

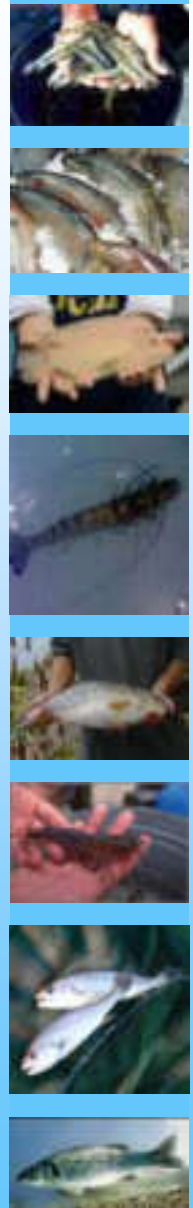
# **PERFOSTIM S/F**

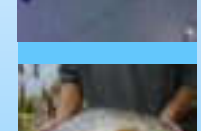
**A NOVEL APPROACH FOR USE OF LACTIC ACID  
BACTERIA *PEDIOCOCCUS ACIDILACTICI* MA18/5M  
AS A PROBIOTIC FEED ADDITIVE IN POSTLARVAE  
AND JUVENILES OF BLACK TIGER SHRIMP  
*PENAEUS MONODON*.**

**NGUYEN THI NGOC TINH, HUYNH THI HONG CHAU, NGUYEN THI KIM VAN, RESEARCH INSTITUTE FOR  
AQUACULTURE N°2, HOCHIMINH CITY, VIETNAM**

**JUN XIE, PEARL RIVER FISHERIES RESEARCH INSTITUTE, CHINESE ACADEMY FISHERIES SCIENCE, GUANGZHOU,  
CHINA**

**VINCENT USACHE, FRANCE.**





# PERFOSTIM S/F

# COMPOSITION

**PERFOSTIM S/F IS AN ORIGINAL FEED ADDITIVE COMPOSED OF TESTED BACTERIA AND ESSENTIAL ELEMENTS :**

## **BACTERIA**

**PEDIOCOCCUS ACIDILACTICI**  
MA 18/5M

- ✓ **COLONIZATION OF DIGESTIVE TRACK**
- ✓ **DECREASE OF PATHOGENS ACTIVITY**
- ✓ **IMPROVEMENT OF THE SURVIVAL RATE**

## **SELENIUM**

- ✓ **LIMITS THE EFFECT OF FREE RADICALS.**
- ✓ **ENZYMATIC ACTIVITY STIMULATION**
- ✓ **IMPROVEMENTS OF GROWTH PERFORMANCES**
- ✓ **DECREASE OF MORTALITY RATE**
- ✓ **FASTER RESPONSE OF THE IMMUNE**

## **VITAMIN E**

## **VITAMIN C**

- ✓ **STIMULATION OF THE IMMUNE RESPONSE.**
- ✓ **INCREASE OF STRESS RESISTANCE.**

# **PERFOSTIM S/F** PROPERTIES

⇒ **PERFOSTIM S/F COULD BE USED DIRECTLY BY THE FARMER FOR THE BENEFIT OF ITS REARING**

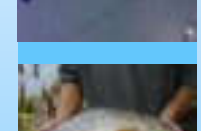
⇒ **PERFOSTIM S/F IS A PROVED ESSENTIAL SUPPLEMENTATION BLEND DEDICATED TO IMPROVE FEED QUALITY AND REARING EFFICIENCY**

⇒ **PERFOSTIM S/F IS ALLOWING :**

✓ **TO STABILIZE GUT BACTERIAL FLORA DURING REARING PHASES**

✓ **TO IMPROVE HEALTH STATUS**

✓ **TO INCREASE GROWTH RATE**



# **PERFOSTIM S/F** **PROPERTIES**

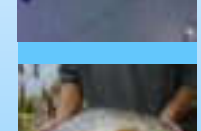
**A STRAIN SELECTED TO IMPROVE THE AQUACULTURE SPECIES PERFORMANCE:**

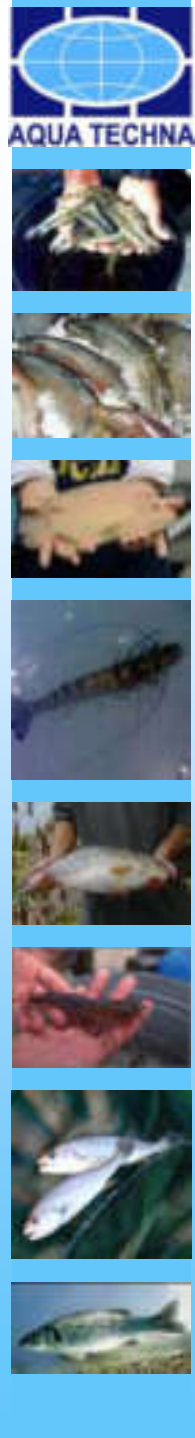
**⇒ IDENTITY:**

- ✓ **PEDIOCOCCUS ACIDILACTICI MA 18/5M,**
- ✓ **DEPOSITED AT THE NATIONAL COLLECTION OF MICROORGANISM (PASTEUR INSTITUTE, FRANCE) N° MA 18/5M.**

**⇒ PROPERTIES:**

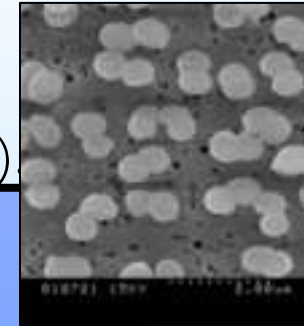
- ✓ **PRODUCES LACTIC ACID L+ FROM CARBOHYDRATE SUBSTRATES (HOMO FERMENTATIVE BACTERIA),**
- ✓ **ABLE TO GROW IN A WIDE RANGE OF PH, TEMPERATURES, OSMOTIC PRESSURES,**
- ✓ **SURVIVES ALL ALONG THE DIGESTIVE TRACT.**



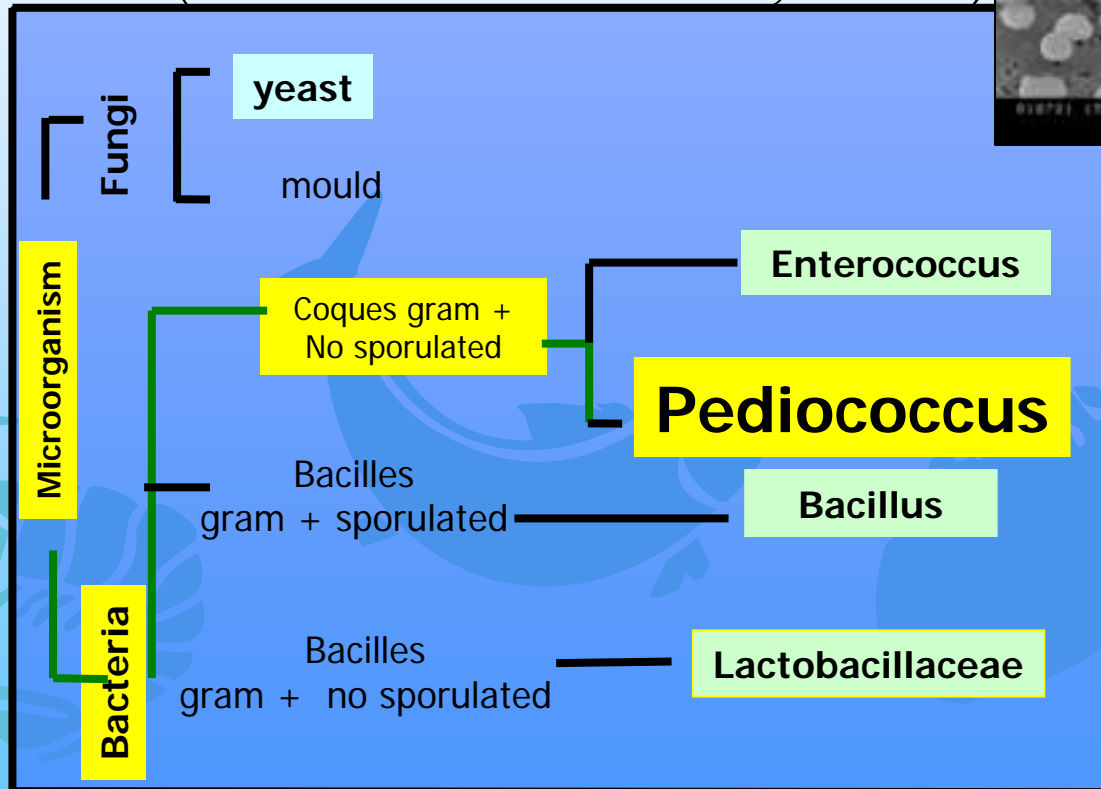


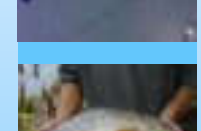
*Pediococcus acidilactici*, strain n°MA 18/5M

Registered at Collection Nationale de Cultures de Microorganismes (CNCM Institut Pasteur, France)



Pediococcus classification  
and other probiotics  
microorganisms

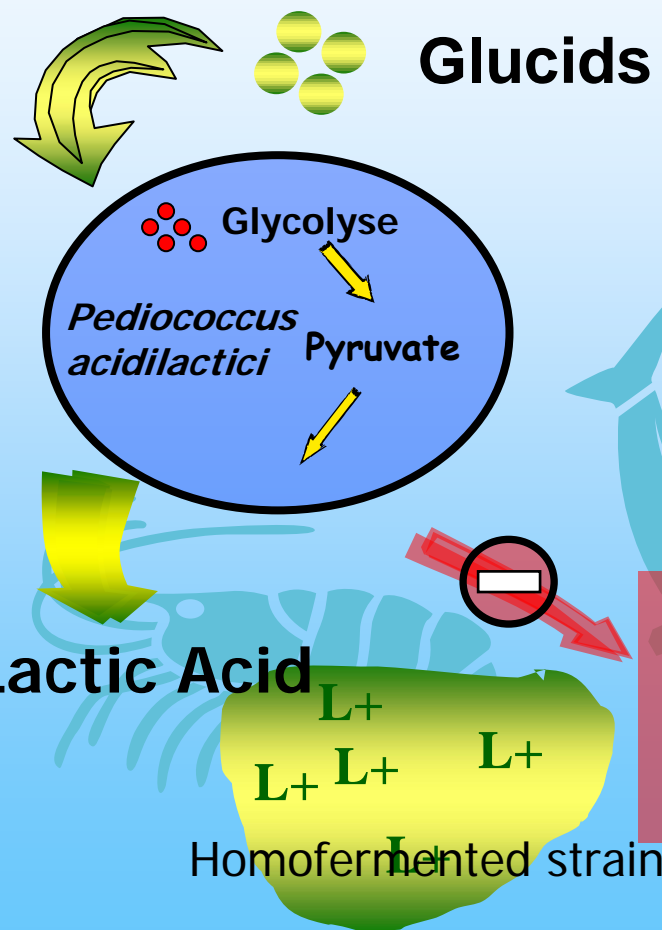




# PERFOSTIM S/F

# PRODUCTION OF LACTIC ACID

Specific sugars transfer to lactic acid by *Pediococcus acidilactici*.



Fermentative profile of <i>Pediococcus acidilactici</i>	
• L-arabinose*	• Rhamnose*
• Ribose*	• N-acetyl glucosamine
• D-xylose*	• Esculine*
• Galactose	• Cellobiose*
• D-glucose	• Trehalose*
• D-fructose	• D-tagatose*
• D-mannose*	

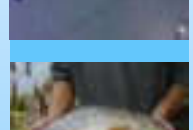
\* not digested by the animal

CO<sub>2</sub> (nutrients lost)\*  
 Ac. Acetic (less palatability)\*

\*: heterofermented bacteria

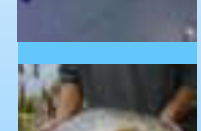
# **PERFOSTIM S/F INTRODUCTION**

**IN ASIA, INTENSIVE SHRIMP FARMING HAS BECOME A BREAK-THROUGH BUSINESS DURING THE PAST DECADES, WITH BLACK TIGER SHRIMP (*PENAEUS MONODON*) BEING ONE OF THE MAJOR CULTURED SPECIES. HOWEVER THE INDUSTRY HAS BEEN DEEPLY CHALLENGED BY DISEASES DUE TO PATHOGENS (FOR INSTANCE *VIBRIO HARVEYI*) AND VIRUSES (MORIARTY, 1999). IN ORDER TO LIMIT THE PROPHYLACTIC USE OF ANTIBIOTICS IN SHRIMP FARMING, THE APPLICATION OF PROBIOTICS (SAFE YEAST & BACTERIA WHICH BENEFICIALLY AFFECT THE HEALTH OF THE HOST) HAS BEEN DEVELOPED IN AQUACULTURE SINCE THE 1980S.**



# **PERFOSTIM S/F** INTRODUCTION

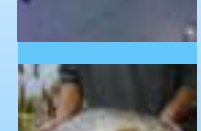
**AS EXAMPLES, STRAINS AS PSEUDOMONAS I-2, (CHYTHANYA ET AL, 2002) AND BACILLUS SUBTILIS BT23 (VASEEHARAN ET AL, 2003) SHOWED ANTAGONISTIC EFFECTS AGAINST VIBRIO HARVEYI. RECENTLY A GROWING NUMBER OF STUDIES REPORTED THE USE OF INNOVATIVE LACTIC ACID BACTERIA (LAB) TO IMPROVE HEALTH AND QUALITY OF FISH AND SHRIMP LARVAE, ALTHOUGH THIS GROUP OF BACTERIA IS NOT DOMINANT IN THE NORMAL INTESTINAL MICROBIOTA OF FISH (RINGO AND GATESOUBE, 1998). SEVERAL REPORTS HAVE ILLUSTRATED THE USE OF LAB IN WATER TREATMENT AND BIOREMEDIATION.**



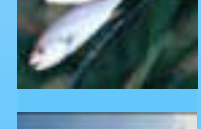
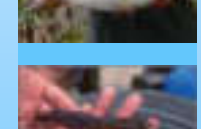
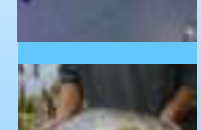
# **PERFOSTIM S/F INTRODUCTION**

**HOWEVER, NO ATTEMPT OF USING LAB AS FEED ADDITIVES IN SHRIMP FARMING HAS BEEN REPORTED SO FAR. OUR OBJECTIVES IN THOSE STUDIES WERE TO:**

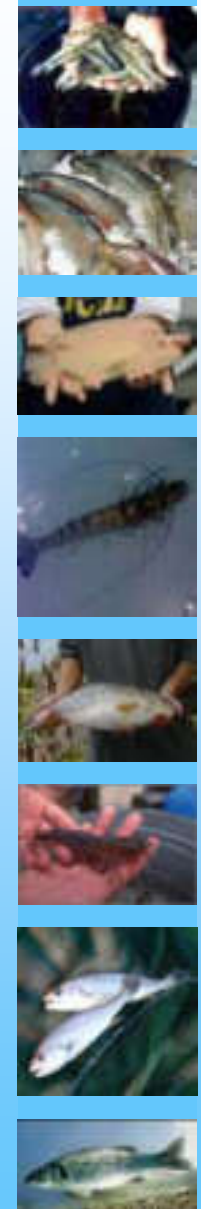
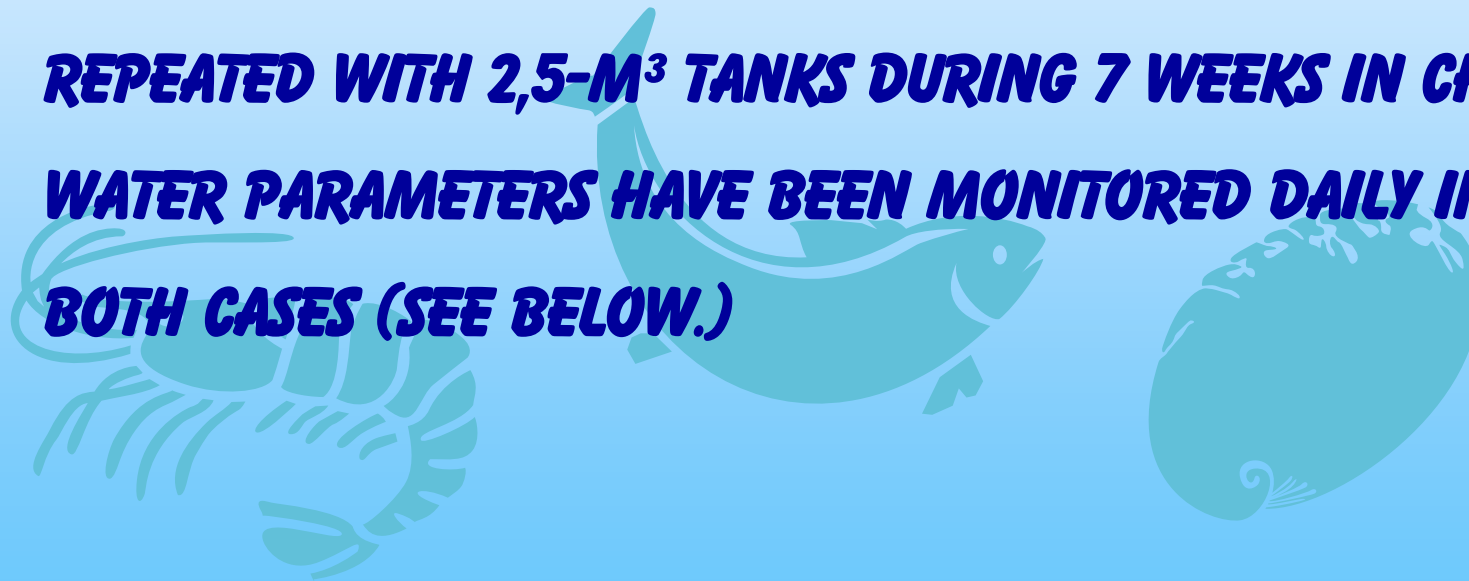
- A) CHARACTERIZE THE APPLICATION OF THE SPECIFIC LAB *PEDIOCOCCUS ACIDILACTICI* AS A PROBIOTIC FEED ADDITIVE AND EVALUATE THE POPULATION OF THE PROBIOTIC IN THE FEED AND IN THE SHRIMP GUT. EVALUATE THE POTENTIAL INTERACTION BETWEEN THE PROBIOTIC POPULATION AND THE TOTAL VIBRIO POPULATION.**
- B) EVALUATE THE POTENTIAL EFFECTS OF THE COMMERCIAL PROBIOTIC STRAIN *P. ACIDILACTICI* SUCCESSFULLY USED ON LIVESTOCK, ON GROWTH AND SURVIVAL OF SHRIMP JUVENILES *PENAEUS MONODON*.**
- C) COMPARE THE POSSIBLE EFFECTS OF A COMMERCIAL PROBIOTIC STRAIN *P. ACIDILACTICI* IN TWO DIFFERENT SHRIMP FARMING CONDITIONS (VIETNAM & CHINA).**

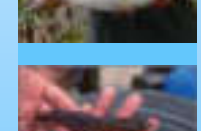


**TRIAL N° 1 HAS BEEN CONDUCTED IN THE RESEARCH INSTITUTE FOR AQUACULTURE N°2, HOCHIMINH CITY, VIETNAM. BLACK TIGER SHRIMP (*PENAEUS MONODON*) PL15 WERE REARED IN 1-M<sup>3</sup> TANKS WITH CONICAL BOTTOM. INITIAL STOCKING DENSITY WAS 200 LARVAE PER TANK. LIGHT AERATION WAS PROVIDED IN THE CENTER OF EACH TANK. THE DEAD LARVAE AND LEFTOVER FEED WERE DAILY SIPHONED OFF, 30% OF WATER WAS RENEWED AFTERWARDS. THE PROBIOTIC PREPARATION TESTED WAS *PEDIOCOCCUS ACIDILACTICI* CNCM MA 18/5M (PERFOSTIM®). THE TWO TREATMENTS WERE DISPATCHED AT RANDOM IN TRIPPLICATES AMONG 6 LARVAL REARING TANKS: 3 CONTROL TANKS & 3 TREATED TANKS WITH A PROBIOTIC DOSE OF 10<sup>6</sup> CFU/G OF FEED. THE PROBIOTIC WAS MIXED WITH THE FEED PRIOR FEEDING. THE TREATED GROUP WAS NOT SUPPLEMENTED WITH ANTIBIOTICS. THE EXPERIMENT LASTED 8 WEEKS. SURVIVAL WAS DETERMINED AT THE END OF EACH MONTH BY COUNTING ALL THE SHRIMP IN EACH TANK. GROWTH RATE WAS DETERMINED EVERY TWO WEEKS BY MEASURING WEIGHT AND LENGTH ON 10 SHRIMPS FROM EACH TANK. SHRIMPS (N=10) WERE ALSO SAMPLED BY MONTH FROM EACH TANK TO DETERMINE *VIBRIO* COUNTS AND *PEDIOCOCCUS ACIDILACTICI* COUNTS IN THE GUT. THE DATA WERE COMPARED BY ONE-WAY ANOVA AT A SIGNIFICANT LEVEL OF 0.05.**



***TRIAL N°2 HAS BEEN CONDUCTED IN CHINA AQUACULTURE  
SCIENCE INSTITUTE SHENGZHEN DAPENG TRIAL CENTER,  
CHINA. THE SAME EXPERIMENTAL DESIGN HAS BEEN  
REPEATED WITH 2,5-M<sup>3</sup> TANKS DURING 7 WEEKS IN CHINA.  
WATER PARAMETERS HAVE BEEN MONITORED DAILY IN  
BOTH CASES (SEE BELOW.)***



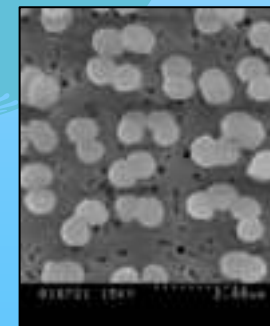


# RESULTS: APPLICATION OF P. ACIDILACTICI IN THE FEED

## PERFOSTIM S/F

<b>IN THE FEED/G</b>	<b>EXPECTED</b>	<b>COUNTED</b>
		<b><math>10^6</math> CFU/G</b>
<b>IN THE GUT /G</b>	<b>EXPECTED</b>	<b>COUNTED</b>
		<b><math>10^4</math>-<math>10^5</math> CFU/G</b>

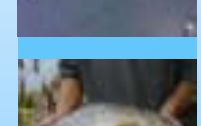
- **PEDIOCOCCUS ACIDILACTICI** MEASURED IN THE FEED IS FOUND AT THE EXPECTED COUNT IN THIS TRIAL.
- **PEDIOCOCCUS ACIDILACTICI** MIXED WITH THE FEED IS FOUND AT A SIGNIFICANT LEVEL IN THE SHRIMP GUT TO EXPRESS ITS METABOLIC FUNCTIONS.



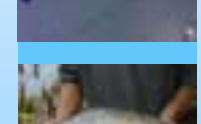
## **RESEARCH INSTITUTE FOR AQUACULTURE N°2, HO CHI MINH CITY, VIETNAM**

### **WATER QUALITY:**

– SALINITY	= 10 – 12 PPT
– TEMPERATURE	= 27°C – 28°C
– PH	= 7 – 7,5
– DO	= 4 – 5 PPM
– NH <sub>3</sub> – N	= < 0,1 PPM

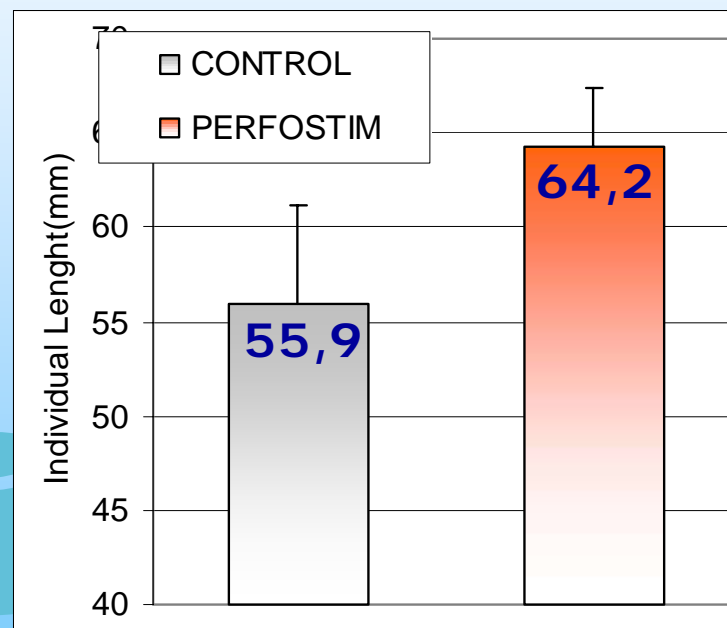
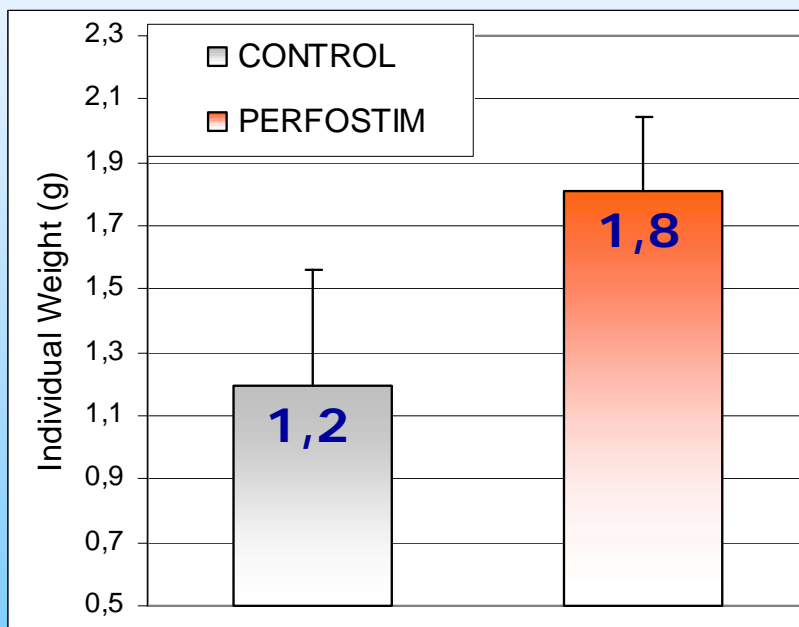


**REARING DENSITY: 450 PL15 / M<sup>3</sup> / REPLICATE**

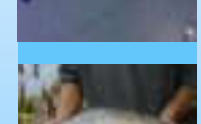


# PERFOSTIM S/F RESULTS: GROWTH

**PERFOSTIM S/F AS FEED ADDITIVE, INCLUDING P. ACIDILACTICI INCREASES THE GROWTH OF P. MONODON JUVENILES IN BOTH WEIGHT AND LENGTH/VIETNAM**

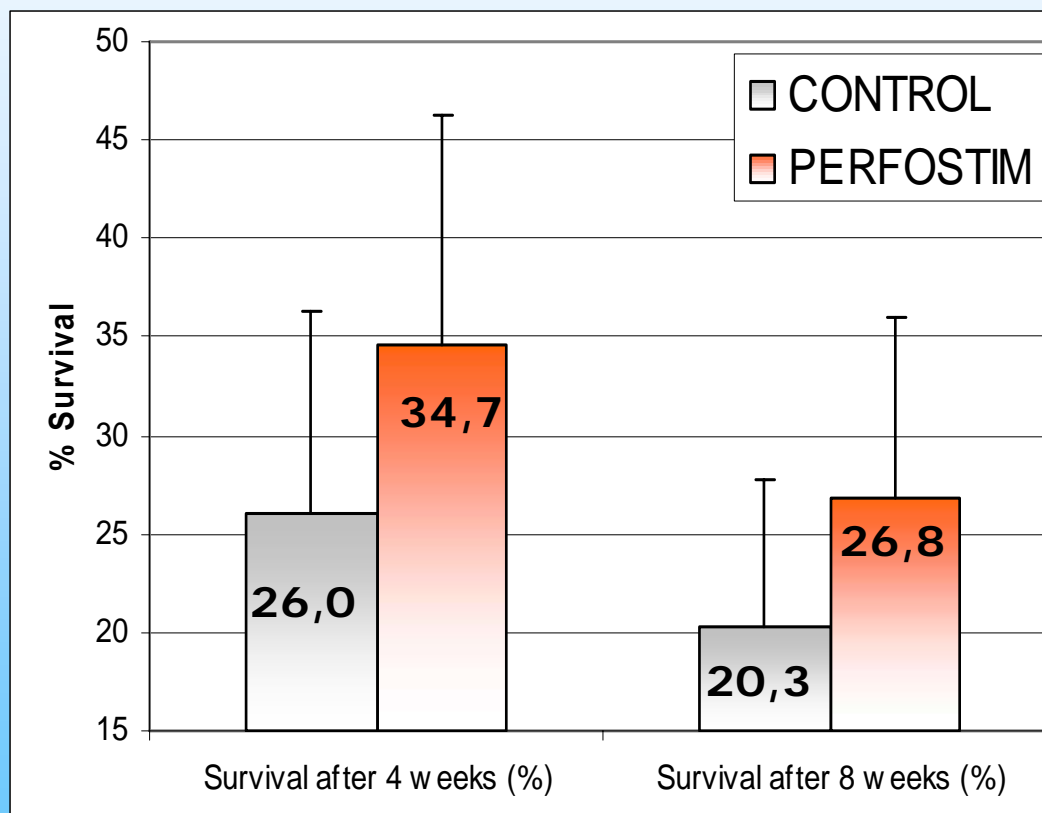


***P. ACIDILACTICI* (PERFOSTIM®) INCREASES SIGNIFICANTLY THE WEIGHT BY 52% ( $P < 0,05$ ) AND INCREASES THE LENGTH BY 15% ( $P > 0,05$ ) OF P. MONODON JUVENILES DURING A 8 WEEK TREATMENT OF PROBIOTIC ADDED IN THE FEED.**

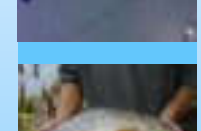


# **PERFOSTIM S/F RESULTS: SURVIVAL**

## **PERFOSTIM INCREASES THE SURVIVAL OF *P. MONODON* JUVENILES VIETNAM**

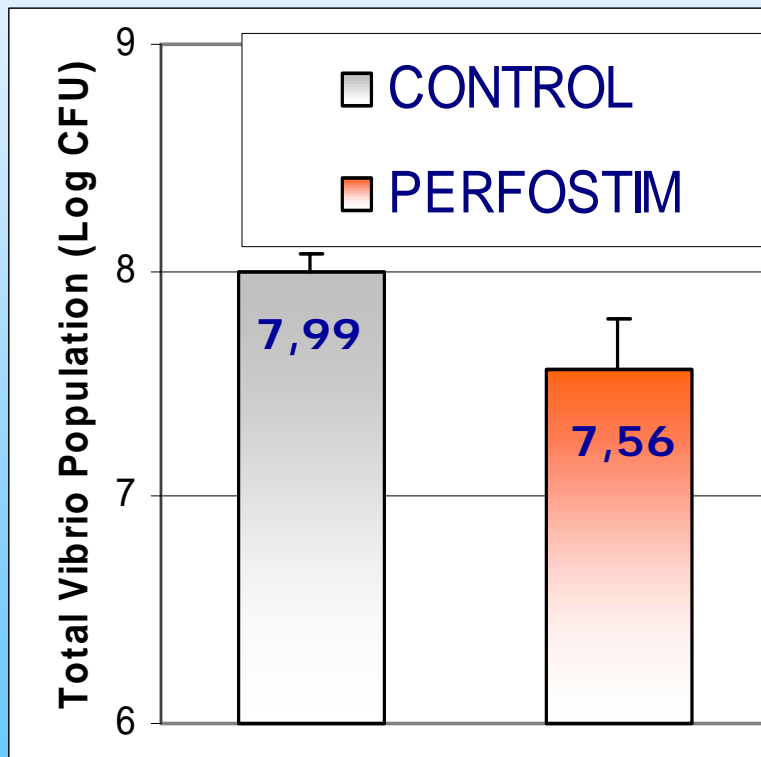


***P. ACIDILACTICI* (PERFOSTIM) INCREASES THE SURVIVAL BY 34% ( $P > 0,05$ ) AND BY 32% ( $P > 0,05$ ) OF *P. MONODON* JUVENILES DURING A 4 WEEK & 8 WEEK TREATMENT RESPECTIVELY.**

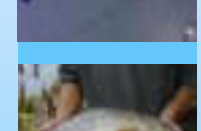


# **PERFOSTIM S/F RESULTS: MICROBIOLOGY**

## **PERFOSTIM DECREASES THE NUMBER OF TOTAL VIBRIO POPULATION IN THE P. MONODON JUVENILES GUT**



***P. ACIDILACTICI- (PERFOSTIM) DECREASES THE NUMBER OF TOTAL VIBRIO POPULATION BY 5,7%(P>0,05) (CFU LOG) IN P.MONODON JUVENILES DURING A 8 WEEK TREATMENT.***



## **AQUACULTURE SCIENCE INST. SHENGZHEN**

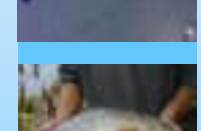
## **DAPENG TRIAL CENTER, CHINA**

### **WATER QUALITY:**

– SALINITY	= 32-34 PPT
– TEMPERATURE	= 19,5°C – 24°C
– PH	= 7 – 7,5
– DO	= 4 – 5 PPM
– NH <sub>3</sub> – N	= < 0,1 PPM

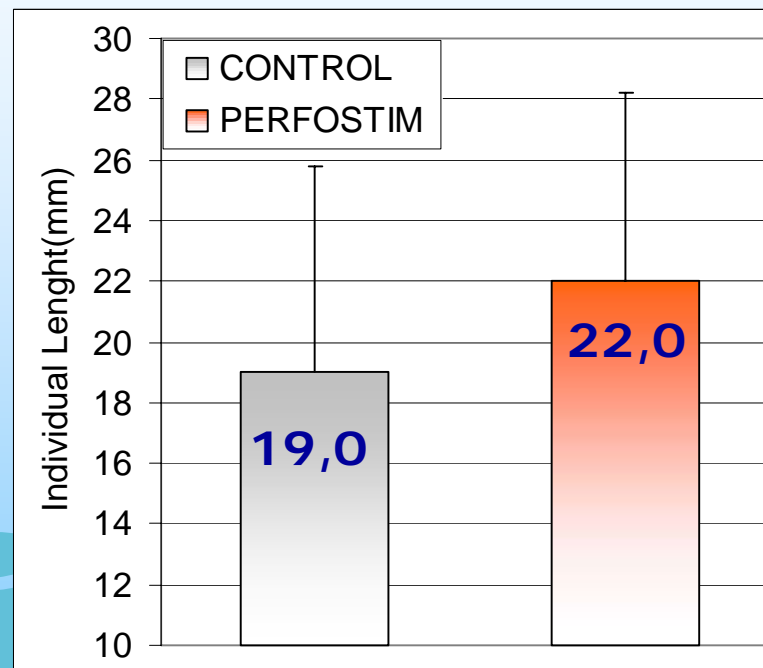
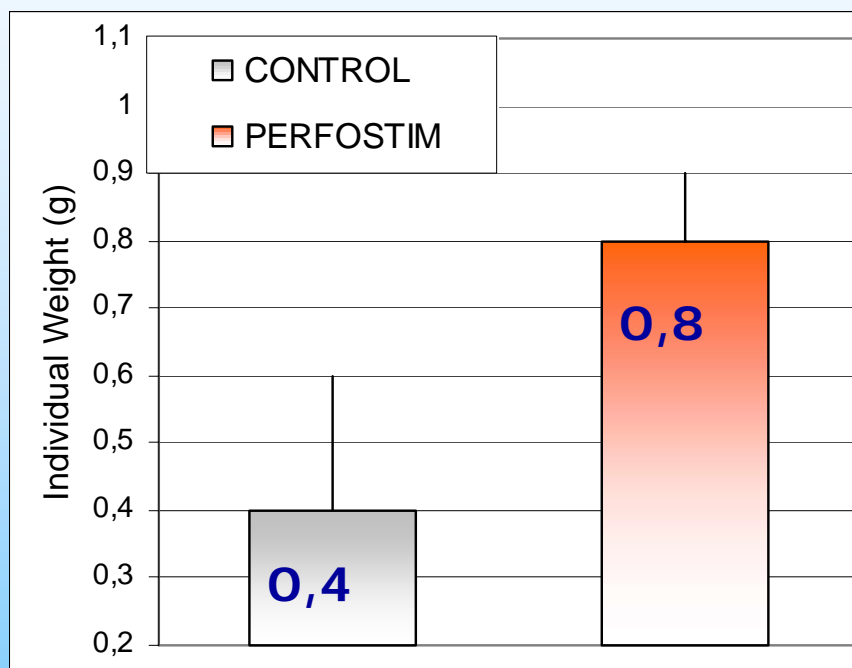


**REARING DENSITY: 2 500 PL15 / M<sup>3</sup> / REPLICATE**

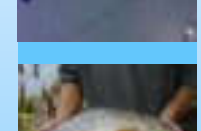


# PERFOSTIM S/F RESULTS: GROWTH

**PERFOSTIM S/F AS FEED ADDITIVE, INCLUDING *P. ACIDILACTICI*, INCREASES THE GROWTH OF *P. MONODON* JUVENILES IN BOTH WEIGHT AND LENGTH/CHINA**



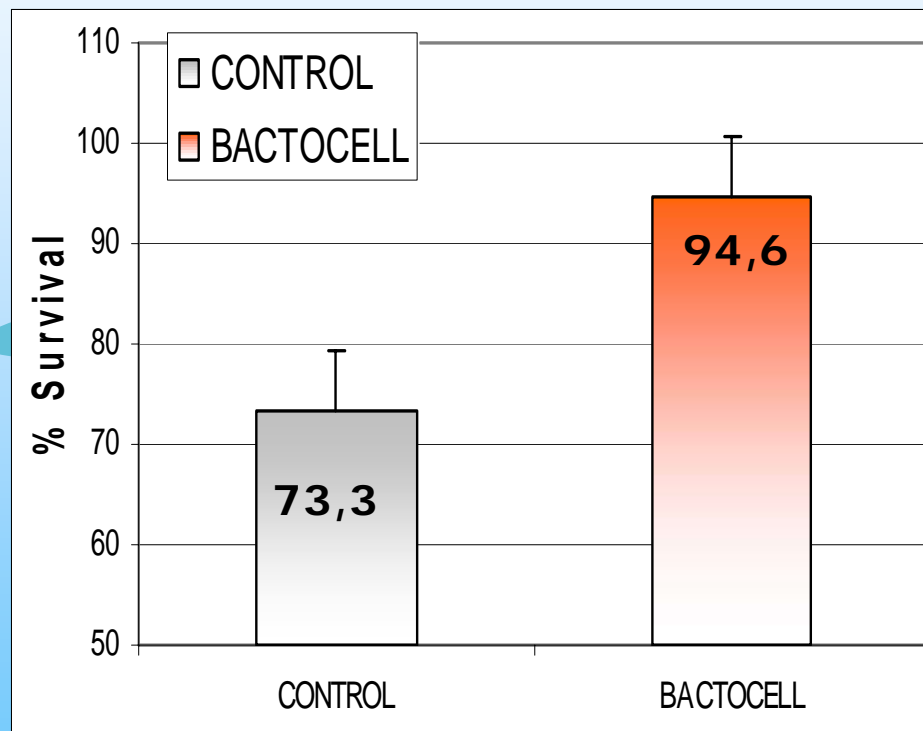
***P. ACIDILACTICI*- (PERFOSTIM®) INCREASES SIGNIFICANTLY THE WEIGHT BY 50% ( $P < 0,05$ ) AND INCREASES SIGNIFICANTLY THE LENGTH BY 16% ( $P > 0,05$ ) OF *P. MONODON* JUVENILES DURING A 7 WEEK TREATMENT OF PROBIOTIC ADDED IN THE FEED.**

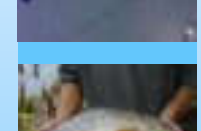


# **PERFOSTIM S/F RESULTS: SURVIVAL**

## **PERFOSTIM INCREASES THE SURVIVAL OF P. MONODON JUVENILES/CHINA**

**P. ACIDILACTICI-  
(PERFOSTIM)  
INCREASES  
SIGNIFICANTLY THE  
SURVIVAL BY  
29%(P<0,05) OF P.  
MONODON JUVENILES  
DURING A 7 WEEK  
TREATMENT.**

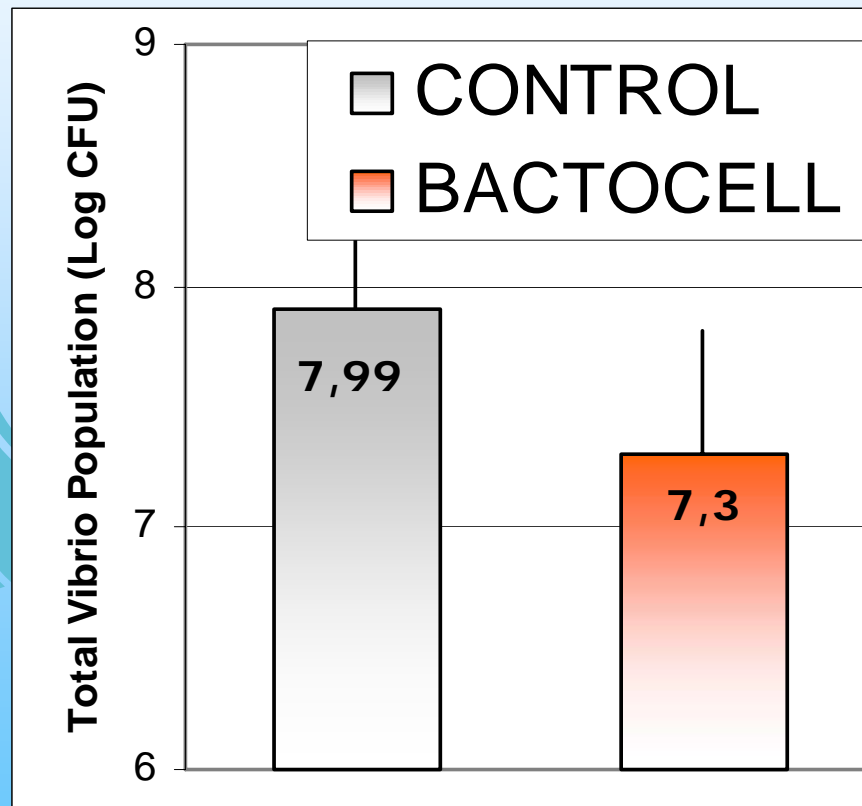




# **PERFOSTIM S/F RESULTS: MICROBIOLOGY**

**PERFOSTIM DECREASES THE NUMBER OF TOTAL VIBRIO POPULATION IN THE P. MONODON JUVENILES GUT /CHINA**

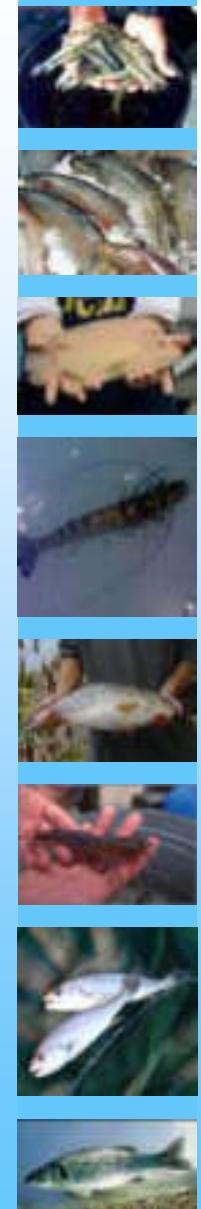
**P. ACIDILACTICI- (PERFOSTIM) DECREASES SIGNIFICANTLY THE NUMBER OF TOTAL VIBRIO POPULATION BY 9,5% ( $P < 0,05$ ) (CFU LOG) IN P.MONODON JUVENILES DURING A 7 WEEK TREATMENT.**



# **PERFOSTIM S/F CONCLUSION**

**IN THIS STUDY, WE DEMONSTRATE THAT :**

- ⇒ **PERFOSTIM INGESTED VIA THE FEED PERMITS THE TRANSIT OF THE PROBIOTIC IN A VIABLE FORM AT A SIGNIFICANT LEVEL IN THE GUT.**
- ⇒ **PERFOSTIM GIVES SIGNIFICANT IMPROVEMENT OF GROWTH PARAMETERS (EXCEPTED "LENGTH" PARAMETER IN VIETNAM TRIAL) AND IMPROVEMENT OF THE SURVIVAL OF SHRIMP JUVENILES PENAEOUS MONODON. THE INCREASE OF SURVIVAL WAS SIGNIFICANT IN CHINA'S TRIAL ( $P < 0,05$ ) AND NON SIGNIFICANT IN VIETNAM'S TRIAL ( $P > 0,05$ )**
- ⇒ **THE PROBIOTIC STRAIN *P. ACIDILACTICI* (PERFOSTIM) GIVES SIMILAR IMPROVEMENT OF GROWTH & SURVIVAL IN TWO DIFFERENT SHRIMP FARMING CONDITIONS (VIETNAM & CHINA). GROWTH PARAMETERS OF THE *P. MONODON* SHRIMP JUVENILES WERE INCREASED BY AN AVERAGE OF 50% IN GROWTH AND 15% IN LENGTH FOR BOTH CONDITIONS. SURVIVAL OF THE *P. MONODON* SHRIMP JUVENILES WAS INCREASED BY AN AVERAGE OF 30% IN BOTH CASES. MOREOVER, THE ADDITION OF PROBIOTIC STRAIN *P. ACIDILACTICI* (PERFOSTIM) IN THE FEED LEADS TO A LOW DECREASE OF THE TOTAL *VIBRIO SPP.* POPULATION IN SHRIMP GUT.**



# **PERFOSTIM S/F CONCLUSION**

**THE INTRODUCTION OF PEDIOCOCCUS ACIDILACTICI (PERFOSTIM) IN SHRIMP FEED GIVES POSITIVE RESULTS IN IMPROVING SURVIVAL AND GROWTH OF THE PENAEUS MONODON SHRIMP LARVAE AND JUVENILES. TO FOLLOW UP THIS STUDY, AN ADAPTED PROTOCOL INCLUDING CHALLENGE TESTS SHOULD BE SET UP IN ORDER TO EVALUATE THE EFFECTS OF THE PROBIOTIC AGAINST SPECIFIC PATHOGENS**

