CENAIM-ESPOL

Secretaría de Educación Superior, Ciencia y Tecnología

Respuesta integral al estudio y control de vibriosis en cultivos de camarón

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XIX CONGRESO ECUATORIANO DE ACUICULTURA

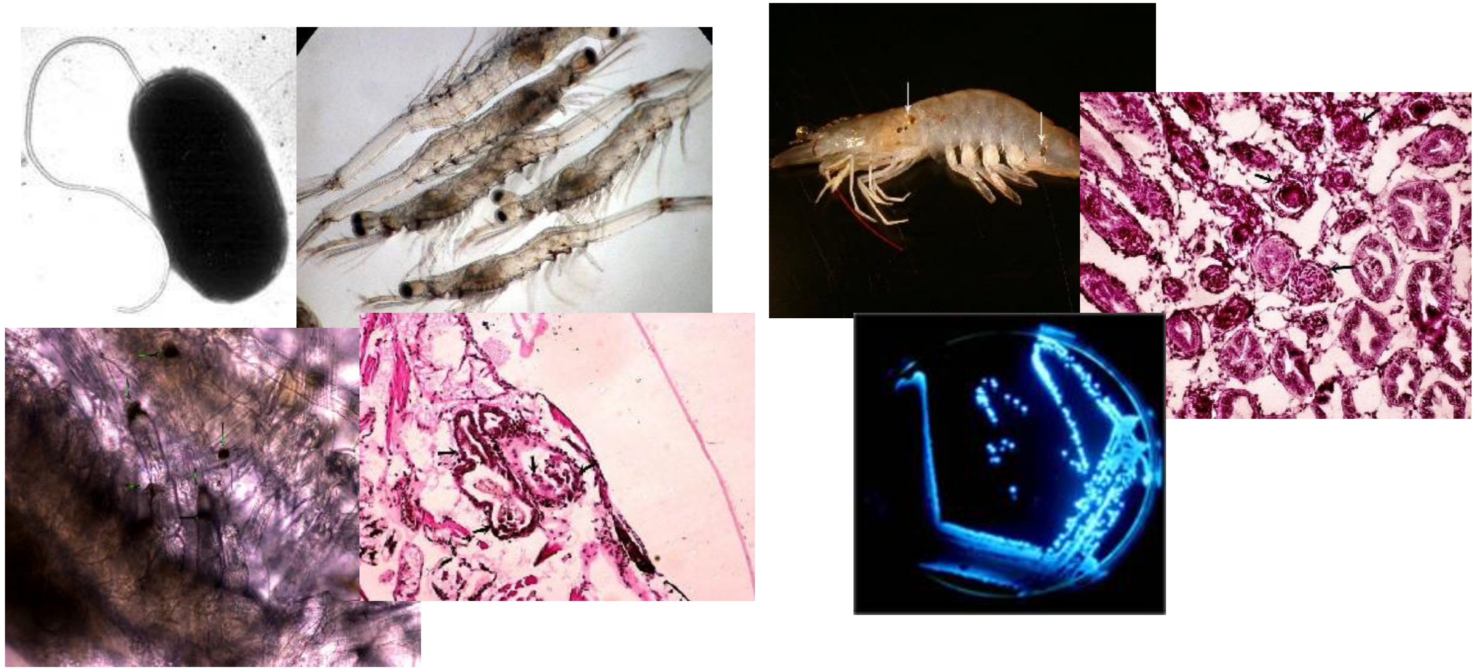
Tecnologías para una producción sustentable

Vibriosis

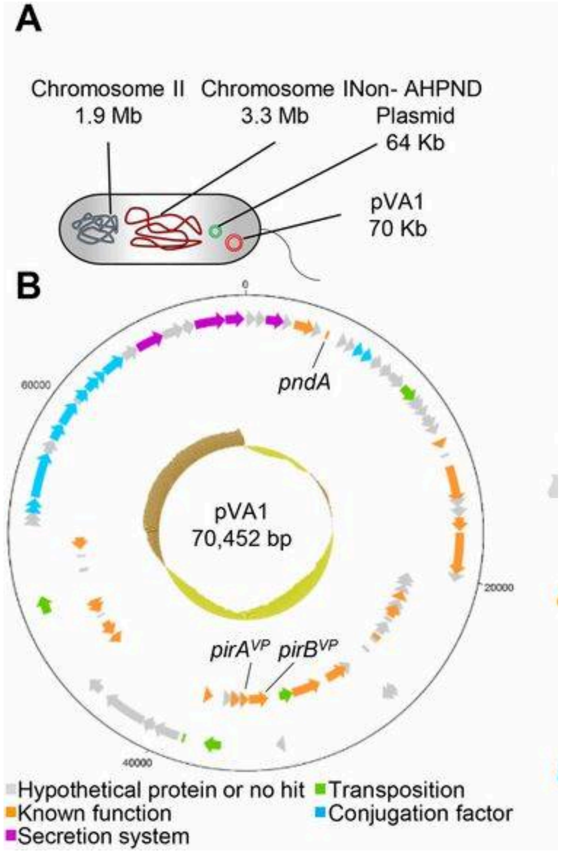
Table 1.
Examples of socio-economic and other impacts of *Vibrio* related diseases in aquaculture system.

Country	*Vibrio* spp. caused disease	Losses and other impacts	Reference
China	*V. fluvialis*	>US$ 120M annual losses between 1990-1992	Wei (2002)
Egypt	*V. anguillarum* *V. alginolyticus* *V. ordalii* *V. harveyi*	Red spot on the ventral and lateral area Swollen and dark skin lesions, necrosis, hemorrhagic areas, exophthalmia and ulcers on the skin surface 50% mortality in Seabass and Seabream	Saad and Atallah (2014)
Indonesia	Luminescent Vibrio	>US$ 100 M in 1991 at shrimp hatcheries	APEC (2000)
Tunisia	*V. parahaemolyticus*	Darkened body color, white nodular skin lesion, and sudden death with haemorrhages in the skeletal muscle of European Seabass	Khouadja et al. (2013)
Mexico	*V. parahaemolyticus*	Acute Hepatopancreatic Necrosis Disease (AHPND) in *L. vannamei* include empty gut, anorexia, lethargy, expanded chromatophores and pale HP with discoloration	Soto-rodriquez et al. (2015)
Thailand	*V. harveyi*	Mass mortalities in *P. monodon*	Groumellec et al. (1995)
Ecuador	*V. harveyi*	Mass mortalities in *P. monodon*	Groumellec et al. (1995)
Japan	*V. carchariae*	Mass mortalities in Japanese abalone *Haliotis diversicolor*	Nishimori et al. (1998)
India	*V. harveyi*	Tail rot, erythemia, and as white patches on the body of seahorses, *Hippocampus kuda*	Raj et al. (2010)
India	*V. parahaemolyticus* *V. alginolyticus* *V. anguillarum* *V. vulnificus*	Poor growth, lethargic movements, red discoloration, and mortality in *Penaeus monodon*	Thakur et al. (2003)
Italy	*V. alginolyticus* *V. anguillarum* *V. harveyi* *V. ordalii* *V. salmonicida* *V. vulnificus*	Mass mortalities in bivalves farm located in Mar Piccolo in Taranto	Cavallo et al. (2012)
West coast of North America	*V. tubiashii*	Reduce the bivalve shellfish larval and seed production. One hatchery in their study estimated a 59% loss in 2007 production.	Elston et al. (2008)

- *Vibrios* spp
- Todas especies camarón penaeidos
- Larvas, postlarvas, juveniles
- Patógenos secundarios y primarios

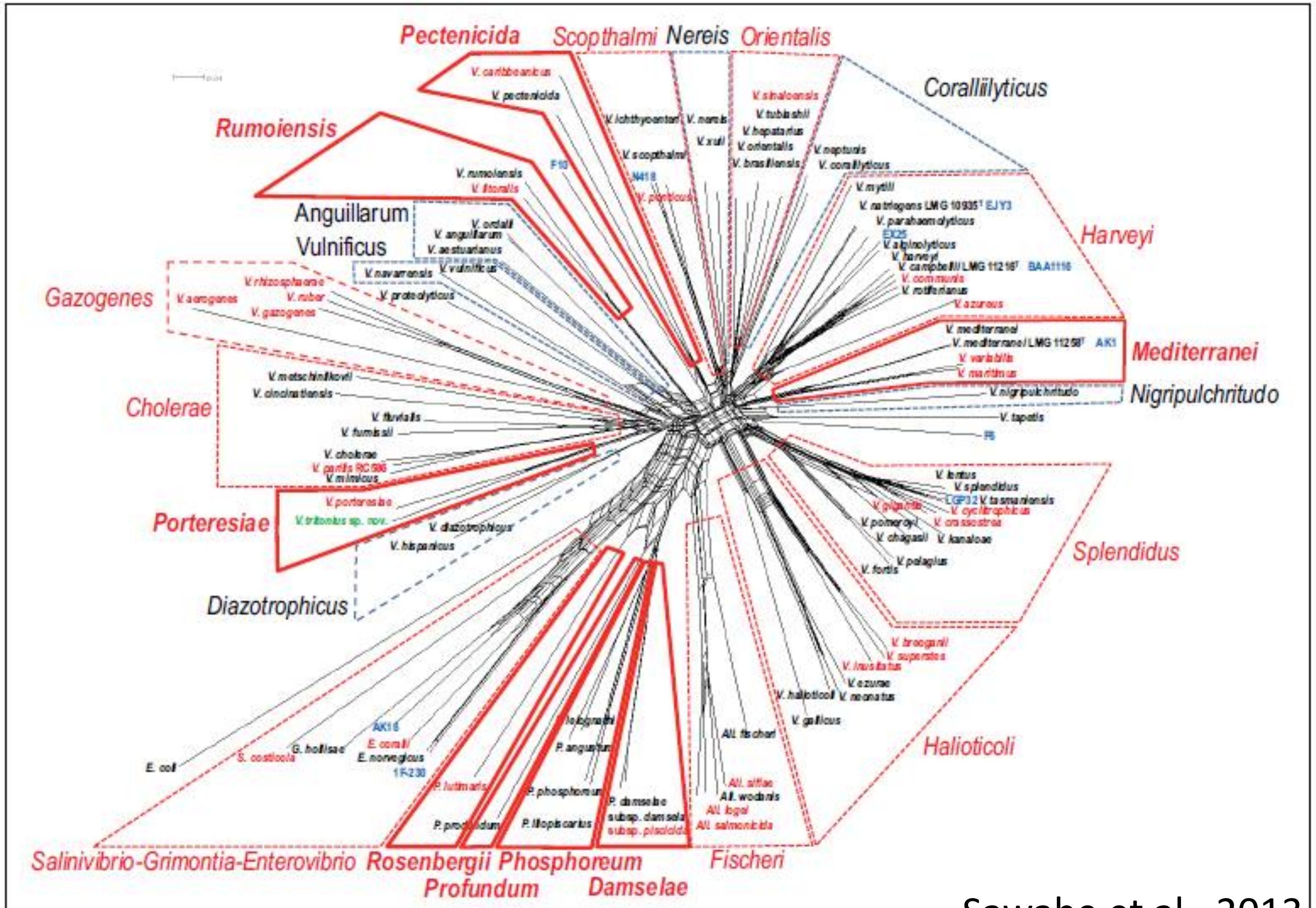


Necrosis aguda hepatopancreática AHPND/EMS-Enfermedad bacteriana más emergente



Lee et al., 2015

Vibrios agrupados en clados



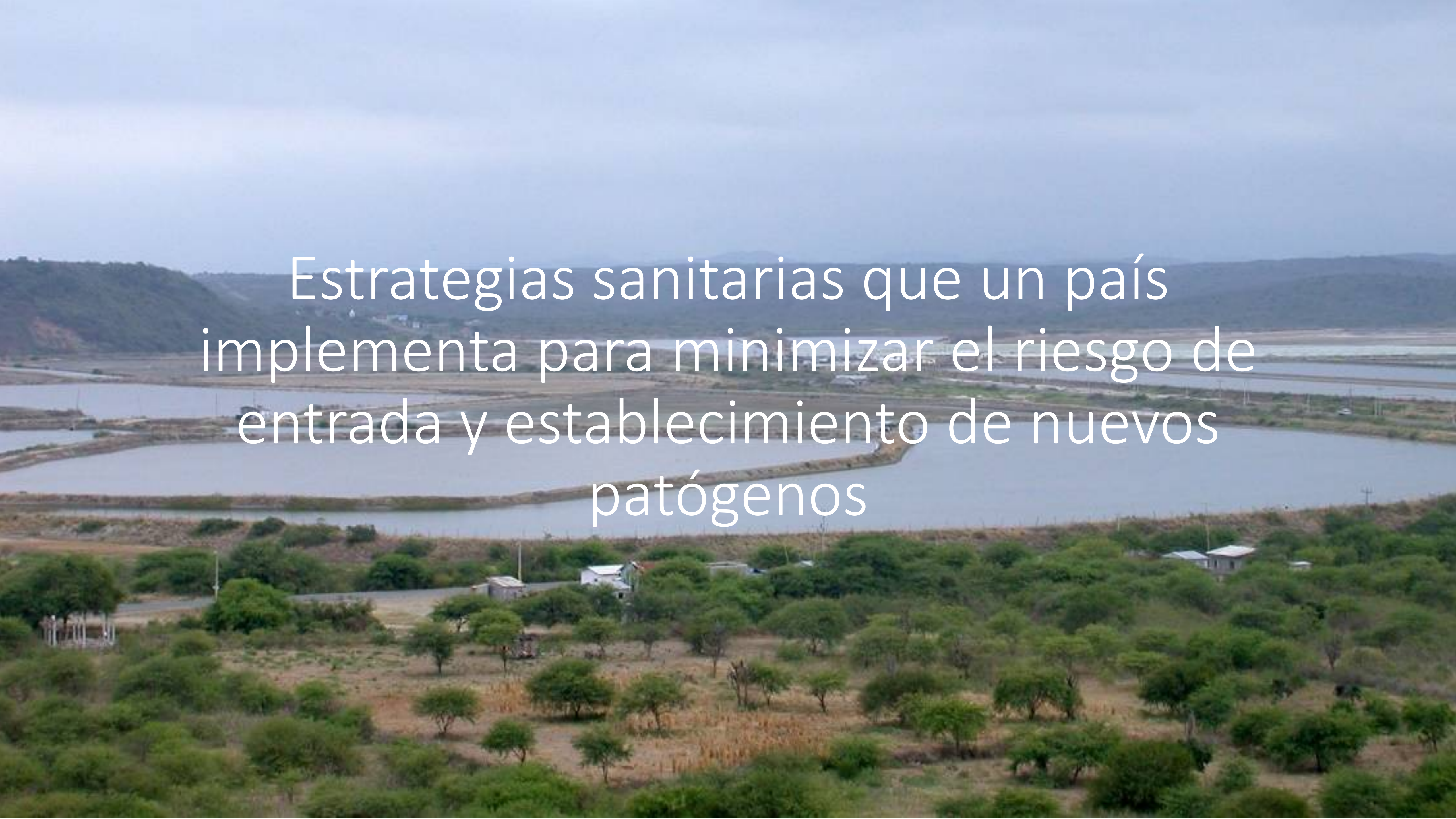
Sawabe et al., 2013

FIGURE 2 | Concatenated split network tree based on eight gene loci. The *ftsZ*, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA*, and *topA* gene sequences from 96 taxa were concatenated, and a tree was reconstructed using the SplitsTree4 program. Clades indicated by a solid red line were the "new" clades proposed in this study. Clades indicated by a dotted red line or by a dotted black line are the clades "emended" and "un-changed," respectively.

Respuesta a problemas emergentes de vibriosis (integral)

- Estrategias para minimizar el riesgo de introducción a un país
- Diagnóstico de la situación
- Estudiar la amenaza
- Estrategias de prevención y control

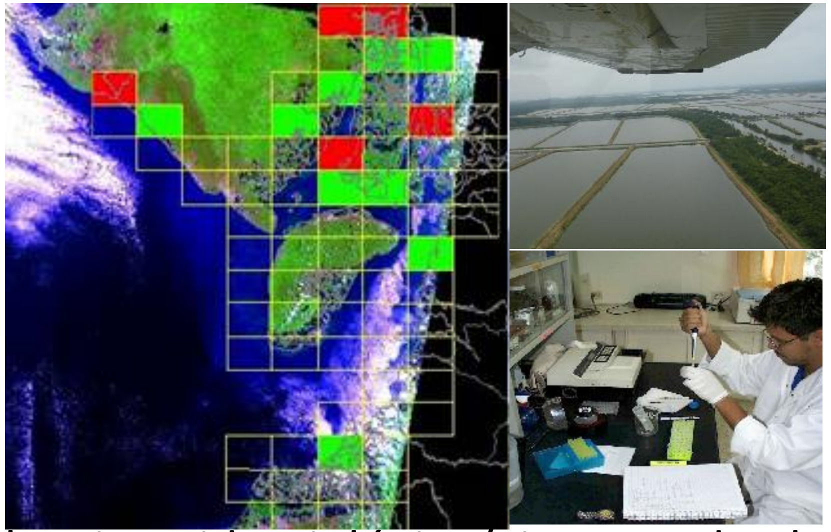
Estrategias sanitarias que un país implementa para minimizar el riesgo de entrada y establecimiento de nuevos patógenos



Disminuir riesgo de introducción patógenos a nivel de país

Análisis de riesgo a la importación

Cuarentena (aislamiento)



Vigilancia epidemiológica/Sistemas de alerta

CENAIM-ESPOL

Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM)

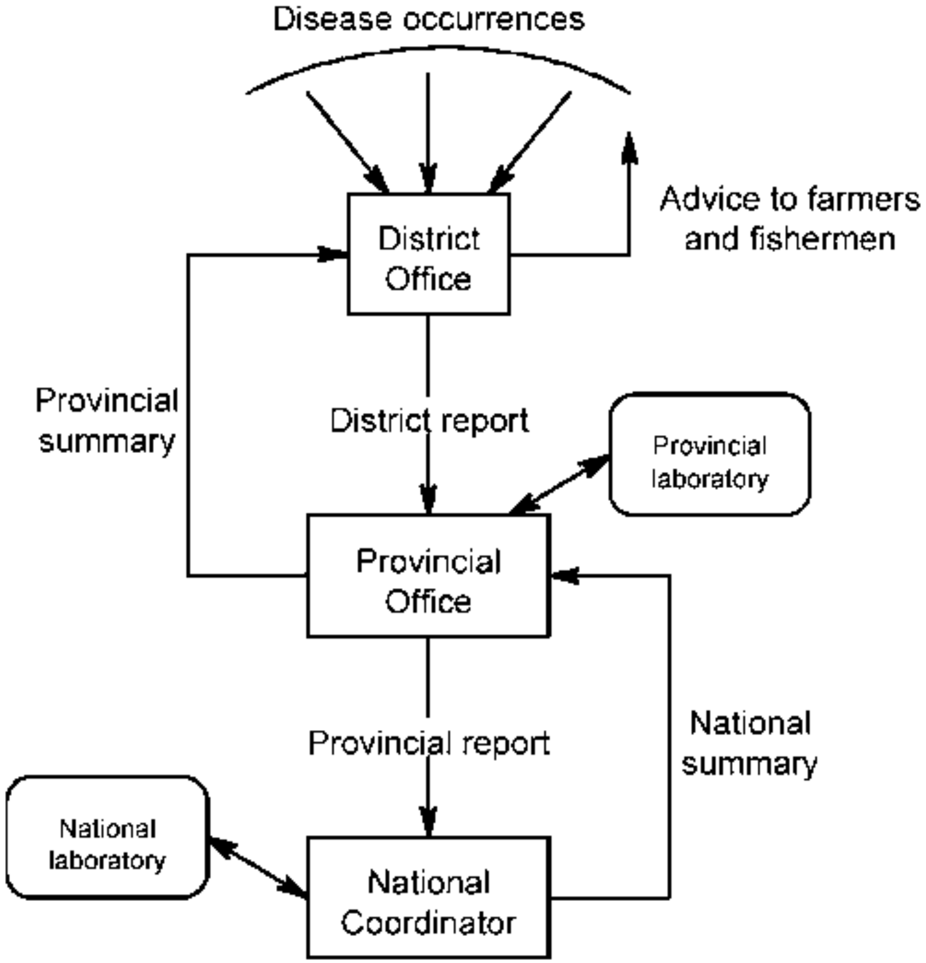
Plan de Contingencia Acuícola (PCA)[1]

Documento sobre la Preparación y Acción ante Epidemias de Camarón Cultivado

Planes de Acción

Vigilancia Epidemiológica y Planes de Acción

Redes de Alerta Sanitaria



- Autoridad sanitaria competente
- Agencias de salud
    - Agencias centrales
    - Agencias locales
- Veterinarios de campo
- Laboratorios de diagnóstico
- Órganos de decisión
- Usuarios finales

Productor concientizado acerca de amenazas (participar de la vigilancia)

Herramientas de diagnóstico

- Implementación de metodología sensible y específica para patógenos emergentes
- Plataforma de herramientas integral: microbiología, histopatología, biología molecular
- Implementación se realice apenas aparezca una amenaza fuera de las fronteras
- Personal muy calificado
- Inter-calibración entre laboratorios nacionales e internacionales

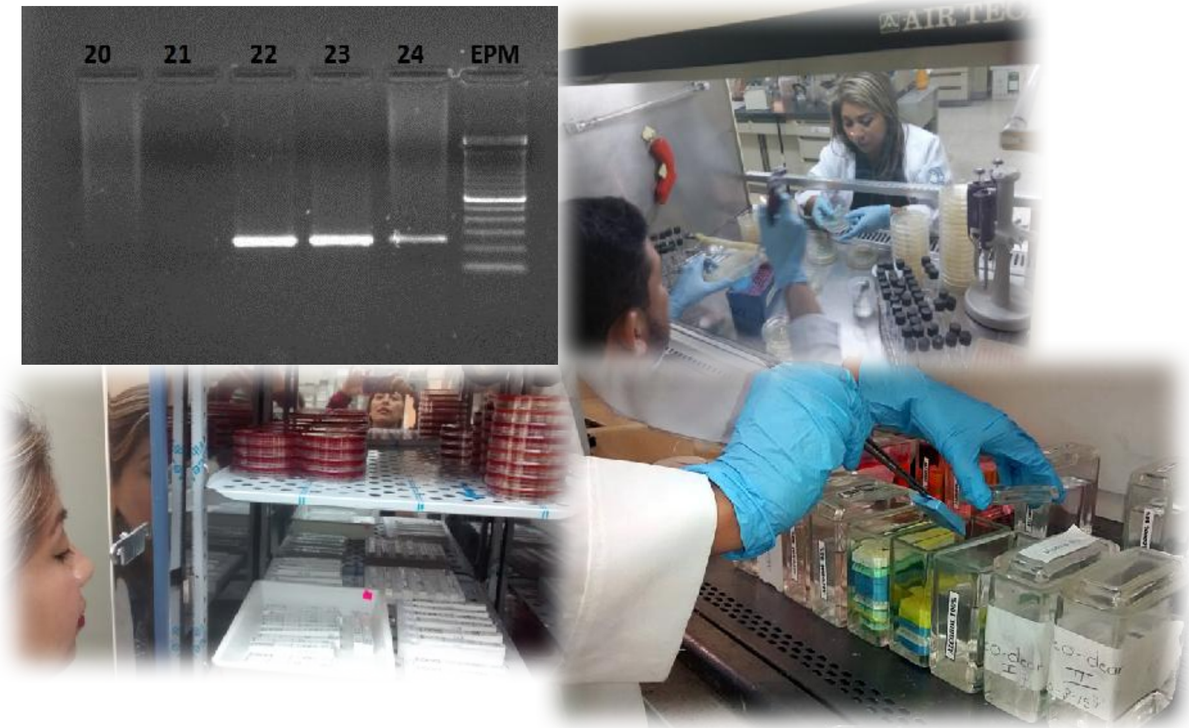
Diagnóstico de la situación

Monitoreo – Colección de datos

- Recogida de datos históricos, clínicos y complementarios (situación epidemiológica)
  - Investigación de brotes – unidades de producción (¿Qué, Cuando, Cuanto, Donde, Porque?)
  - Información clínica (edad, tamaño, signos externos)
  - Manejo (densidades, alimentación, supervivencias, crecimiento, días de cultivo, productos usados, protocolo de manejo)
  - Ambiente (parámetros ambientales, vectores, clima)
  - Bioseguridad
  - Localidad, extensión, afectación contigua/separada
  - Documentación fotográfica

Monitoreo – Colección de muestras

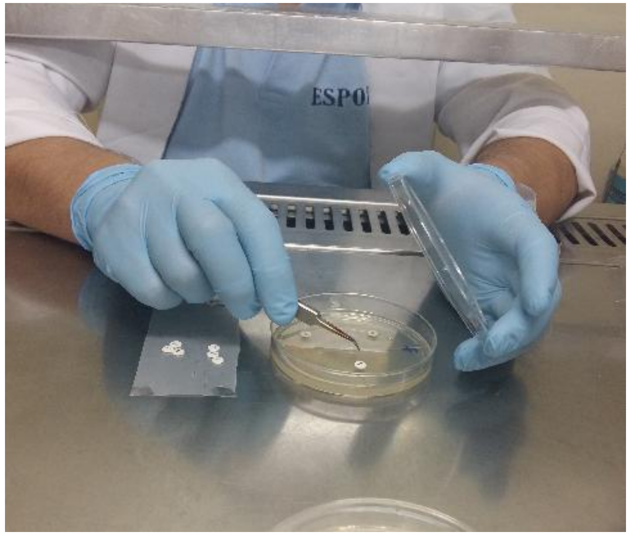
- Estudios de detección, prevalencia
- Identificación patógeno/enfermedad
  - Plataforma integral de métodos de diagnóstico (microbiología, histopatología, biología molecular)



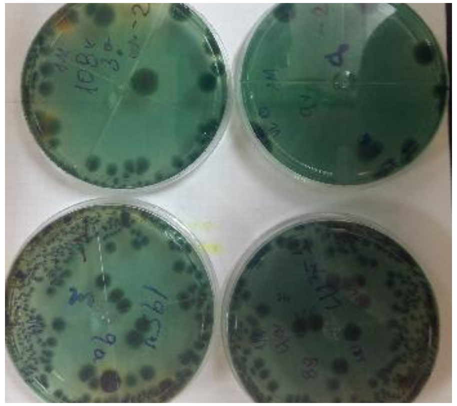
Estudiar la amenaza

Caracterización microbiológica

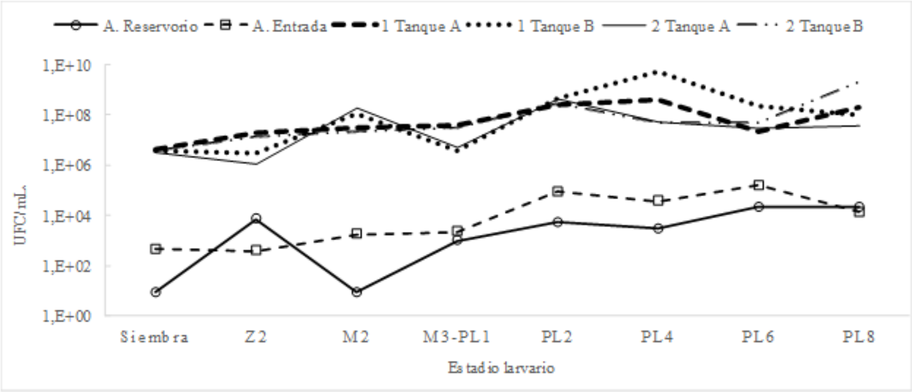
- Cargas bacterianas (camarón/agua/suelo/insumos/tanques)
- Identificación bacteriana (técnicas bioquímica)
- Aislamiento de cepas



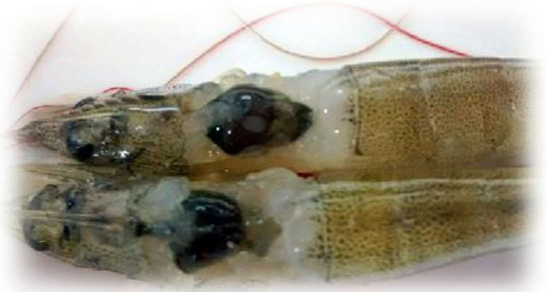
Carga bacteriana

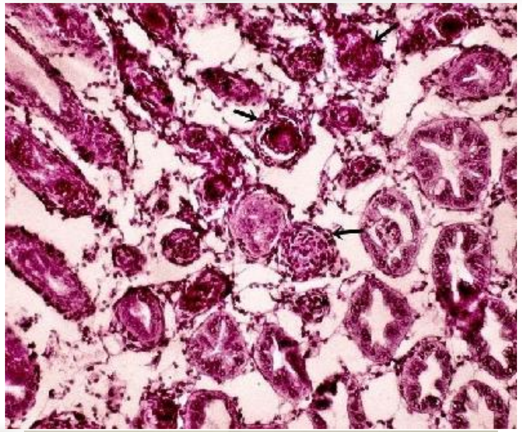


Carga bacteriana en el sistema



Caracterización patológica





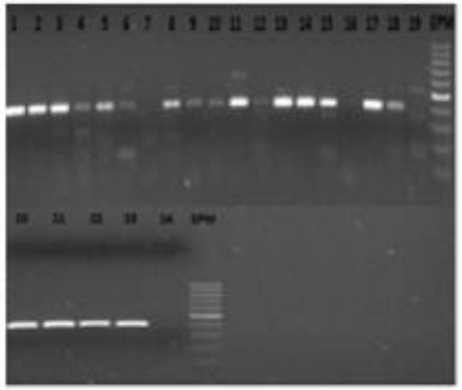
Caracterización *in situ* de la patología

- Observar manifestaciones clínicas y de enfermedad
- Análisis en fresco (implementado por productores)
- Determinar características y severidad de lesiones histológicas

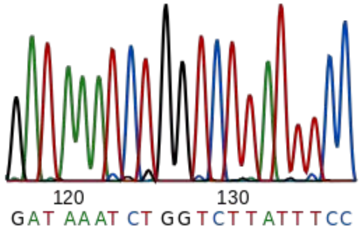
Caracterización molecular

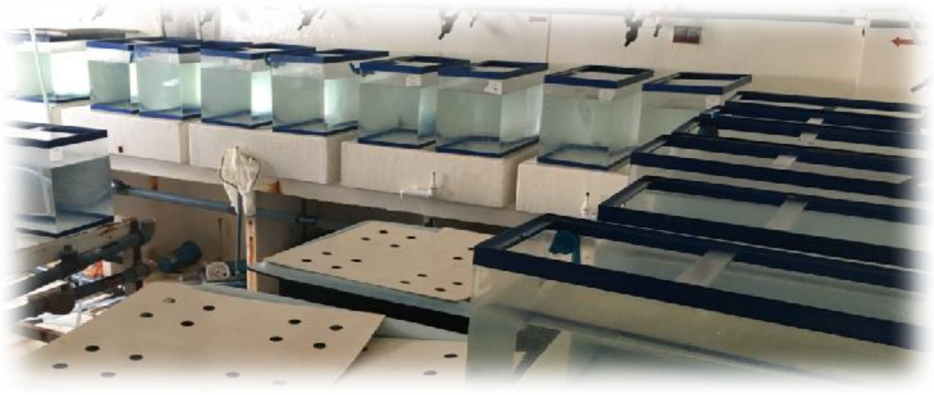
- Identificación del patógeno o patogenicidad con primer específicos
- Secuenciación (16S rADN, MLSA) para identificación molecular de la especie
- Mejoramiento del diagnóstico
- Origen, propagación y evolución del patógeno
- Cepas circulantes y relación con letalidad

Amplificación de genes específicos



Secuenciación



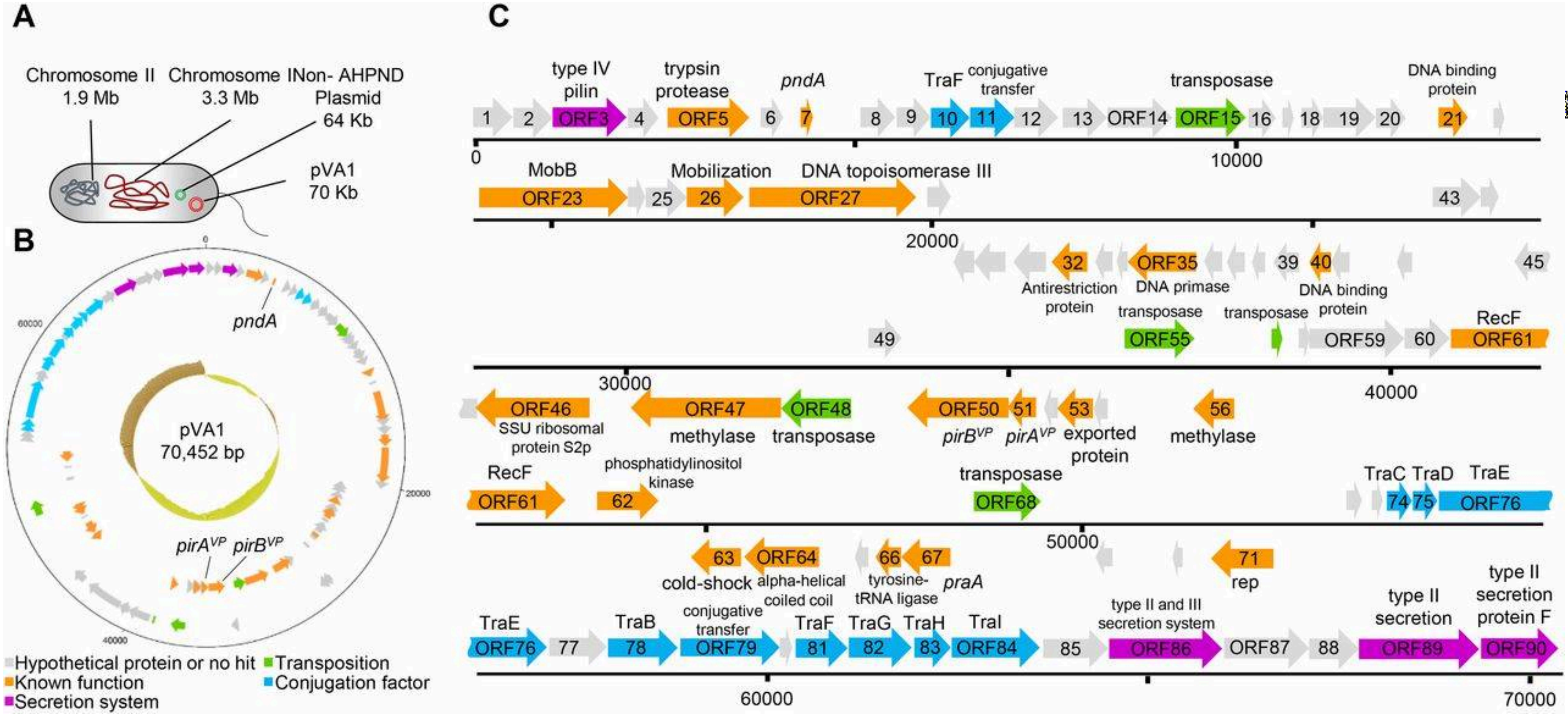


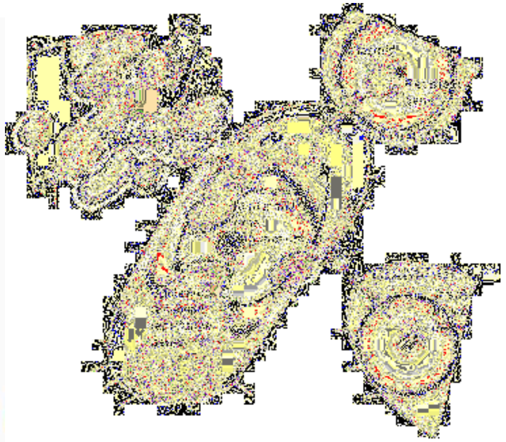
Pruebas de desafío

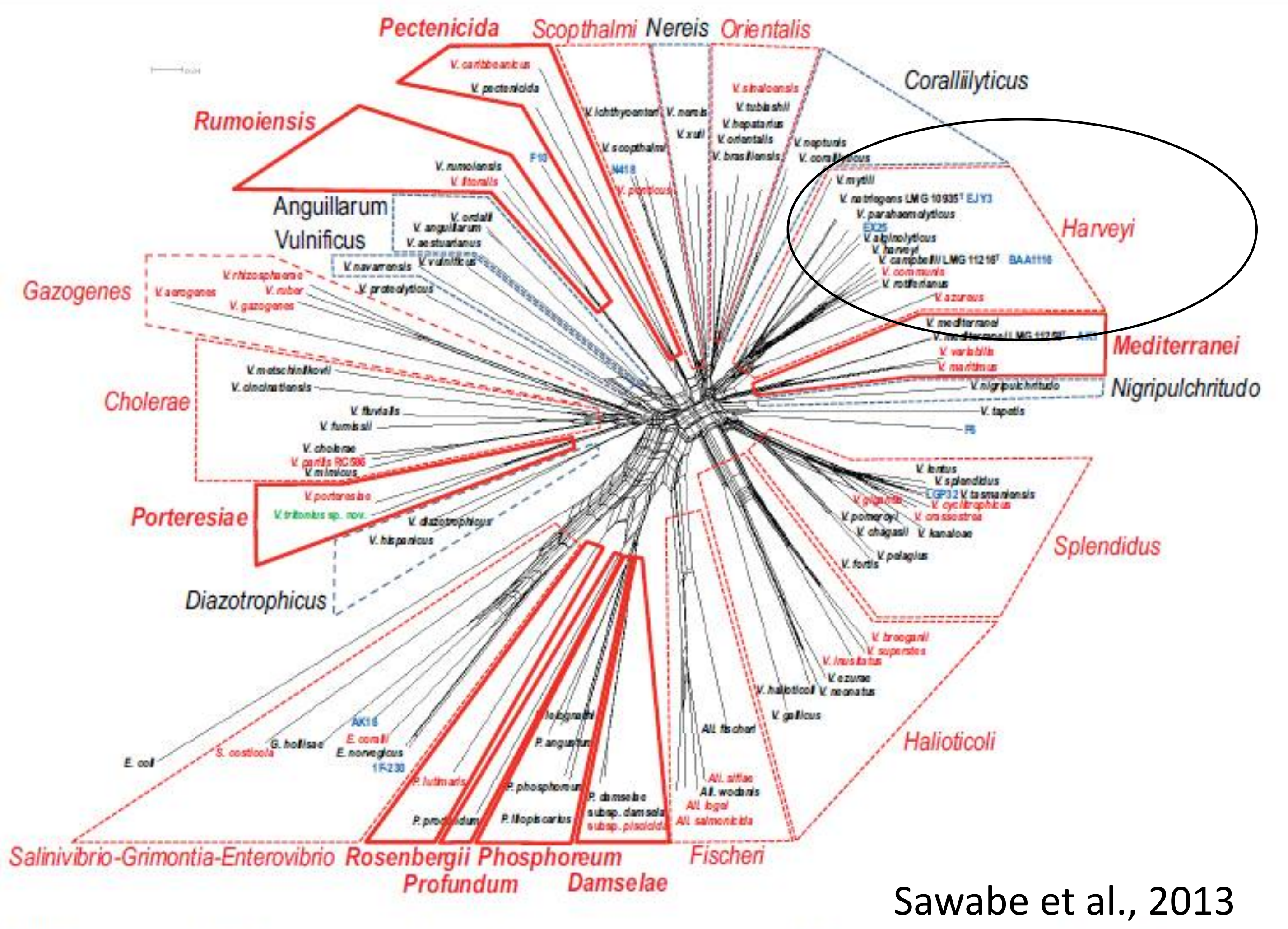
Estudios genómicos – Genoma completo (NGS)

- Genoma completo y comparación de genomas de distintos aislados geográficos
  - Diagnóstico
  - Identificación de la especie
  - Origen, propagación y evolución
  - Estudio *in silico* de comportamiento de cepas a determinados medios
  - Identificación de factores de virulencia (invasión, cause enfermedad y evada defensa huésped)

Estudios genómicos – Transferencia horizontal de genes







Sawabe et al., 2013

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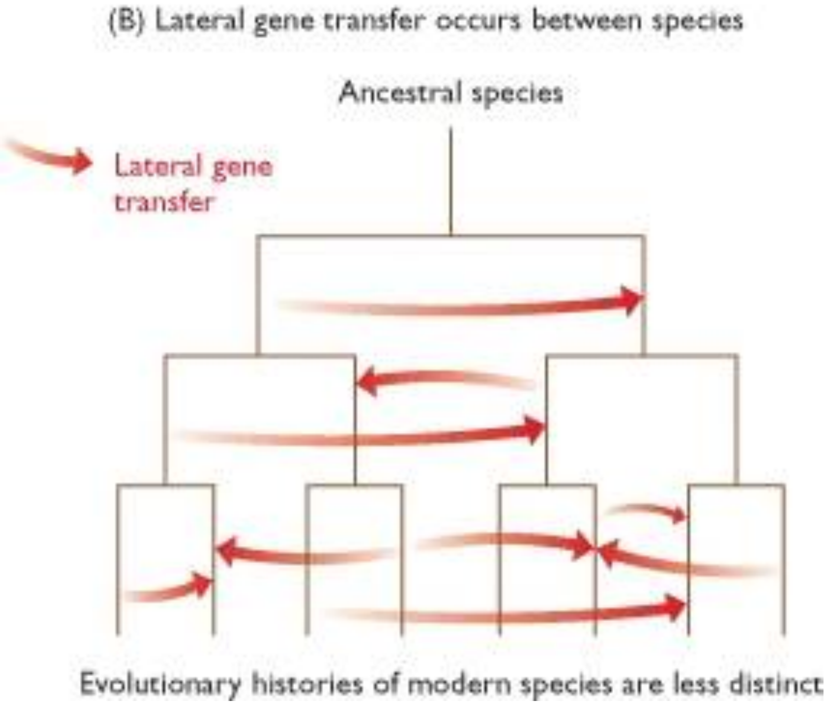
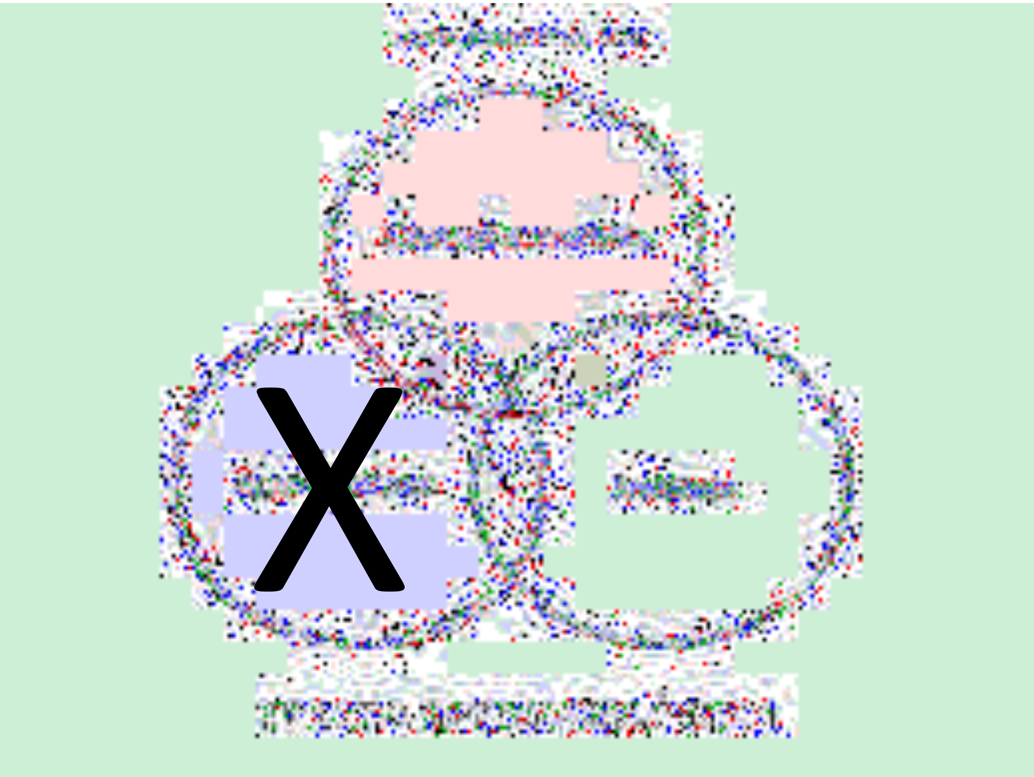



Control

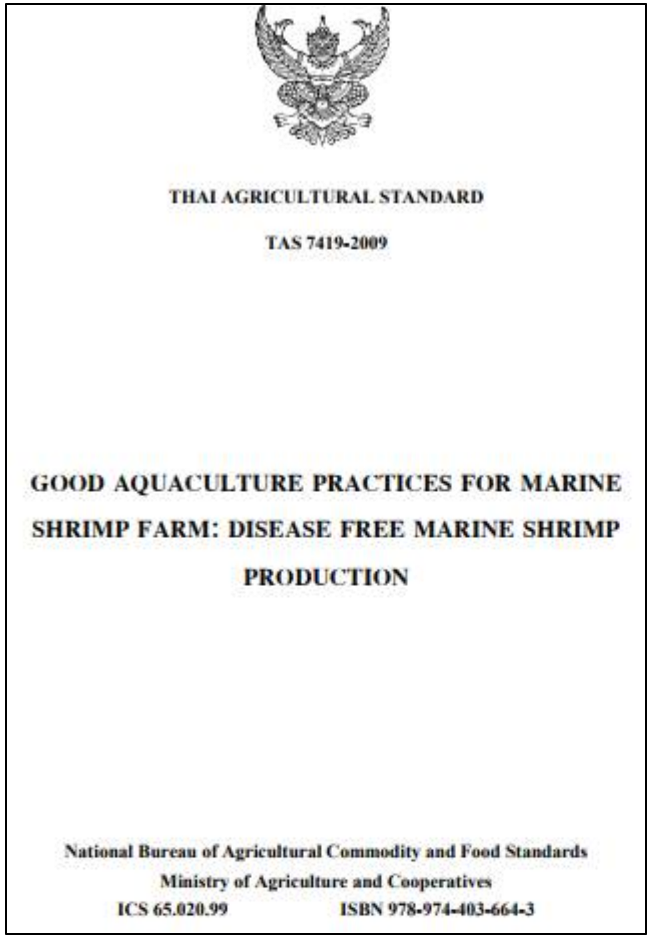
Bioseguridad



Minimizar riesgo de entrada, dispersión y salida de patógenos

Adopción de buenas prácticas de manejo

- Guía para productores
- Todos las etapas de práctica de la camaronera en laboratorios y nursery
  - Localización
  - Manejo de animales
  - Uso y almacenamiento de productos,
  - Manejo de efluentes
  - Sanidad
  - Cosecha, colecta y manejo post cosecha previa a la transportación



THAI AGRICULTURAL STANDARD

TAS 7419-2009

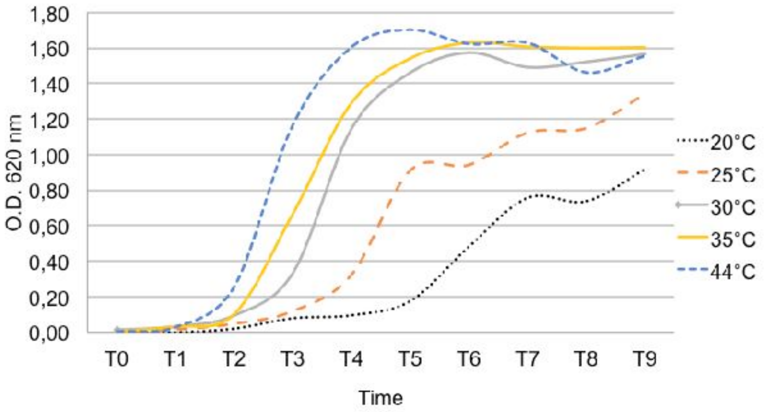
GOOD AQUACULTURE PRACTICES FOR MARINE SHRIMP FARM: DISEASE FREE MARINE SHRIMP PRODUCTION

National Bureau of Agricultural Commodity and Food Standards
Ministry of Agriculture and Cooperatives
ICS 65.020.99 ISBN 978-974-403-664-3

Pruebas *in vitro*

- Comportamiento de cepas a distintas condiciones ambientales
- Pruebas de sensibilidad de patógenos a agentes terapéuticos y no terapéuticos (antibiogramas y MIC)

Comportamiento a distintas condiciones ambientales

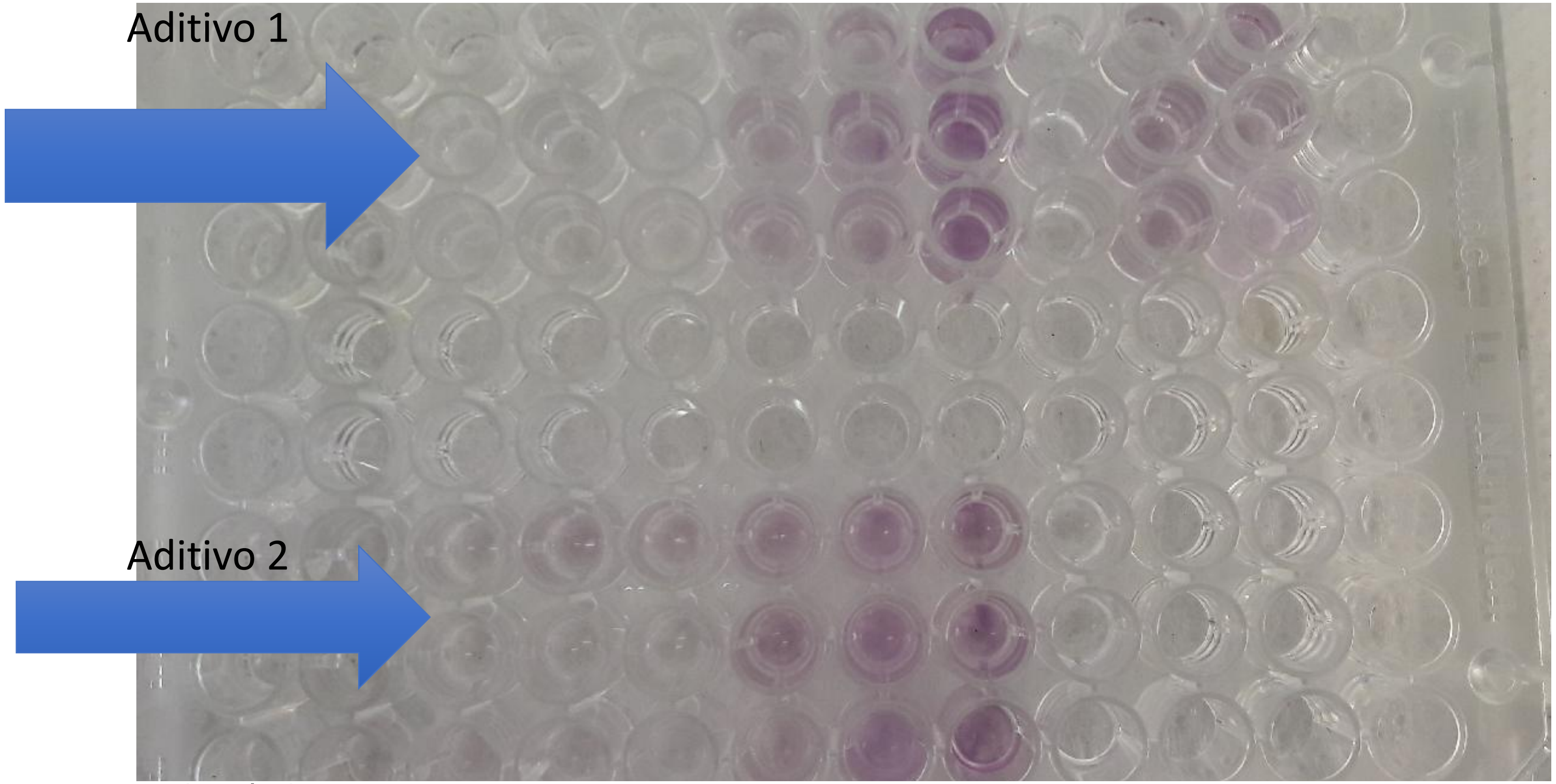


Toxicidad de productos

De mayor a AAC Menor concentración

Más tóxico Menos tóxico

Ensayo de viabilidad celular (actividad metabólica) por reducción del compuesto MTT

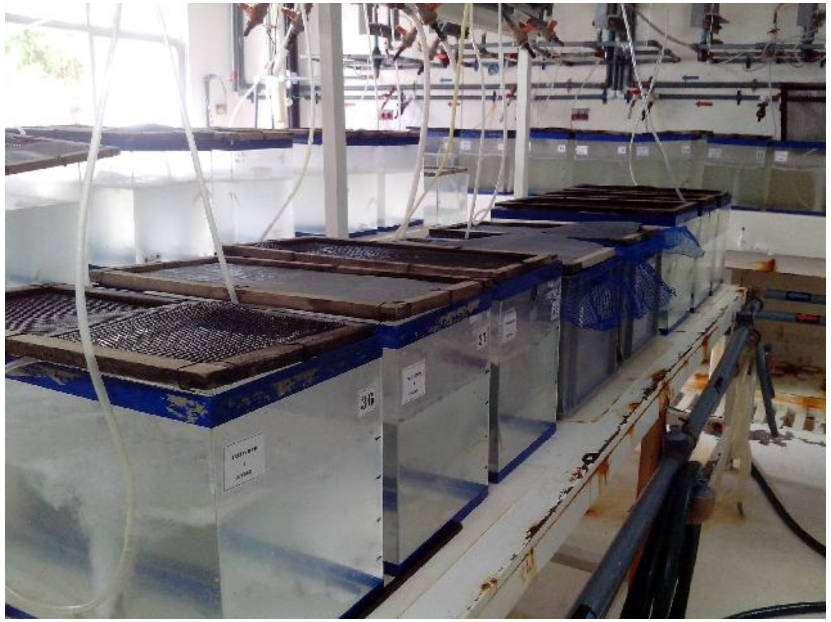


Domínguez y Rodríguez, 2017 (en elaboración)

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Pruebas de desafío (Challenge test) – Pruebas *in vivo*

- Comprobación de postulados de Koch con cepas aisladas
- Estandarización de pruebas de desafío (reproducibles en tiempo)
- Validación de agentes terapéuticos con cepas aisladas y distintas por criterios microbiológicos, genómicos y patológicos
- Relación entre cepas genéticamente distintas y letalidad
- Pruebas de resistencia de animales a la enfermedad



Antibióticos
Método de control tradicional

- Antibióticos usados en forma rutinaria y como profilaxis tiene importantes desventajas
  - Resistencia bacteriana a antibióticos
  - Transmisión horizontal de genes de resistencia a antibióticos
    - Bacterias acuícolas
    - Patógenos humanos
  - Asociado a aparición de cepas virulentas
  - Muchos casos es inefectivo en producción

Table 1

The different classes of antibiotics used in aquaculture, their importance for human medicine and examples of (multi)resistant pathogenic bacteria isolated from aquaculture settings.

Drug class	Importance for human medecine[a]	Example	Resistant bacteria	Multiple[b] resistance?	Isolated from	Reference
Aminoglycosides	Critically important	Streptomycin	*Edwardsiella ictulari*	Yes	Diseased striped catfish (*Pangasianodon hypophthalmus*), Vietnam	[6]
Amphenicols	Important	Florfenicol	*Enterobacter* spp. and *Pseudomonas* spp.	Yes	Freshwater salmon farms, Chile	[7]
Beta-lactams	Critically important	Amoxicillin	*Vibrio* spp., *Aeromonas* spp. and *Edwardsiella tarda*	Yes	Different aquaculture settings, Australia	[8]
Beta-lactams	Critically important	Ampicillin	*Vibrio harveyi*	Yes	Shrimp farms and coastal waters, Indonesia	[9]
Fluoroquinolones	Critically important	Enrofloxacin	*Tenacibaculum maritimum*	Yes	Diseased turbot (*Scophthalmus maximus*) and sole (*Solea senegalensis*), Spain and Portugal	[10]
Macrolides	Critically important	Erythromycin	*Salmonella* spp.	Yes	Marketed fish, China	[11]
Nitrofurans	Critically important	Furazolidone	*Vibrio anguillarum*	Yes	Diseased sea bass and sea bream, Greece	[12]
Nitrofurans	Important	Nitrofurantoin	*Vibrio harveyi*	Yes	Diseased penaeid shrimp, Taiwan	[13]
Quinolones	Critically important	Oxolinic acid	*Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp.	Yes	Pond water, pond sediment and tiger shrimp (*Penaeus monodon*), Philippines	[14]
Sulphonamides	Important	Sulphadiazine	*Aeromonas* spp.	Yes	Diseased katla (*Catla catla*), mrigel (*Cirrhinus mrigala*) and punti (*Puntius* spp.), India	[15]
Tetracyclines	Highly important	Tetracycline	*Aeromonas hydrophila*	Yes	Water from mullet and tilapia farms, Egypt	[16]
Tetracyclines	Highly important	Oxytetracycline	*Aeromonas salmonicida*	Yes	Atlantic salmon (*Salmo salar*) culture facilities, Canada	[17]

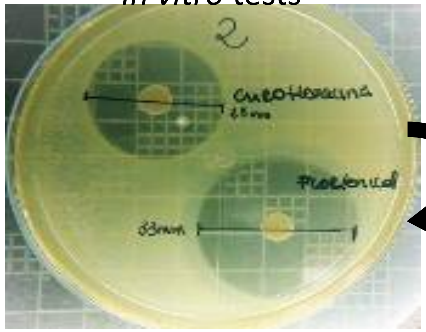
[a] On the basis of World Health Organisation Expert Consultations on 'Critically Important Antimicrobials for Human Medicine' [18*].
[b] Resistance to antibiotics belonging to different classes in at least one of the isolates.

Defoirdt et al. 2011

Búsqueda de nuevos probióticos

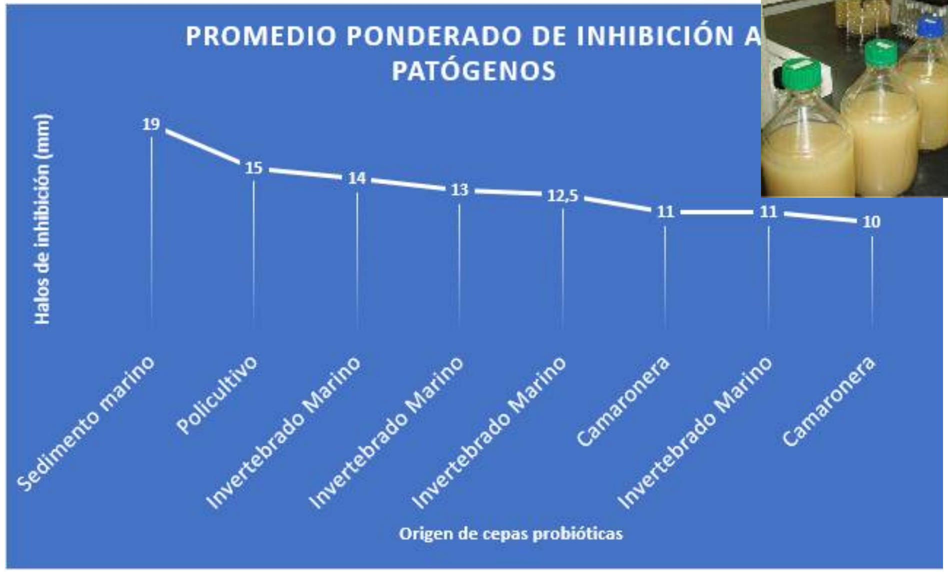
- Cribado de cepas bacterianas marinas, fuentes de compuestos bioactivos antagonistas a patógenos acuáticos
- Bacterias benéficas aislada de sistemas de producción (*Bacillus*, tecnología a punto)
- Bacterias efectivas en otras enfermedades

In vitro tests



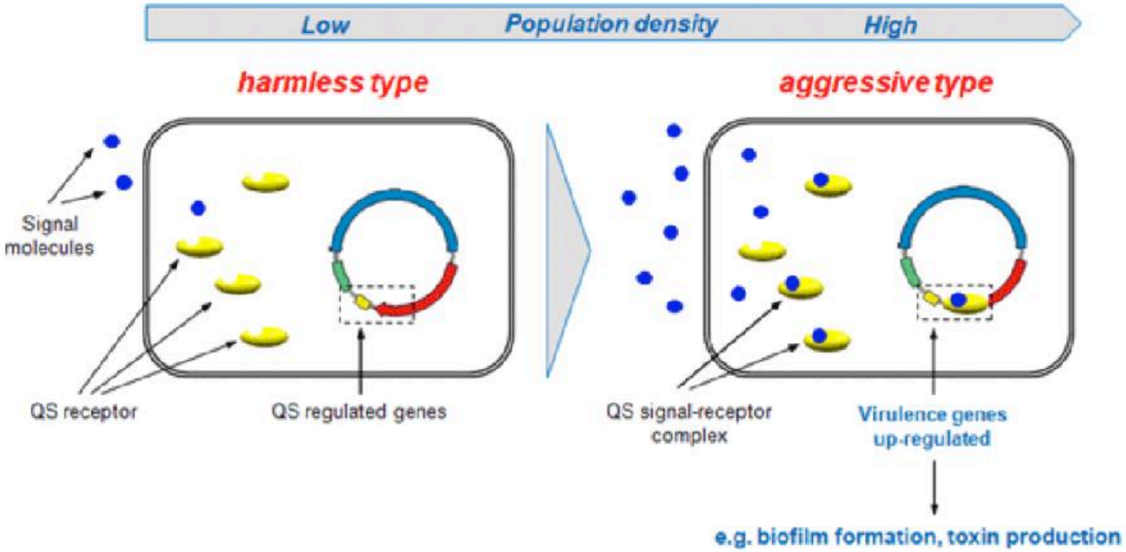
In vivo tests

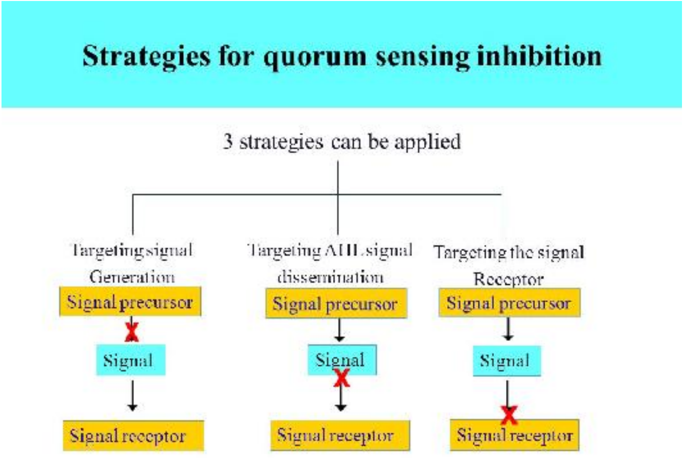
Field experiments



Inhibición de expresión de genes de virulencia a través de inhibición quorum sensing

Quorum sensing





Quorum quenching

Natural compound(s)	Source	QS activity	Ref.
Furanone/ 2(5H)-Furanone/	Macroalga (*Delisea pulchra*)	Mimics AHL signal by occupying the binding site on putative regulatory protein which results in the disruption of QS-mediated gene regulation. Inhibit biofilm formation in *Aer. hydrophila*	[109, 17]
		Repress LuxR protein dependent expression of P(luxI)-gfp(ASV) reporter fusion. Inhibit virulence factor in *E. coli* XL-1.	[110]
(5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone.	Macroalga (*Delisea pulchra*)	Disrupts QS-regulated bioluminescence in *V. harveyi* by interacting with Hfq protein. Inhibit swarming motility and biofilm formation in *E. coli*	[111, 112]
Ajoene (1-Allyldisulfanyl-3-(prop-2-ene-1-sulfinyl)-propene)	Garlic extract (*Allium sativum*)	Blocks the QS-regulated productions of rhamnolipid resulting in phagocytosis of biofilm. Targets Gac/RSM part of QS and lowers the expression of regulatory RNAs in *P. aeruginosa* PAO1	[113, 114]
Iberin (1-Isothiocyanato-3-(methylsulfinyl)propane)	Horseradish extract (*Armoracia rusticana*)	Inhibit expression of QS-regulated *lasB-gfp* and *rhlA-gfp* genes responsible for virulence factor in *P. aeruginosa*	[115]
Sulforaphane (1-Isothiocyanato-4-(methylsulfinyl)butane)	Boroccoli	Reduce the expression of lasI-luxCDABE reporter in *P. aeruginose*	[116]
Erucin (4-methylthiobutyl isothiocyanate)	Boroccoli	Reduce the expression of lasI-luxCDABE reporter in *P. aeruginose*	[116]
Naringin (4'5-diOH-Flavone-7-rhgluc)	Citrus extract	Decrease the QS mediated biofilm formation and swimming motility in *Y. enterocolitica*	[18]
Naringenin (4',5,7-Trihydroxyflavanone)	Malagasy bark extract (*Combretum albiflorum*)	Reduces production of pyocyanin and elastase in *P. aeruginosa* PAO1. Also inhibit 3-oxo-C12-HSL and C4-HSL synthesis driven by *lasI* and *rhlI* genes	[117, 118]
Taxifolin/ Distylin (dihydroquercetin)	Malagasy plant extract (*Combretum albiflorum*)	Reduces production of pyocyanin and elastase in *P. aeruginosa* PAO1	[117]
Morin (2',3,4',5,7-Pentahydroxyflavone)	Grapefruit (*Artocarpus heterophyllus*)	Inhibit LasR and RhlR dependent protease, elastase and hemolysin in *P. aeruginosa* PAO1	[119, 120]
Patulin/ Clavacin (4-Hydroxy-4*H*-furo[3,2-c]pyran-2(6*H*)-one)	*Penicillium sp.*	Targets the RhlR and LasR proteins. Down-regulates QS genes for biofilm formation and virulence in *P. aeruginosa*	[121]
Penicillic acid (3-Methoxy-5-methyl-4-oxo-2,5-hexadienoic acid)	*Penicillium sp.*	Down-regulates QS genes for biofilm formation in *P. aeruginosa*	[121]
Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	Vanilla beans extract (*Vanilla planifolia Andrews*)	Interfere with AHL receptors. Inhibit C4-HSL, C6-HSL, C8-HSL, 3-oxo-C8-HSL. Inhibit biofilm formation in *Aer. hydrophila*	[122, 123, 16]
Agrocinopine B ([(3S,4R,5R)-3,4,5,6-tetrahydroxy-2-oxohexyl] [(2R,3S,4S)-3,4,5-trihydroxy-1-oxopentan-2-yl] hydrogen phosphate)	Crown gall cells	Control conjugation of pTiC58 by regulating expression of the arc operon in *A. tumefaciens*	[124]
L-canavanine (L-α-Amino-γ-(guanidinooxy)-n-butyric acid)	Seed exudates (*Medicao sativa*)	Inhibit the expression of QS-regulated phenotype exopolysaccharide II production in *Si. meliloti*	[125]
Gamma-aminobutyric acid (GABA) (4-Aminobutanoic acid)	Plants (*Arabidopsis sp.*)	Induce the expression of attKLM operon to stimulate inactivate 3-oxo-C8-HSL by *A. tumefaciens* lactonase AttM	[126, 127]
Rosmarinic acid (R-O-(3,4-Dihydroxycinnamoyl)-3-(3,4-dihydroxyphenyl) lactic acid)	Sweet basil (*Ocimum basilicum*)	Inhibit protease, elastase, hemolysin production, biofilm formation and virulence factor in *P. aeruginosa*	[119, 108, 128]
Salycilic acid (2-Methyl-5-tert-butylsalicylic acid)	Plant phenolic secondary metabolite	Inhibit the expression of vir regulon in *A. tumefaciens*. Also stimulates AHL-lactonase expression which degrades AHLs.	[129]
Chlorogenic acid (3-Caffeoylquinic acid)	Plant extract (*Moringa oleifera*)	Inhibit QS-regulated violacein production in *C. violaceum* 12472	[130]
Allin (2-Amino-3-[prop-2-ene-1-sulfinyl]-propionic acid)	Garlic extract (*Allium sativum*)	Inhibit QS-regulated gene expression by interacting with receptors in *P. aeruginosa* and make biofilm sensitive to antibiotics.	[113, 131]
Ursolic acid (3beta-Hydroxyurs-12-en-28-oic acid)	Plant extract (*Sambucus chinensis*)	Inhibit biofilm formation by suppressing cysteine synthesis in *E. coli*	[132, 133]
Ellagic acid (Benzoaric acid)	Fruit extract of *Terminalia chebula Retz.*	Down-regulate the expression of virulence gene in *P. aeruginosa* PAO1. Reduces biofilm formation and swarming motility in *B. cepacia*	[134, 135]
α-Hydroxybutyric acid (2-hydroxy-butanoic acid)	*Arabidopsis exudates*	Induce the expression of attKLM-lacZ fusion in *A. tumefaciens*	[136]
Epigallocatechin gallate (Epigallocatechol)	Green tea (*Camellia sinensis* L.)	This compound has gallic acid moiety and specifically block AHL-mediated biofilm formation in *Sta. aureus* and *B. cepacia*. Inhibit transfer of conjugative R plasmid in *E. coli*	[135, 137-139]
Pyrogallol (1,2,3-Trihydroxybenzene)	Plant extract (*Punica granatum*)	Inhibit AI-2 mediated bioluminescence in *V. harveyi*	[140, 141]
Cinnamon oil/ Cynnamaldheyde (trans-Cinnamaldehyde)	*Cinnamomum zeylanicum*	Interfere with AI-2 based QS and decreases the DNA-binding ability of LuxR protein to reduce virulence in *V. spp*. Reduces LuxR-mediated transcription from the PluxI promoter which influences biofilm formation in *P. aeruginosa*	[142, 143]
Furocoumarin/ Psoralen (7H-Furo[3,2-g][1]benzopyran-7-one)	Grapefruit juice and extract (*Psoralea corylifolia* L.)	The structural resemblance of furan moiety results in QS-mediated inhibition of biofilm formation in *E. coli*. Inhibit QS-mediated swarming motility in *P. aeruginosa* PAO1	[144, 145]
Urolithin (3,8-Dihydroxy-benzo[c]chromen-6-one)	Ellagitannin-rich extract from Pomegranate	Inhibit C6-HSL and 3-oxo-C6-HSL associated biofilm formation in *Y. enterocolitica*. Inhibit QS-mediated swarming motility in *E. coli*	[146, 147]

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Biocontrol of Luminous Vibriosis in Shrimp Aquaculture: A Review of Current Approaches and Future Perspectives

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ABSTRACT
Healthy shrimp culture system is always in harmony with the ecology of the pond environment. This can be manipulated by developing a dense heterotrophic bacterial community that takes care of waste generated in the system through in situ bioremediation. Considering the importance to reduce an occurrence of luminous vibriosis in shrimp aquaculture, countless studies have been carried out with an objective to screen anti-vibrio biological agents, which can be used as an alternative to antibiotics. In such studies, microalgae, bacteriophage, and probiotic bacteria have been found to have potential benefits in reducing vibriosis. Eco-based shrimp farming, green water technology, bio-floc technology, phage therapy, and integrated multi-trophic aquaculture (IMTA), since their inception, hold a promising alternative to antibiotics in the near future. This article seeks to secure all the available information on different biological agents, their involvement in lowering *Vibrio* load, and strategies to control *Vibrio* infection in shrimp aquaculture.

KEYWORDS
Bio-floc; green water technology; probiotics; microalgae; phage therapy

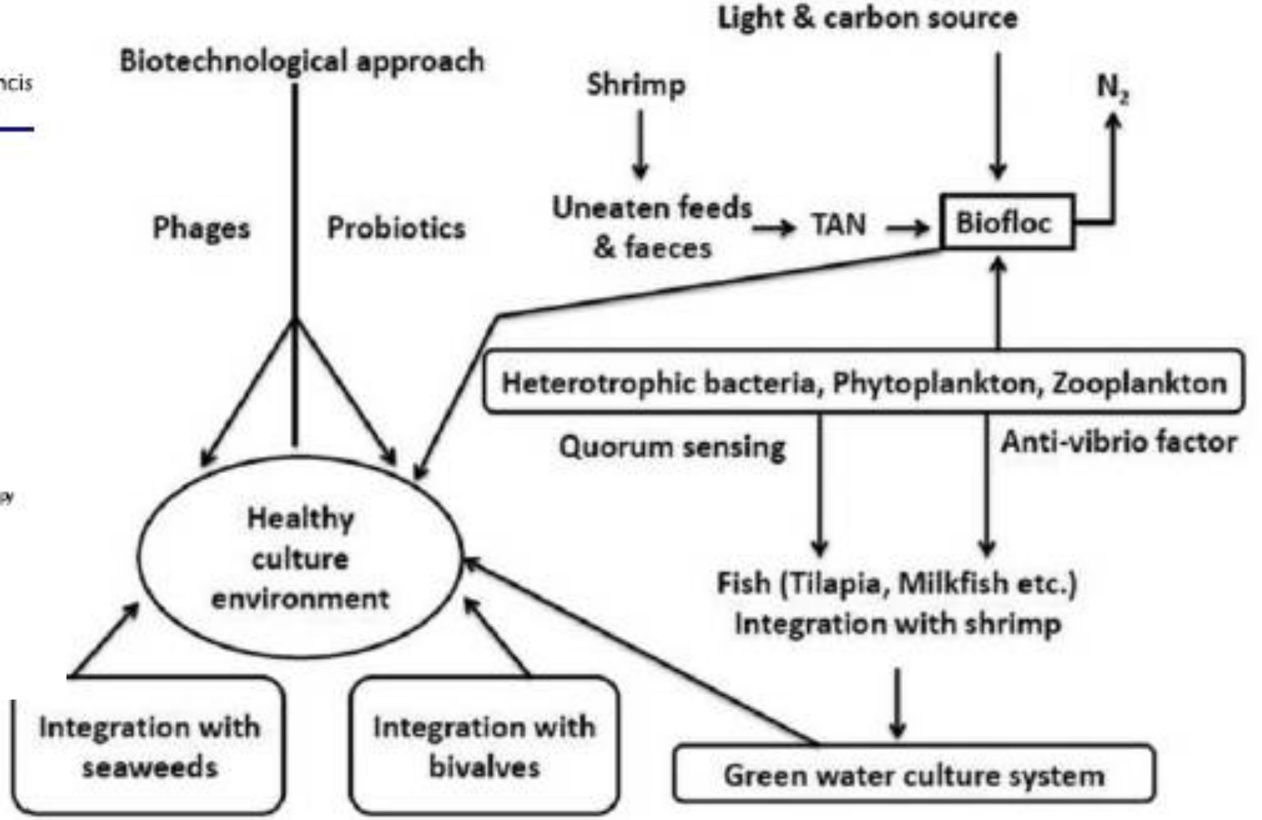

Figure 2. Schematic of integrated and biotechnological approaches to sustain healthy shrimp culture environment.

Integración de hidrobiontes y acuacultura multitrófica integrada en varios esquemas de combinación a sistemas de cultivo
Crecimiento de microorganismos benéficos (bioflocs y probióticos)

Conclusiones

- Colaboración conjunta entre los involucrados para minimizar los impactos de enfermedades bacterianas
- Es necesario conocer al enemigo para elaborar métodos de control efectivos
- Bioseguridad y buenas prácticas de manejo pueden ser inmediatamente implementadas
- Estrategias son insuficientes si son aplicadas aisladamente
- Aplicación conjunta de varias estrategias podría obtener un mejor resultado de prevención y control