Mitigation of AHPND based on phenotype switching in Vibrio parahaemolyticus

12th Annual Workshop of the National Reference Laboratories for Crustacean Diseases
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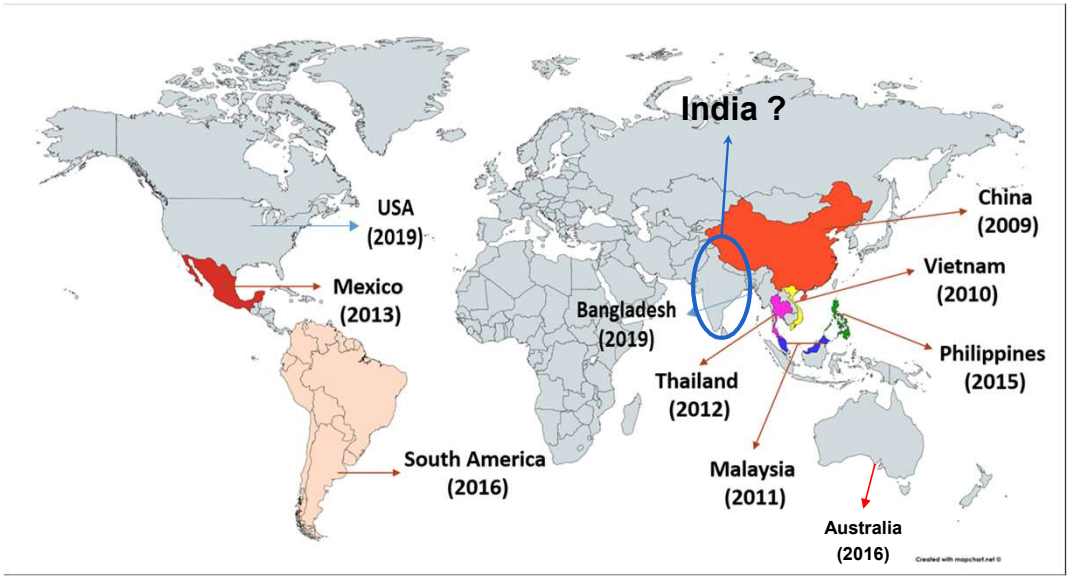
FACULTY OF BIOSCIENCE ENGINEERING

Artemia Reference Center

The bacterial disease, acute hepatopancreatic necrosis disease (AHPND)



Epidemiology of AHPND

Mortalities (upto 100%) within 20-30 days of stocking

- ✓ ~60 % drop in shrimp production
- ✓ Global loss of $ 43 billion in the last 10 years

Still a major challenge for development of sustainable shrimp aquaculture

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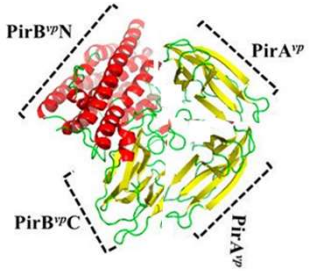
Causative agent of AHPND

Vibrio parahaemolyticus

pVA1 plasmid (63-70 kb) → Encodes → Binary PirA and PirB toxins



PirA and PirB toxins → Primary virulence factor of AHPND-causing bacteria

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Artemia franciscana model system

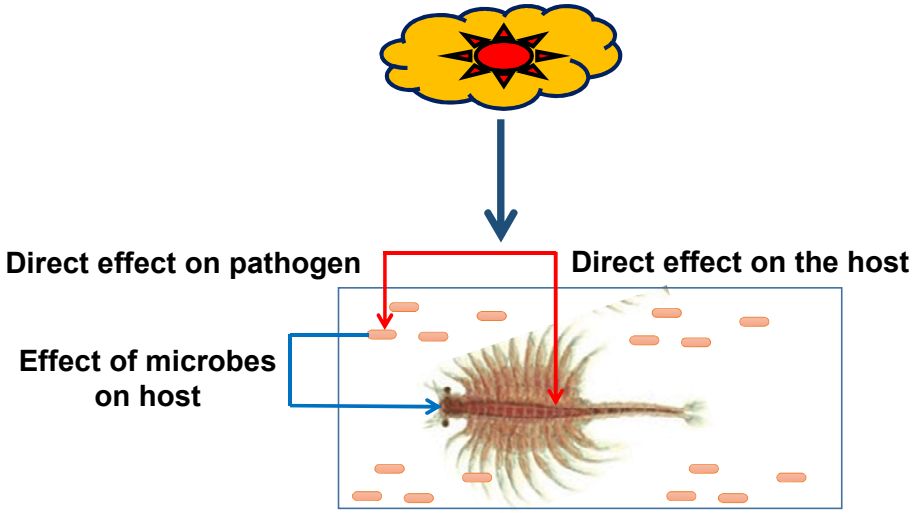
Shares high homology with shrimps and other crustaceans genomes

Low space & cost requirement

Well characterized developmental stages

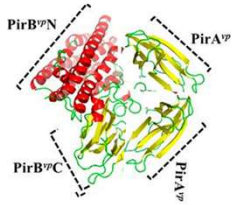
Established molecular techniques

Gnotobiotic (germ-free) animal model system



Infection model for AHPND was developed

V. parahaemolyticus

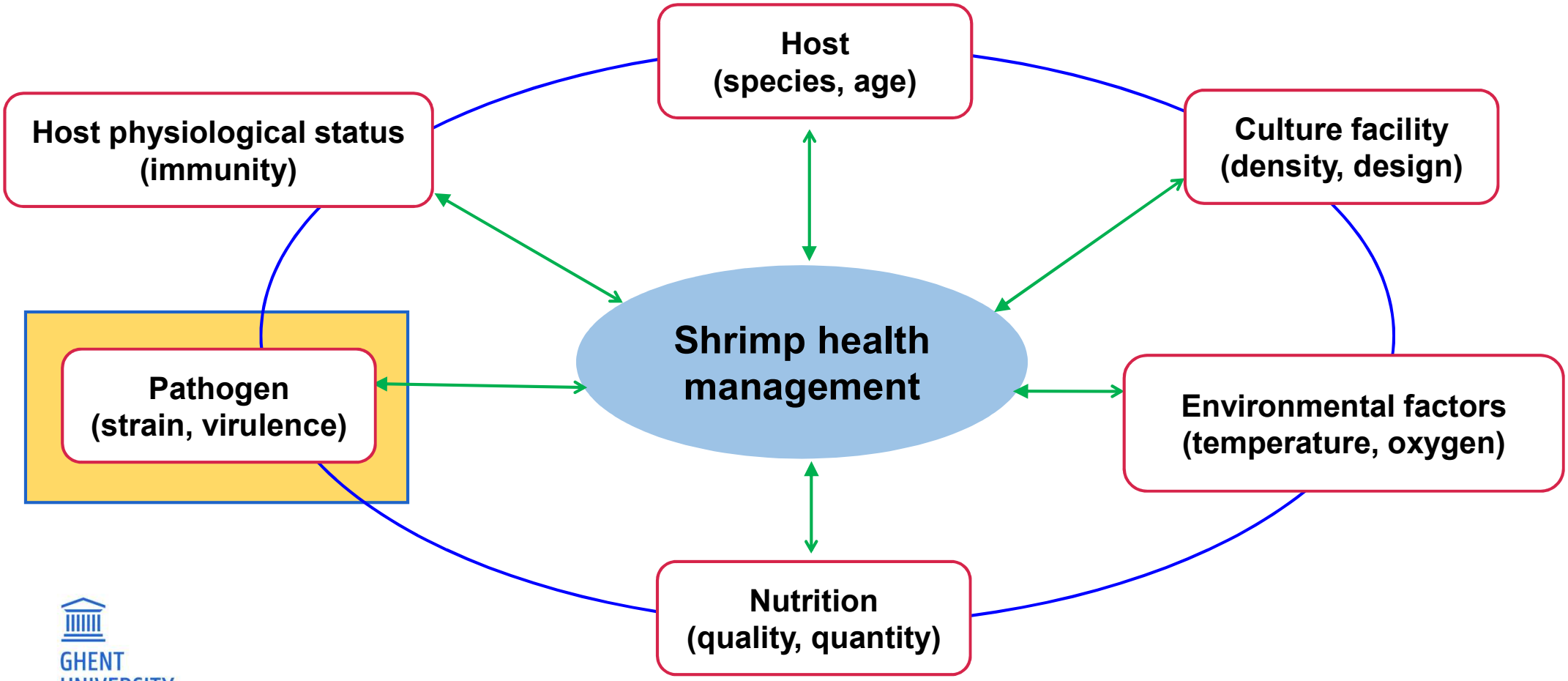


PirA^{VP} and PirB^{VP} toxins

Artemia franciscana

Macrobrachium rosenbergii *Litopenaeus vannamei*

Mitigation strategies



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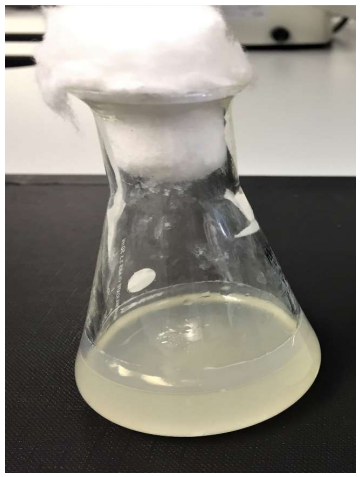
environmental microbiology

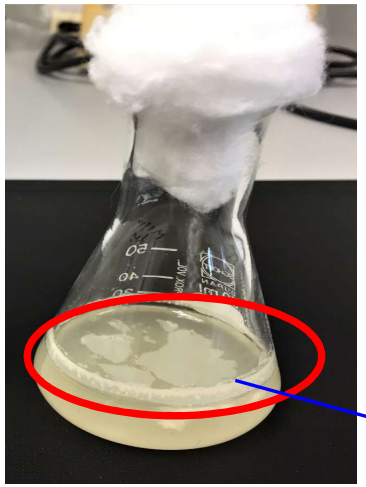
Environmental Microbiology (2020) **00**(00), 00–00 doi:10.1111/1462-2920.14903

Environmental conditions steer phenotypic switching in acute hepatopancreatic necrosis disease-causing *Vibrio parahaemolyticus*, affecting $PirA^{VP}$/$PirB^{VP}$ toxins production

Vikash Kumar[1,2] *, Suvra Roy[1,2], Kartik Baruah[1,3], Delphi Van Haver[4,5,6], Francis Impens[4,5,6] and Peter Bossier[1]



120 rpm



110 rpm

High flocculation and biofilm formation

***V. parahaemolyticus* (AHPND strain) incubated overnight in marine broth**

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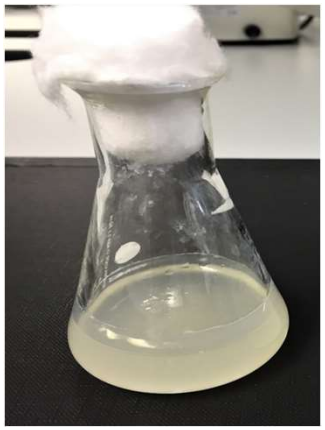
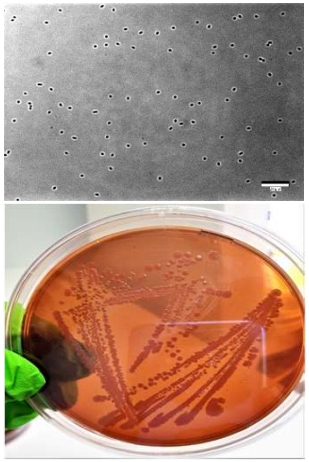
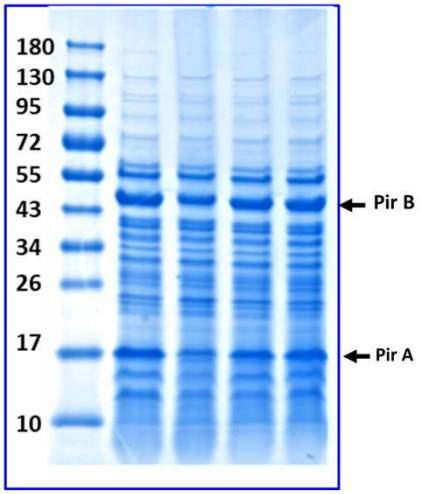




Red colonies

Secreted proteins



Virulent gene expression

Toxicity to shrimp

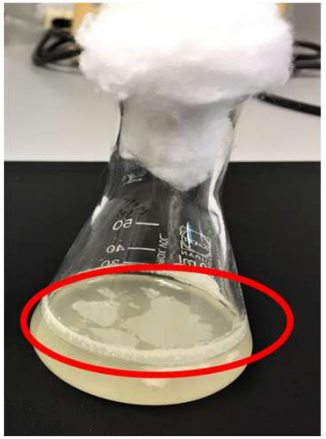
Tolerance to antibiotics

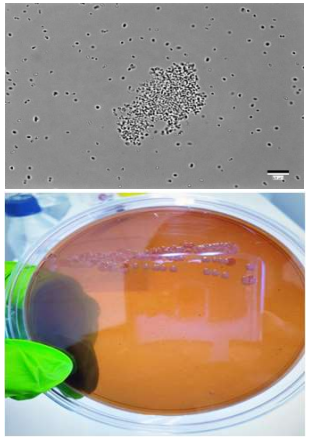
Alkaline phosphatase gene expression

120 rpm

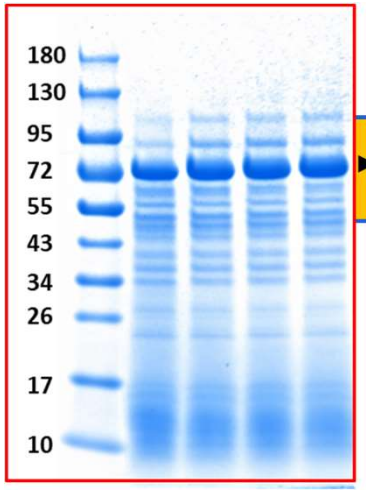
Phenotype switching

110 rpm





Purple colonies



PirAB toxin

Virulent gene expression

Toxicity to shrimp

Tolerance to antibiotics

Alkaline phosphatase gene expression

A new phenomenon - phenotype switching (Aquaculture system)

- **Shaking condition regulates phenotype status of AHPND strain**

Virulent phenotype

⇩

PirA & PirB toxins

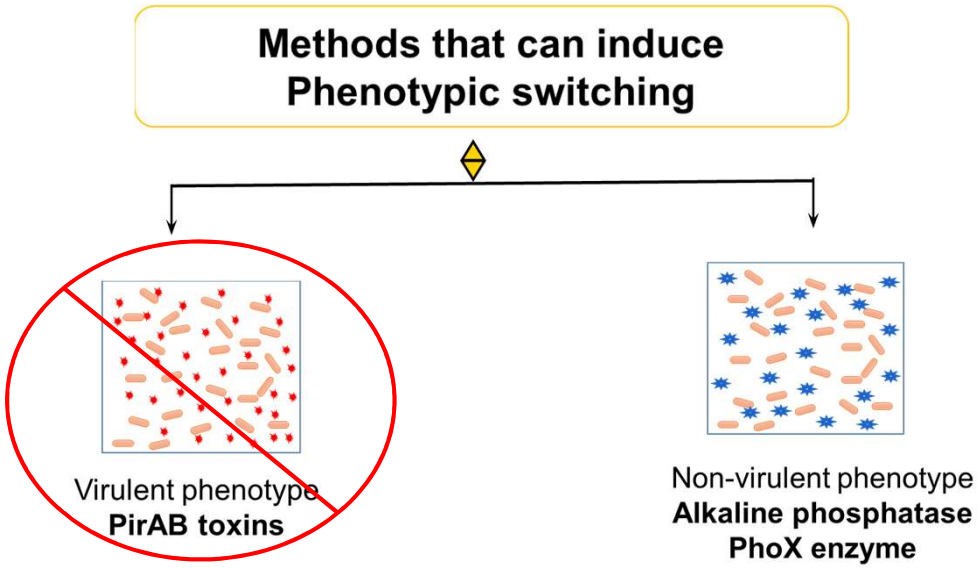
Non-virulent phenotype

⇩

Alkaline phosphatase

⇩

Measurement of **PirAB and AlkPhoX gene can be used as marker** to monitor the phenotype status of AHPND strain



Promising approach to manage AHPND

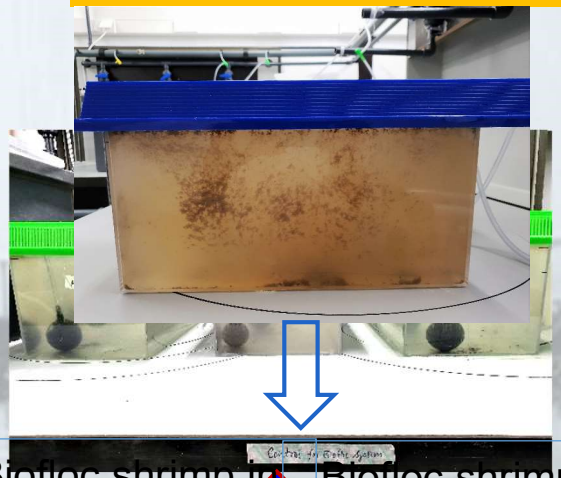
Biofloc system

known aquaculture technology

Promising method to mitigate AHPND in shrimps

Hostins et al. 2019

Revisited the story to unravel the mechanism



Biofloc shrimp in seawater

Biofloc shrimp in biofloc water

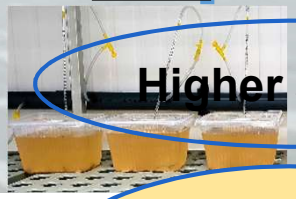
Seawater shrimp in biofloc water

Group 1

Group 2

Group 3

Group 4



Higher survival

Control shrimp + Clear seawater + *V.p.* M0904

Control shrimp + Clear seawater + *V.p.* M0904

Biofloc shrimp + Biofloc water + *V.p.* M0904

Mechanism was not known

Survival

Gene expression

frontiers in Microbiology

Biofloc-based enhanced survival of *Litopenaeus vannamei* upon AHPND-causing *Vibrio parahaemolyticus* challenge is partially mediated by reduced expression of its virulence genes

ORIGINAL RESEARCH
published: 24 June 2020
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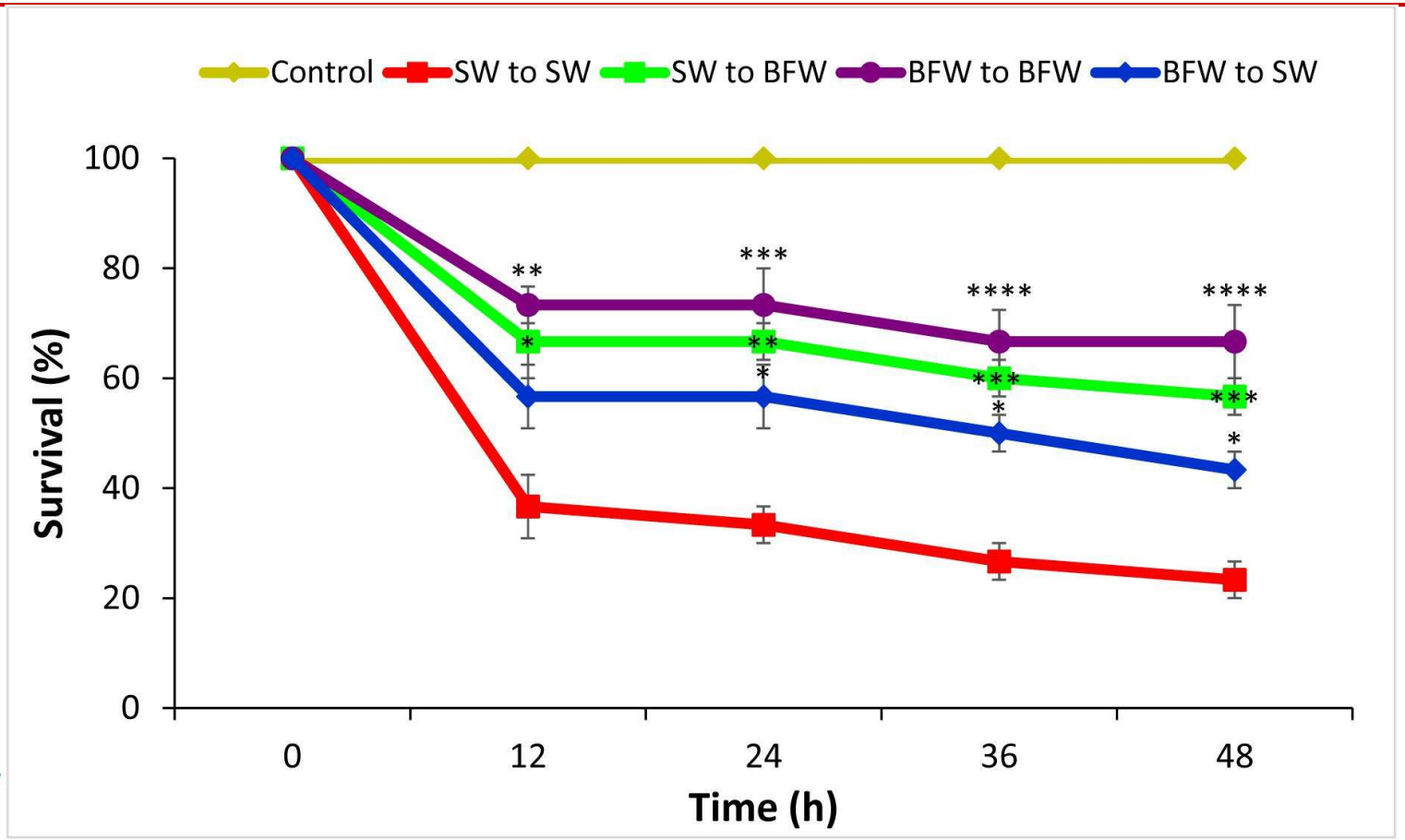
Vikash Kumar[1,2], Mathieu Wille[1], Tânia Margarida Lourenço[1] and Peter Bossier[1]*



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frontiers in Microbiology

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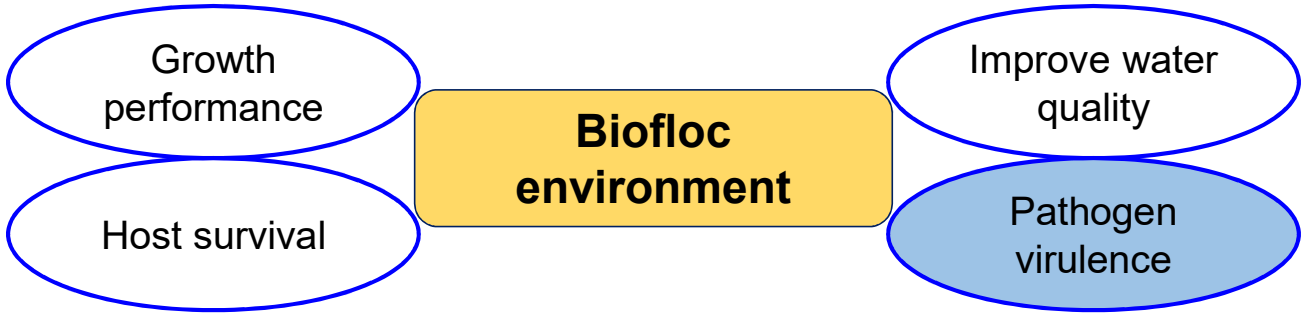
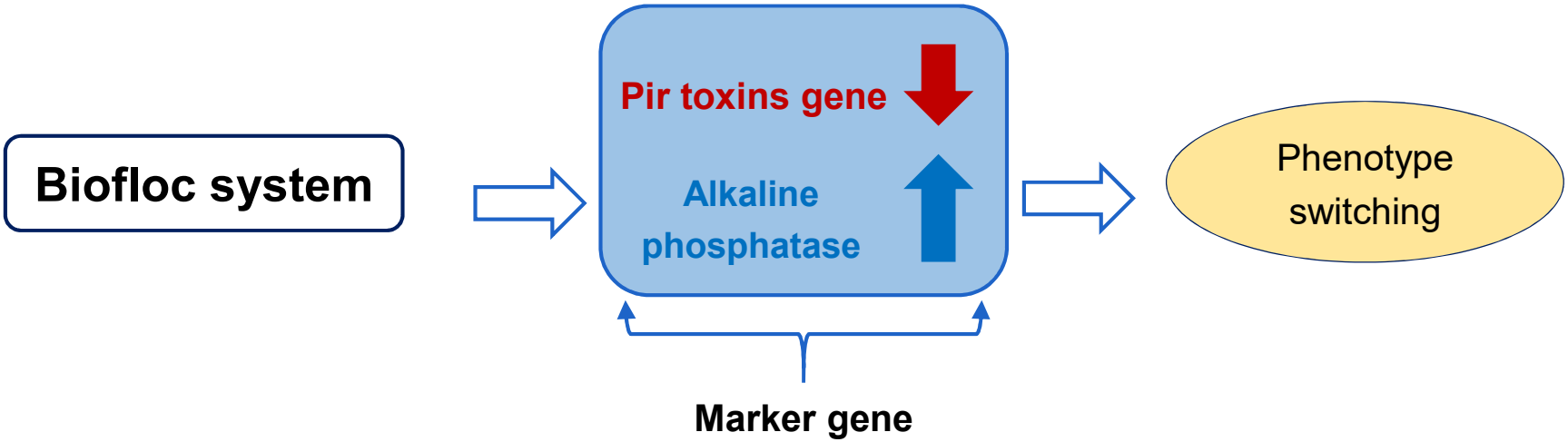




Cellulose

Hemicellulose

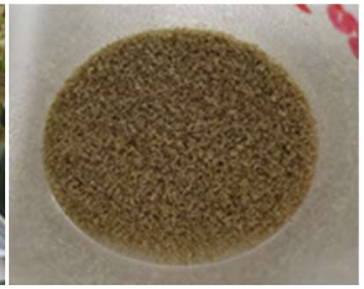
Lignin

Bamboo powder

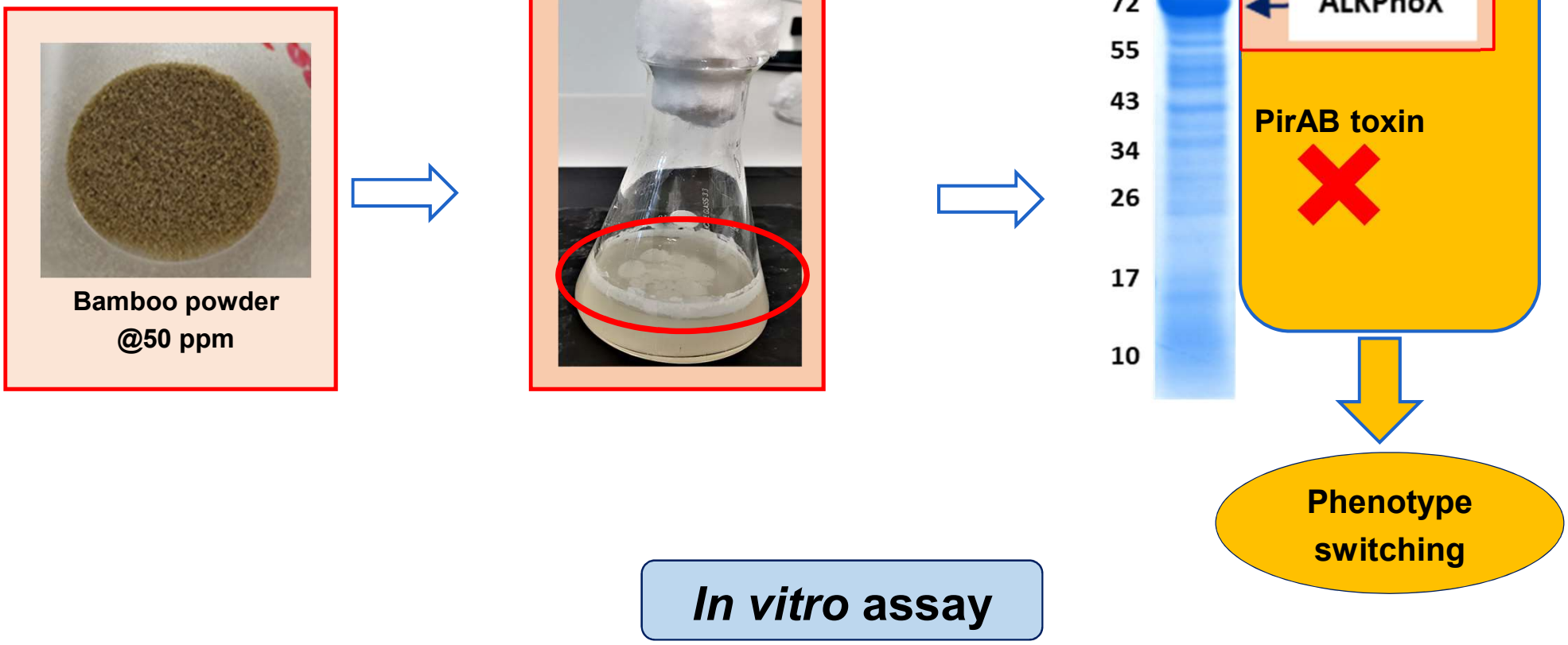
Rich source of carbon

well-established biofilm carrier

Bamboo



Bamboo powder

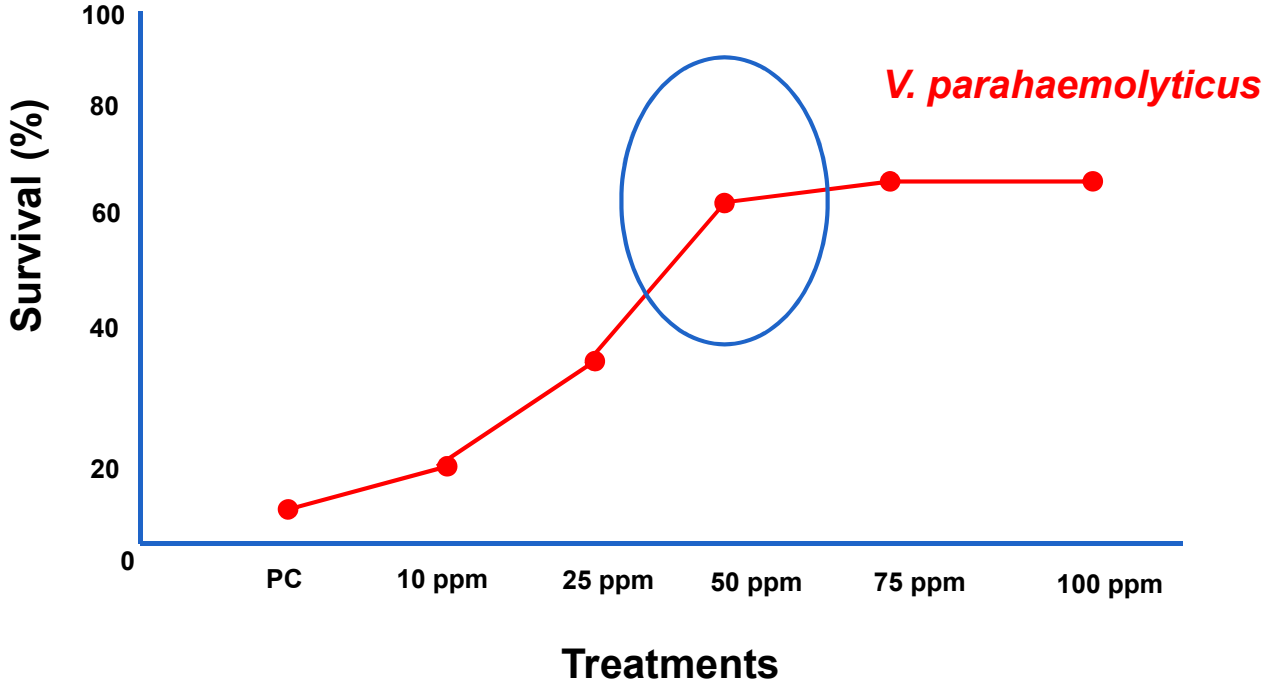

Secreted proteins
180
130
95
72
55
43
34
26
17
10
Phenotype switching marker protein
ALKPhoX
PirAB toxin
Bamboo powder @50 ppm
Phenotype switching
In vitro assay

Bamboo powder induces phenotype switching and protects brine shrimp against AHPND-causing *Vibrio parahaemolyticus* strains

Bamboo powder

Parahaemolyticus
M0904

Survival of brine shrimp



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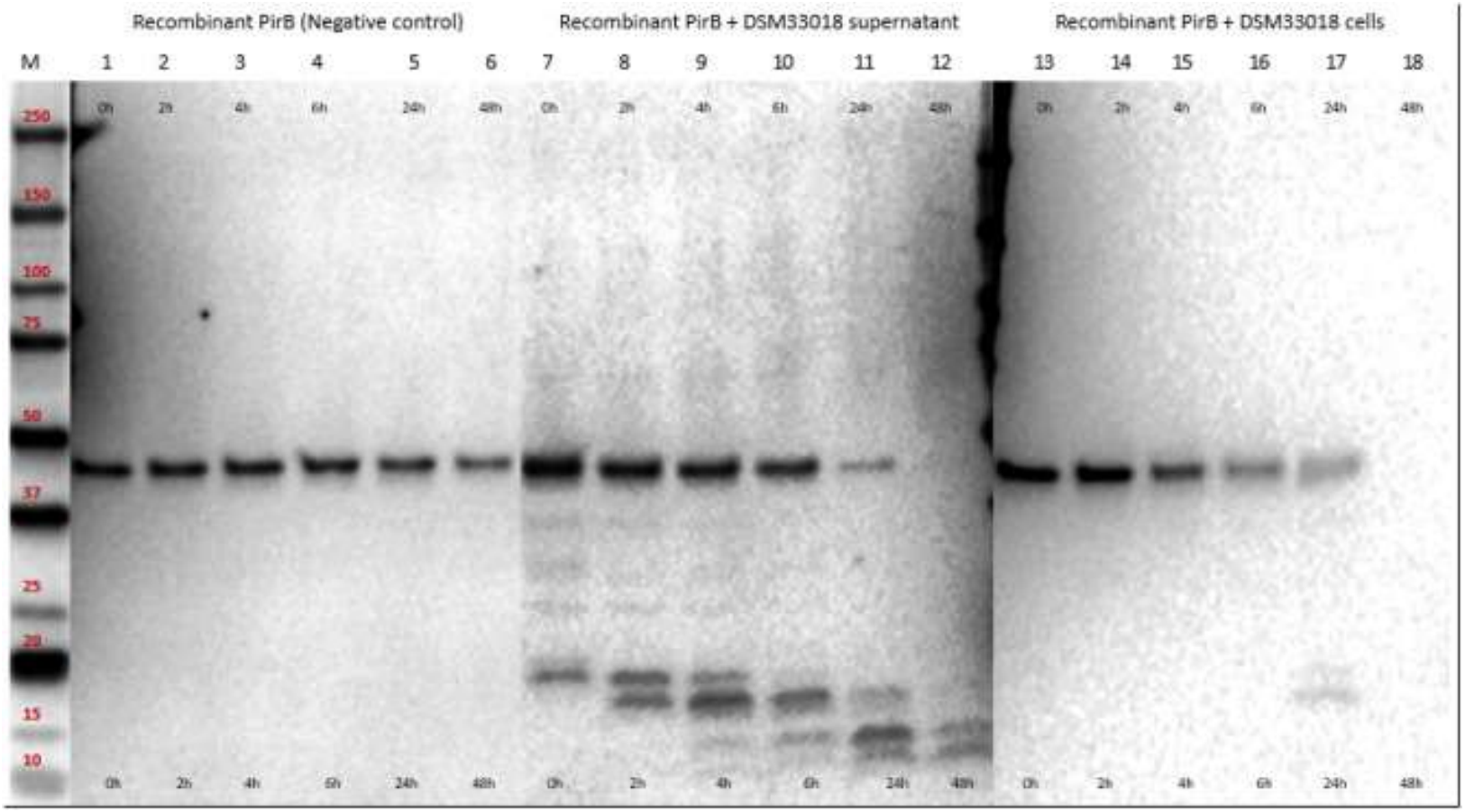
TOXIN DEGRADATION BY BACILLUS STRAIN

WESTERN BLOT ON PIRB DEGRADATION



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Conclusion

- *V. parahaemolyticus* AHPND strain displaying two distinct phenotypes in response to shaking conditions:
  - planktonic virulent and
  - biofilm non-virulent
- Biofloc environment and bamboo powder can be used to induce phenotype switching and protect shrimp against AHPND strains:
  - strategies that can be implemented by small scale farmers provided knowledge on phenotype switching is validated under field circumstances
- Bacillus strains, through secreted proteases, can degrade the toxin

Take home message

AHPND bacteria display phenotype switching, influencing the virulence

Biofloc system & bamboo powder are promising method to induce phenotype switching

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Lab. of Aquaculture & ARC
Ghent University

All data based on PhD of Vikash Kumar

ICAR grantee

PhD defense on Aug 25th 2020

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Thank you for the attention!