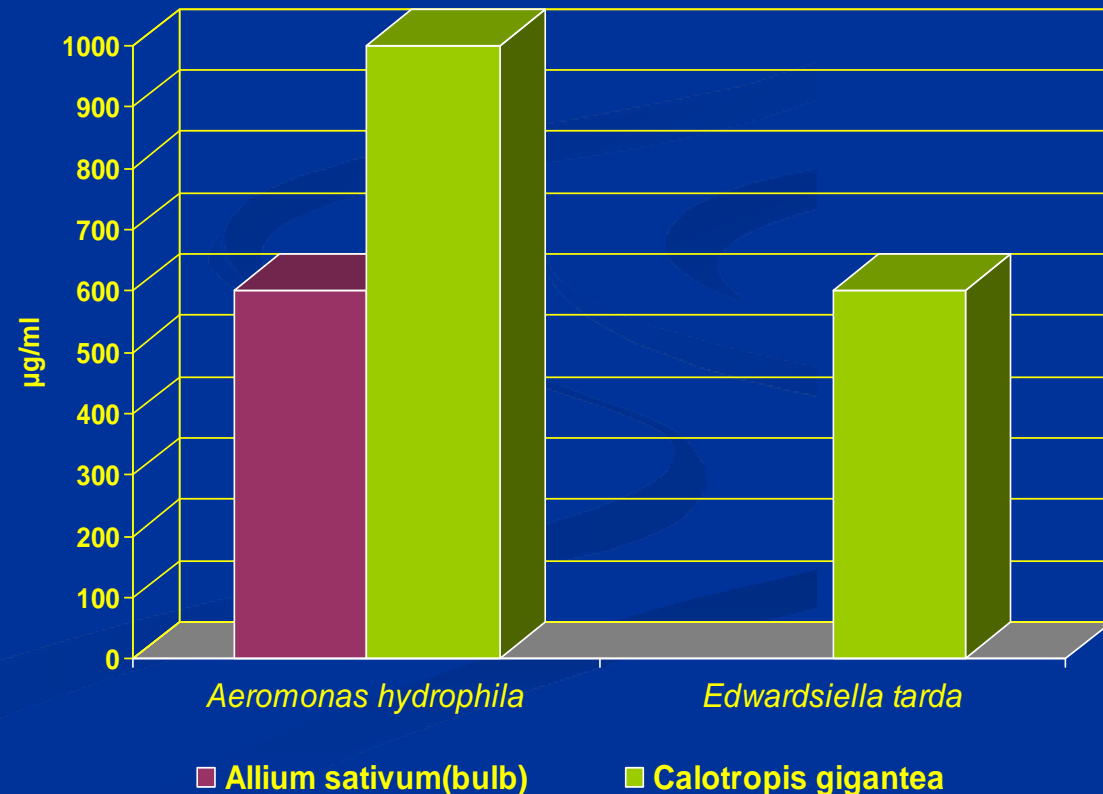
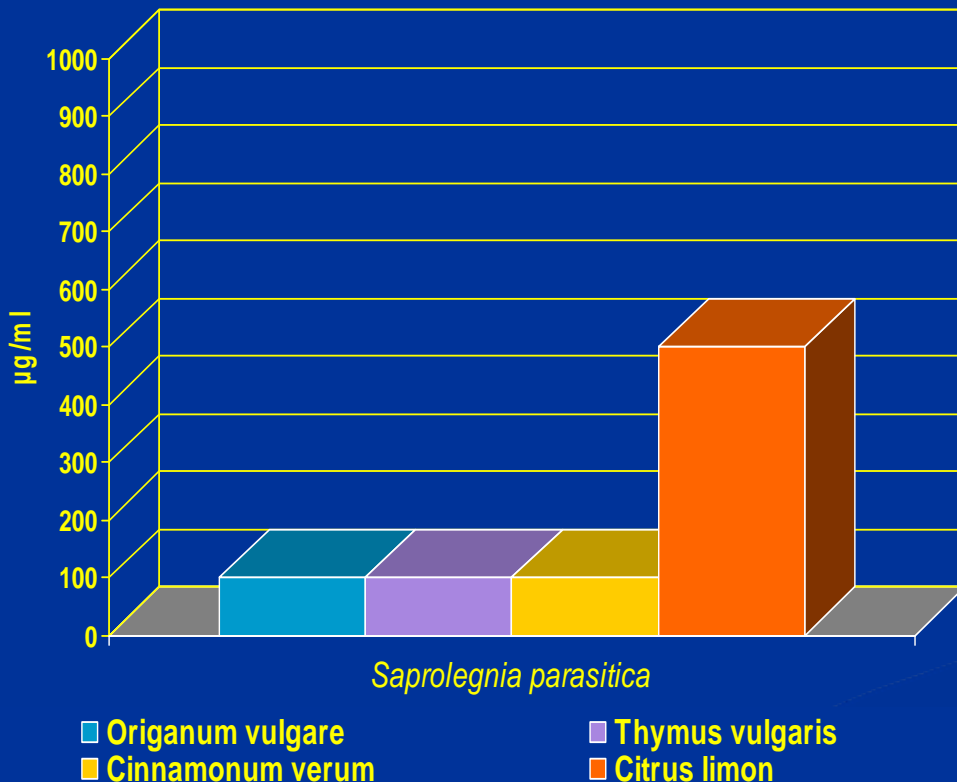


## Antibacterial properties of essential oils against *Saprolegnia parasitica* (Tampieri et al, 2003)

## Antibacterial properties of plant extracts against fish pathogens (Munizzaman et al, 2004)

- ⇒ *In vitro* trial
- ⇒ Minimum inhibitory concentrations of EO

- ⇒ *In vitro* trial
- ⇒ Minimum inhibitory concentrations of plant extracts against fish pathogenic bacteria ( $2 \cdot 10^7$  CFU /ml)

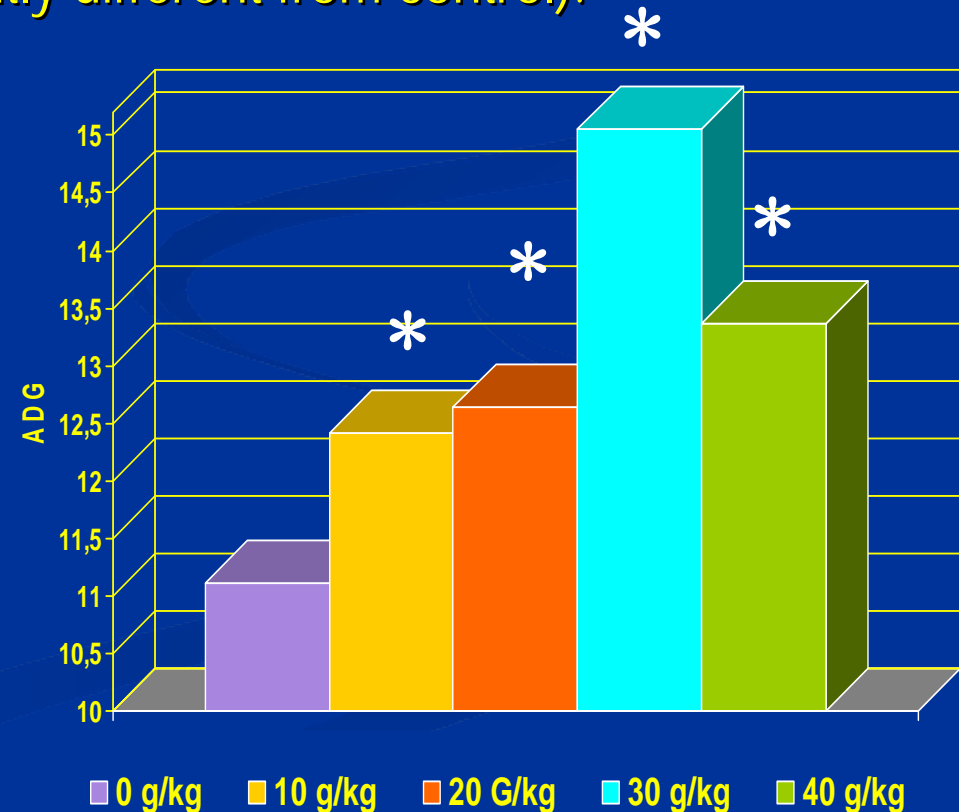
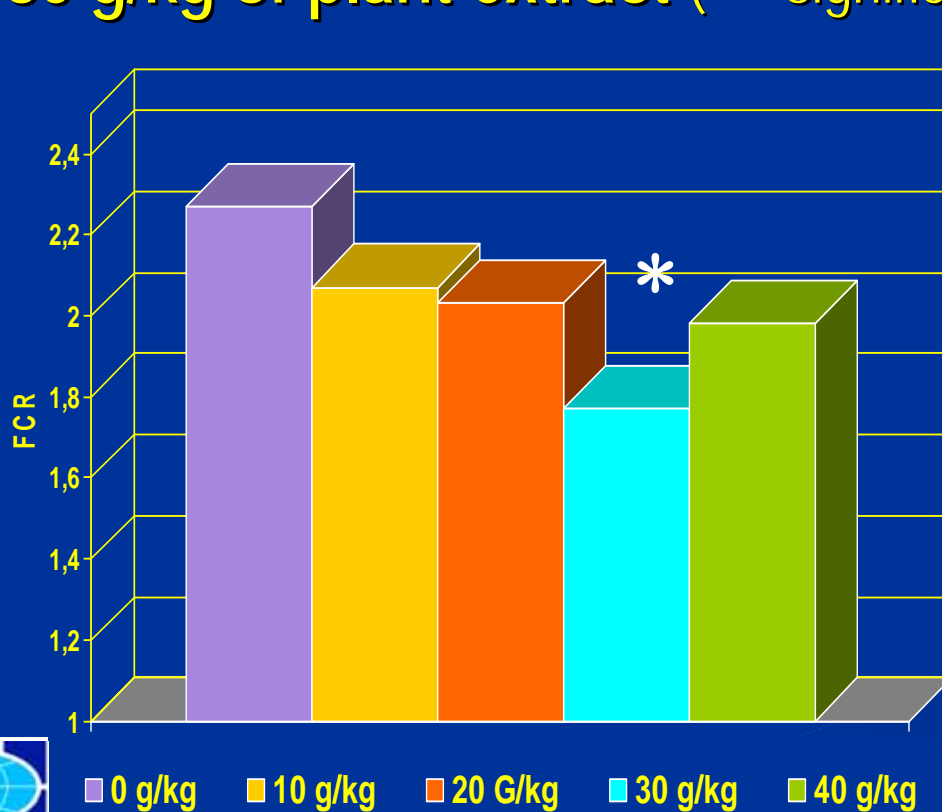


# Effect of plant extract on growth performance and survival of Tilapia



(Shalaby et al, 2005)

- ⇒ Test of a plant extract incorporated in fish feed at 4 concentrations during 90 days.
- ⇒ Improving of Feed Conversion Ratio and of Average Weight Gain with 30 g/kg of plant extract (\* = significantly different from control).



# Aquaviance efficiency against 4 fish pathogenic bacteria strains

- ⇒ Trial in collaboration with the National Veterinary School of Nantes
- ⇒ Study of Aquaviance antibacterial activity against 4 fish pathogenic bacteria strains and 1 reference strain for international antibiograms and the Techna reference strain :
  - ✓ *Edwardsiella tarda*
  - ✓ *Yersinia ruckeri*
  - ✓ *Aeromonas hydrophila*
  - ✓ *Vibrio anguillarum*
  - ✓ *Escherichia coli* ESCH1
  - ✓ *Escherichia coli* O78K80



# Characteristics of the tested bacteria

	Description	Reservoirs / Predisposing factors	Gross pathology
<i>Edwardsiella tarda</i> (Edwardsiellosis)	Gram- bacteria, motile, aero-anaerobic	In freshwater, high temperature, poor water quality, high organic content, high stocking density / In fish, reptiles and mammals	Spiralling movements, malodorous, gaseous lesions, mortality
<i>Vibrio anguillarum</i> (Vibriosis)	Gram- bacteria, motile, aero-anaerobic	In farm, aquarium, wild marine and estuarine fishes / Overfeeding, stress, instability of water temperature	Haemorrhages & ulcerations of the skin, fin, tail and in internal organs, mortality  Higher mortality in young fishes
<i>Yersinia ruckeri</i> (Enteritic Redmouth Disease)	Gram- bacteria, motile	Fish in fresh and seawater / Poor water quality, high density, water temperatures	Darkening & haemorrhages of the mouth, skin, anus & fins, mortality
<i>Aeromonas hydrophila</i> (Motile Aeromonas Septicemia)	Gram- bacteria, motile, aero-anaerobic	In farm and wild fishes / In worldwide brackish, fresh, estuarine, marine, chlorinated & unchlorinated water	Lack of appetite, swimming abnormalities, pale gills, skin ulcerations, haemorrhagic septicemia, mortality

# Aquaviance efficiency against 4 fish pathogenic bacteria strains



⇒ Determination of the MIC and the MBC for each strains of bacteria with different concentrations of Aquaviance:

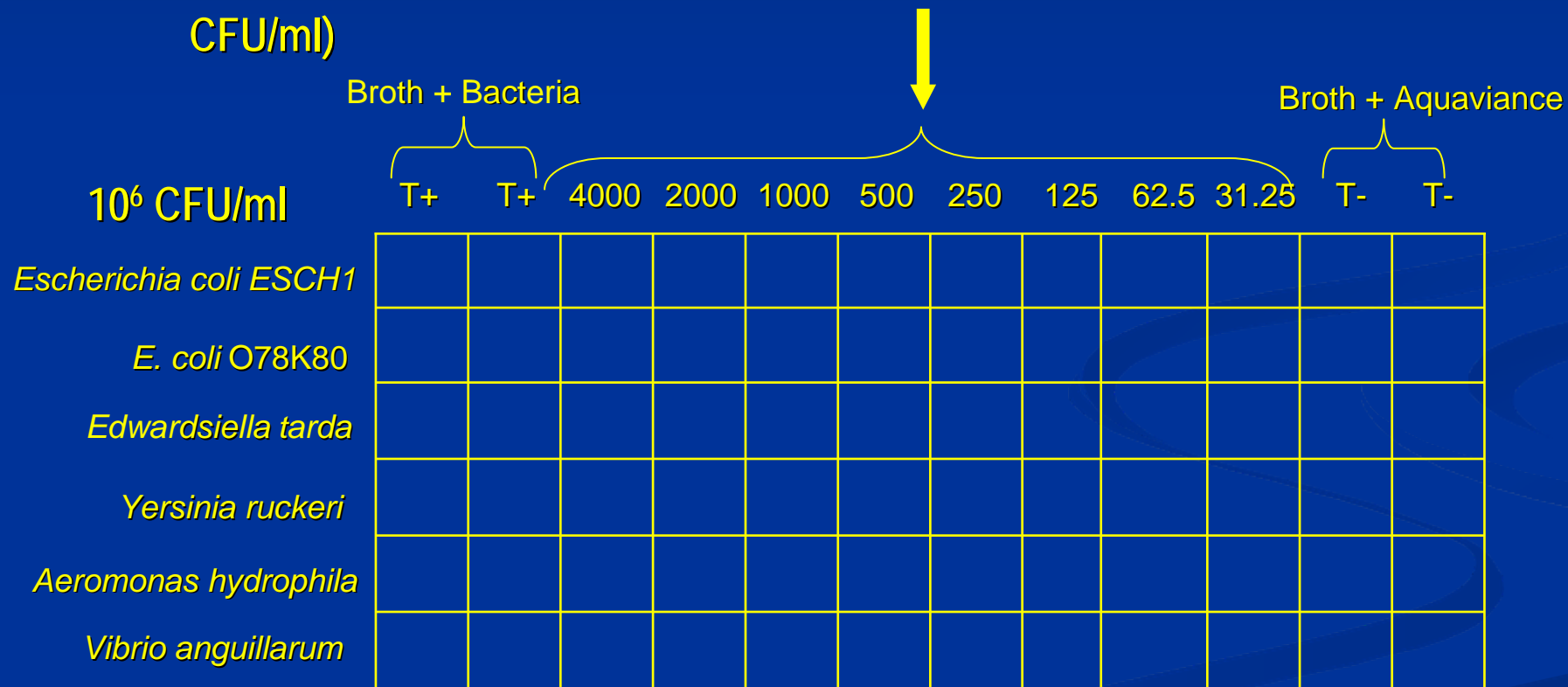
⇒ Definitions:

- ✓ MIC (Minimum Inhibitory Concentration) = the lowest concentration of Aquaviance to inhibit the growth of microorganisms up to 24h incubation.
- ✓ MBC (Minimum Bactericidal Concentration) = the lowest concentration of Aquaviance at which the initial inoculum is killed.

# Aquaviance efficiency against 4 fish pathogenic bacteria strains: method

## ⇒ Material and Method

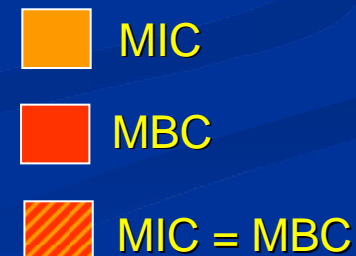
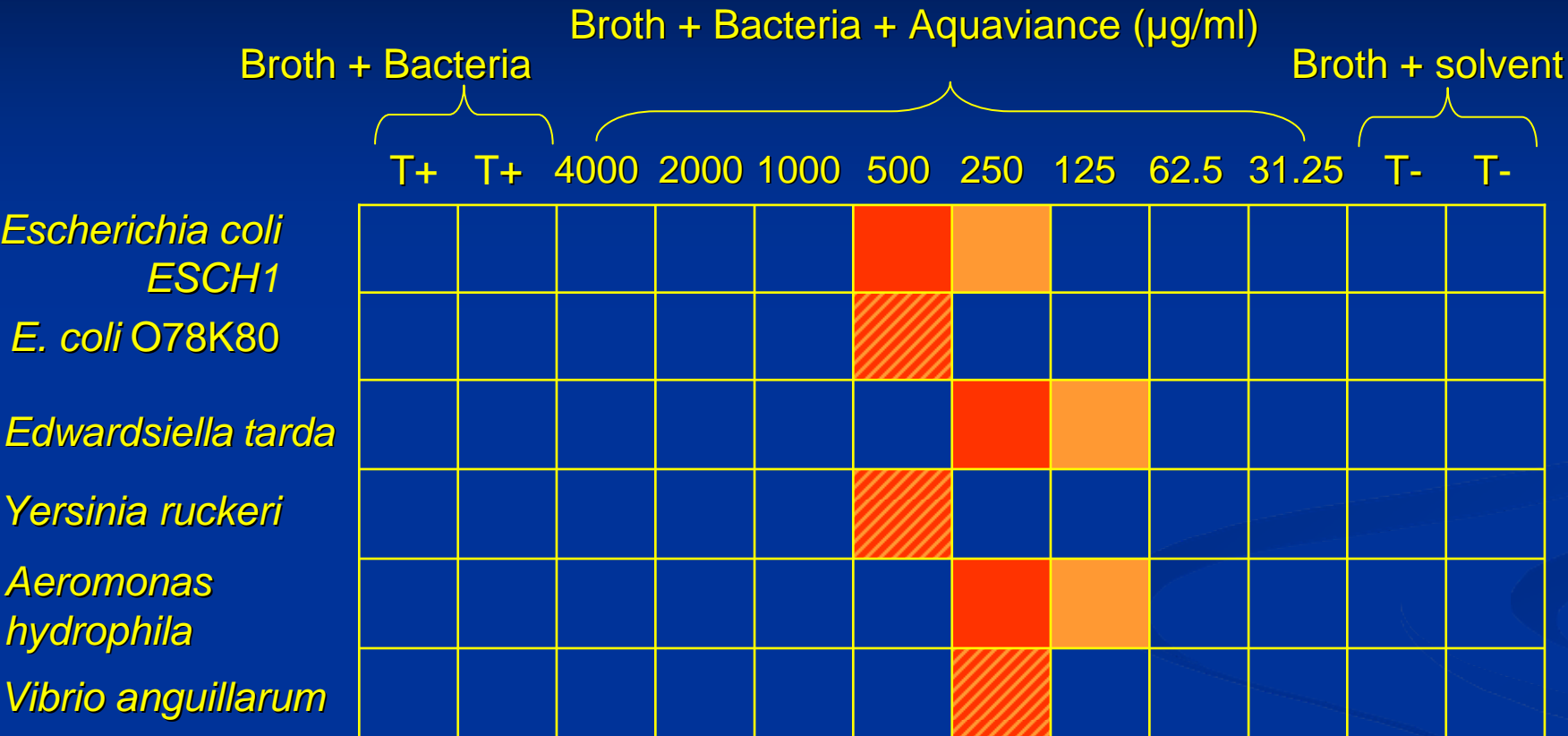
- ✓ Test of 8 concentrations of Aquaviance (from 4000µg/ml to 31.25 µg/ml ) in 96-well plates
- ✓ 50 µl of each concentration of Aquaviance + 50 µl of inoculum (10<sup>6</sup> CFU/ml)



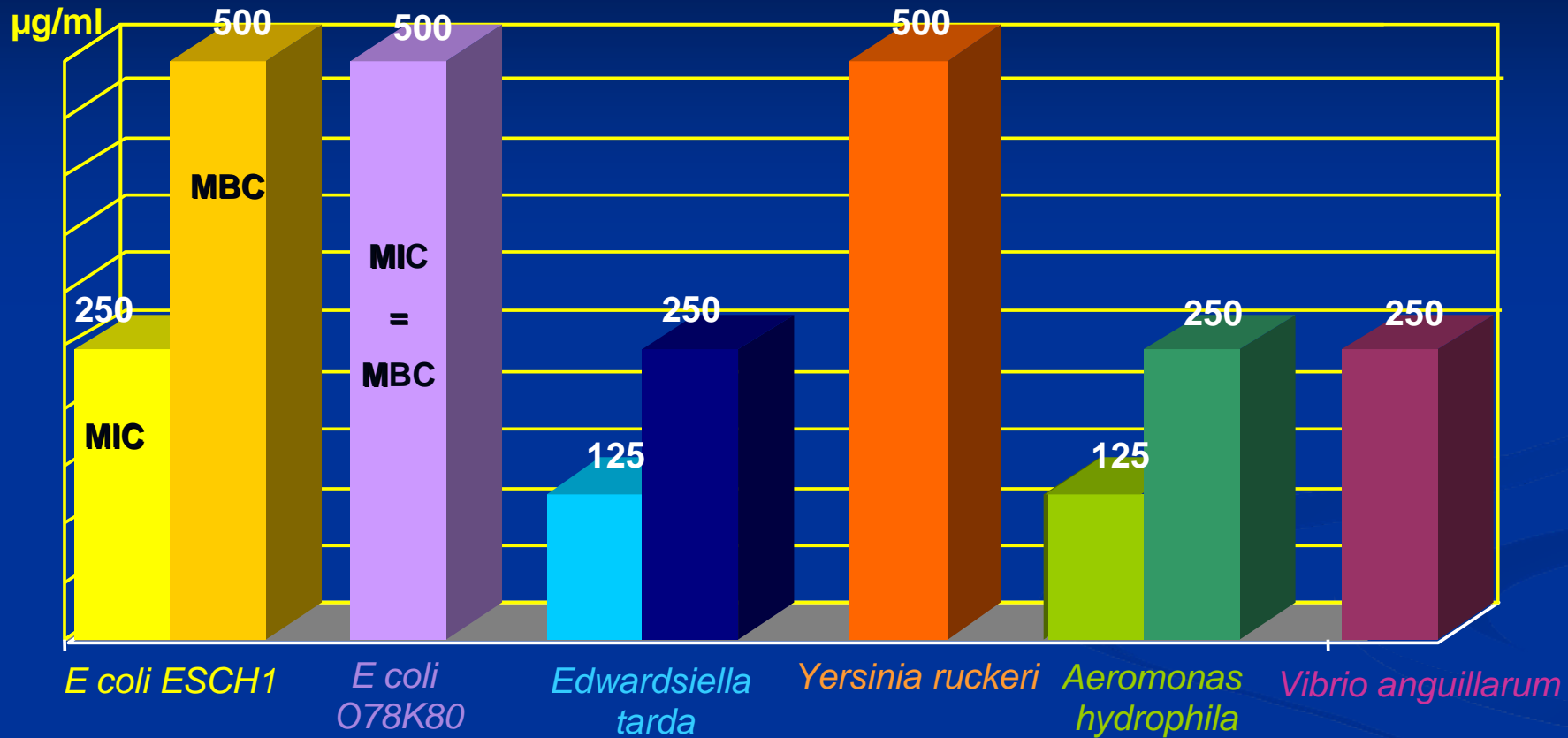
- ✓ Incubation during 24 hours at 22°C



# Aquaviance efficiency against 4 fish pathogenic bacteria strains : results

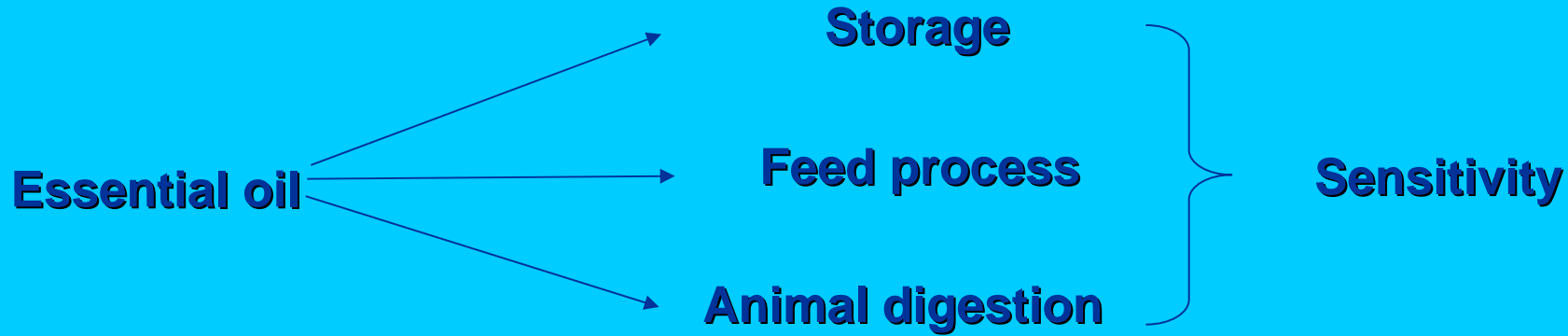


# Aquaviance efficiency against 4 fish pathogenic bacteria strains

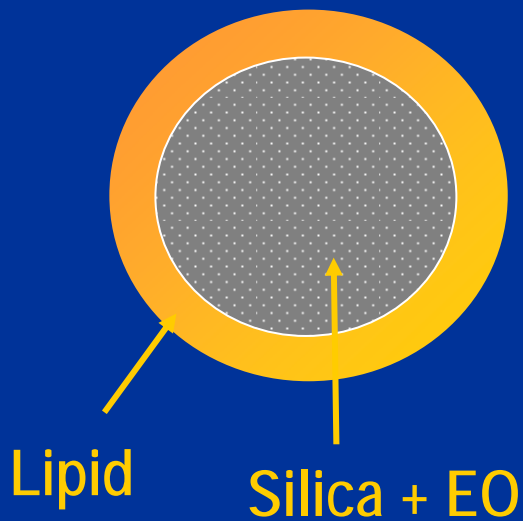


- Aquaviance effect is proven against the tested pathogenic bacteria
- All MIC, MBC are inferior to 500 µg/ml
- The most sensitive strains are *Edwardsiella tarda*, *Aeromonas hydrophila* and *Vibrio anguillarum*

# Aquaviance : an innovative technology

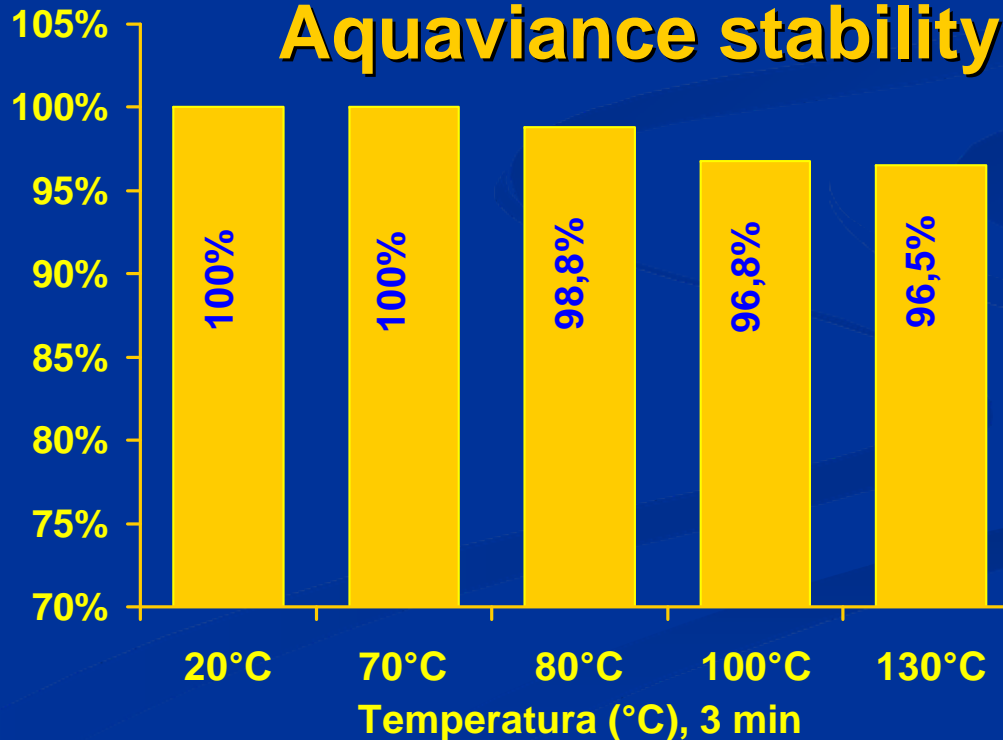


## Encapsulation



%Residual de sustancia activa

## Aquaviance stability



# Essential Oils

## Analytical control

Essential Oil

Additive

Premix

Feed

1- Extraction

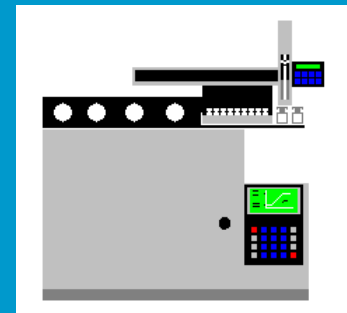
Hydrodistillation

Extraction  
with solvent



2- Dosage

GC  
FID or MS



# CONCLUSION

⇒ Alternative to Growth Promotors

⇒ Target

- ✓ Performances (weight, FCR)
- ✓ Health status

⇒ Key points

- ✓ Stimulate de enzymatic secretion
- ✓ Increase the feed intake
- ✓ Maintain the flora balance under normal conditions
- ✓ Improve the fish health status.





**THANK  
YOU!**

