Food and Agriculture Organization of the United Nations

International Technical Seminar on Understanding Antimicrobial Resistance & Biosecurity in Aquaculture

Alternatives to Antimicrobials in Aquaculture

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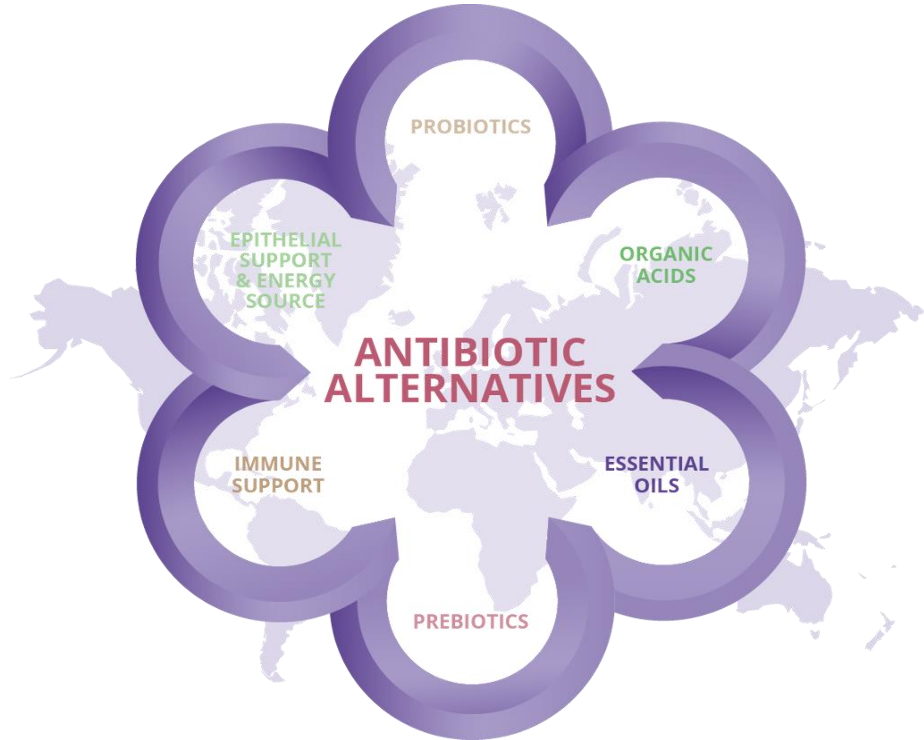
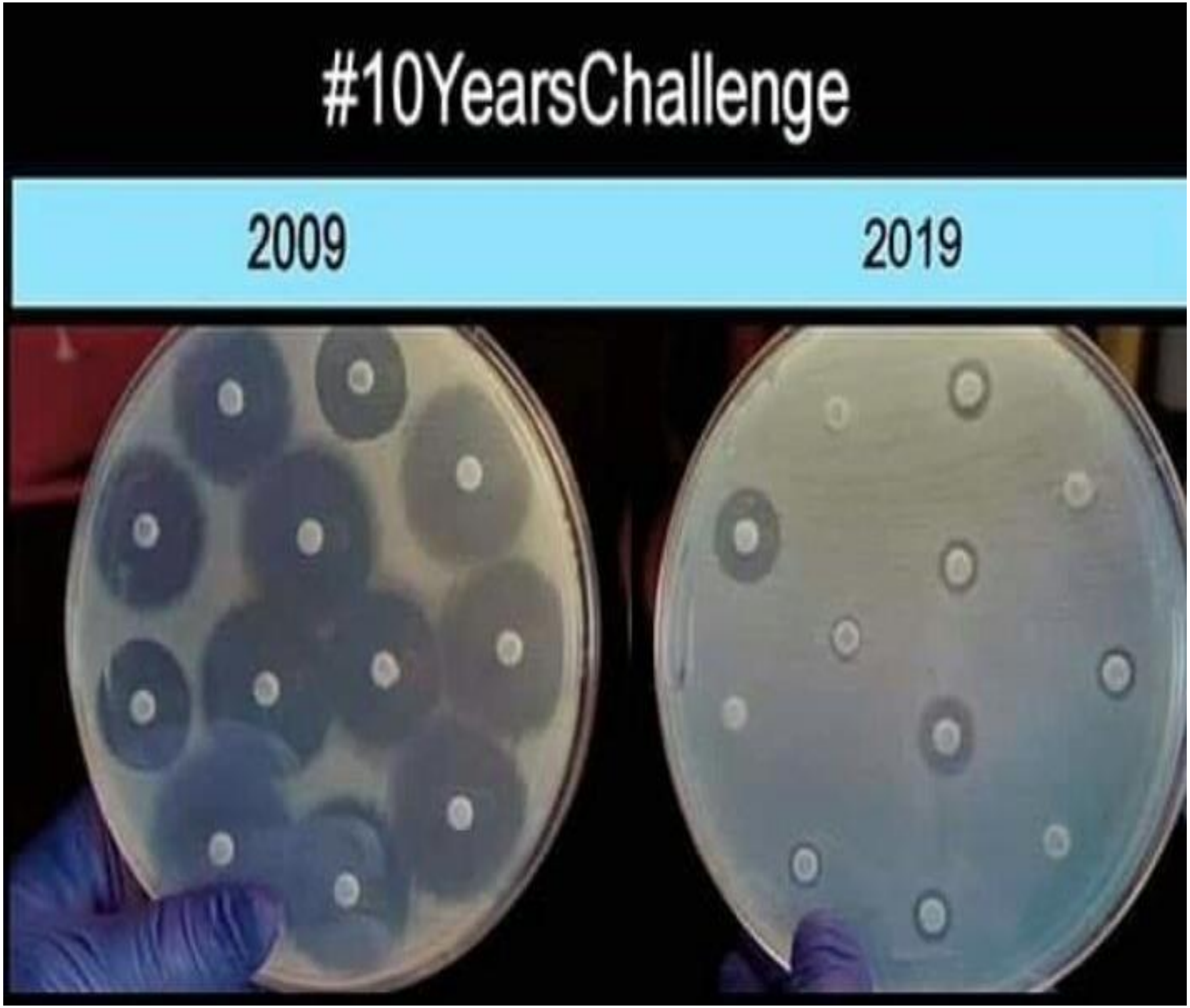
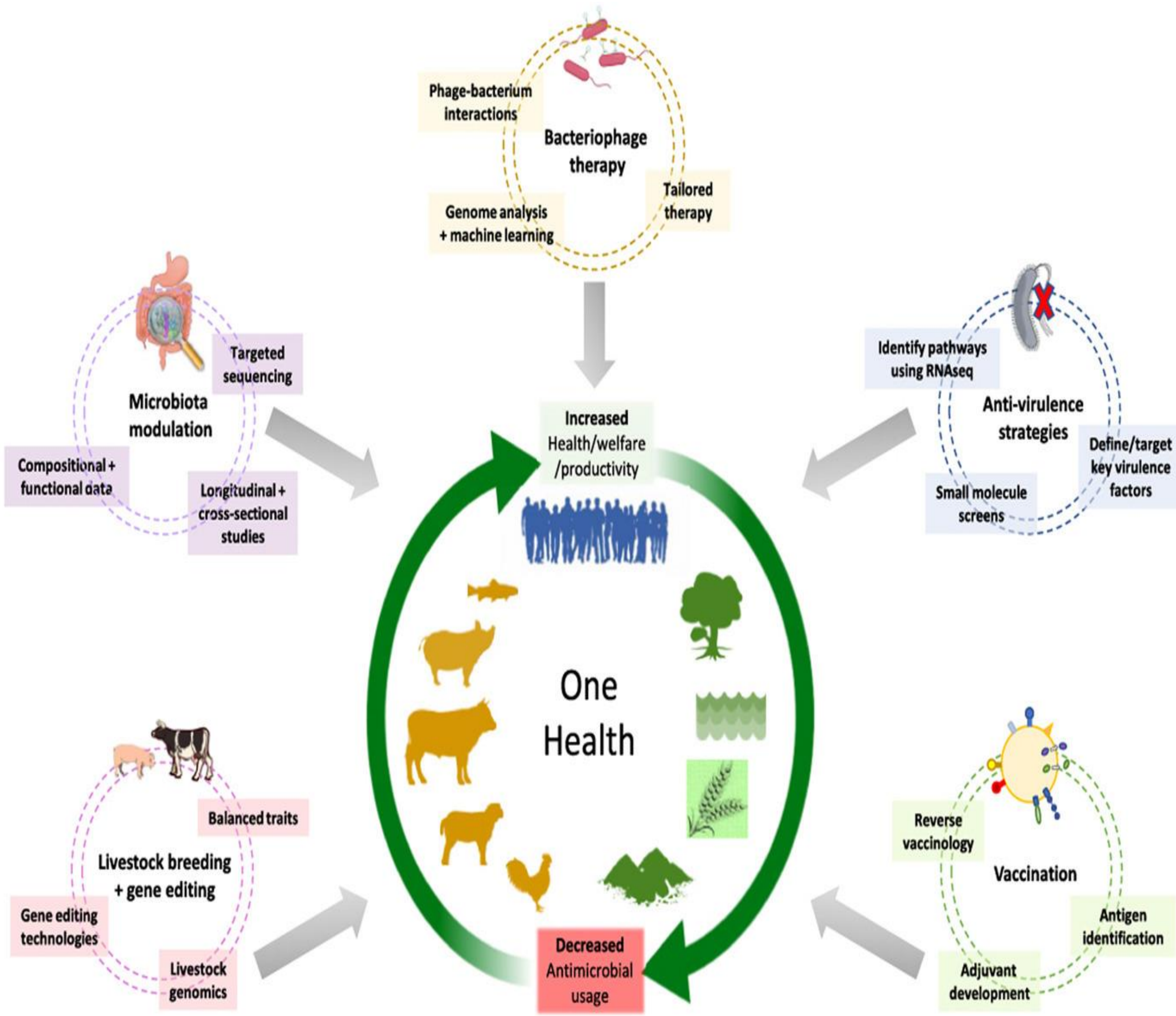



#10YearsChallenge
2009
2019

WHAT WILL REPLACE OUR FAILING ANTIBIOTICS?



ANTIBIOTIC

- Secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms
- Antibiotics are effective only against bacteria
- They are ineffective against viruses, & parasites or most true fungi
- Disease prevention measures - Proactive
- Eg: Regular monitoring of water, larval tanks for bacterial counts, levels of luminous / other bacteria
- Chemotherapy- Reactive
- Only after finding definite evidence of bacterial disease
- Backed up by data on antibiotic sensitivity of causative agent

BACTERIAL RESISTANCE TO ANTIMICROBIAL AGENTS

Use of antibiotics in aquaculture results in

Prolonged persistence of drugs in products intended for human consumption

Release of drugs/metabolites to aquatic environment.

Prolonged and indiscriminate use causes

Ineffectiveness of the drug due to the development of resistance and tolerance by the bacteria.

Transfer of antibiotic resistance from aquaculture system to water borne human pathogens.

When do we consider a microorganism RESISTANT?

A bacteria can be termed resistant, if it has the ability to function, survive or persist in the presence of higher conc. of an antimicrobial agent than the members of the population from which it emerged (Smith, 1994)

Intrinsic resistance

Expressed by chromosomal genes

Extrinsic resistance

Acquired, selective pressure exerted on bacteria during antibiotic administration

Results from

Mutation in chromosomal region

Acquisition of plasmids and transposons

WHY ARE WE LOOKING FOR ALTERNATIVES ?

Emergence of resistant pathogens

Build up of pathogens

Chemical residues in shrimp meat

Destroys useful microorganisms

Effectivity in seawater - questionable

Chemicals overused to compensate ineffectivity

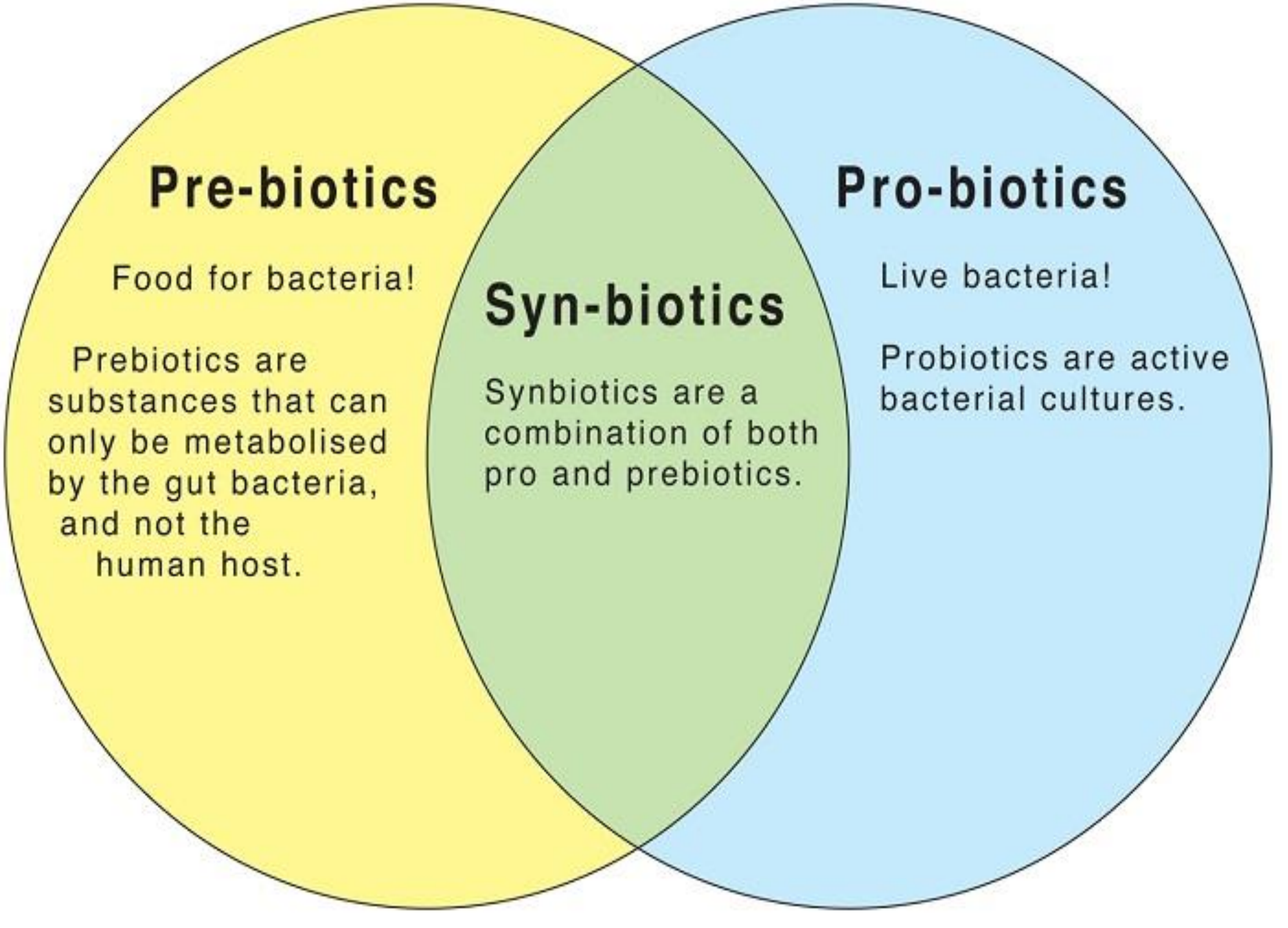
Environmental deterioration due to residues

- **Diseases are many and our interventions are even more**
- **Sometimes rational, most often irrational**
- **Driven by avaracious individuals for quick & maximum returns**
- **No end to greed - disease is an outcome.**

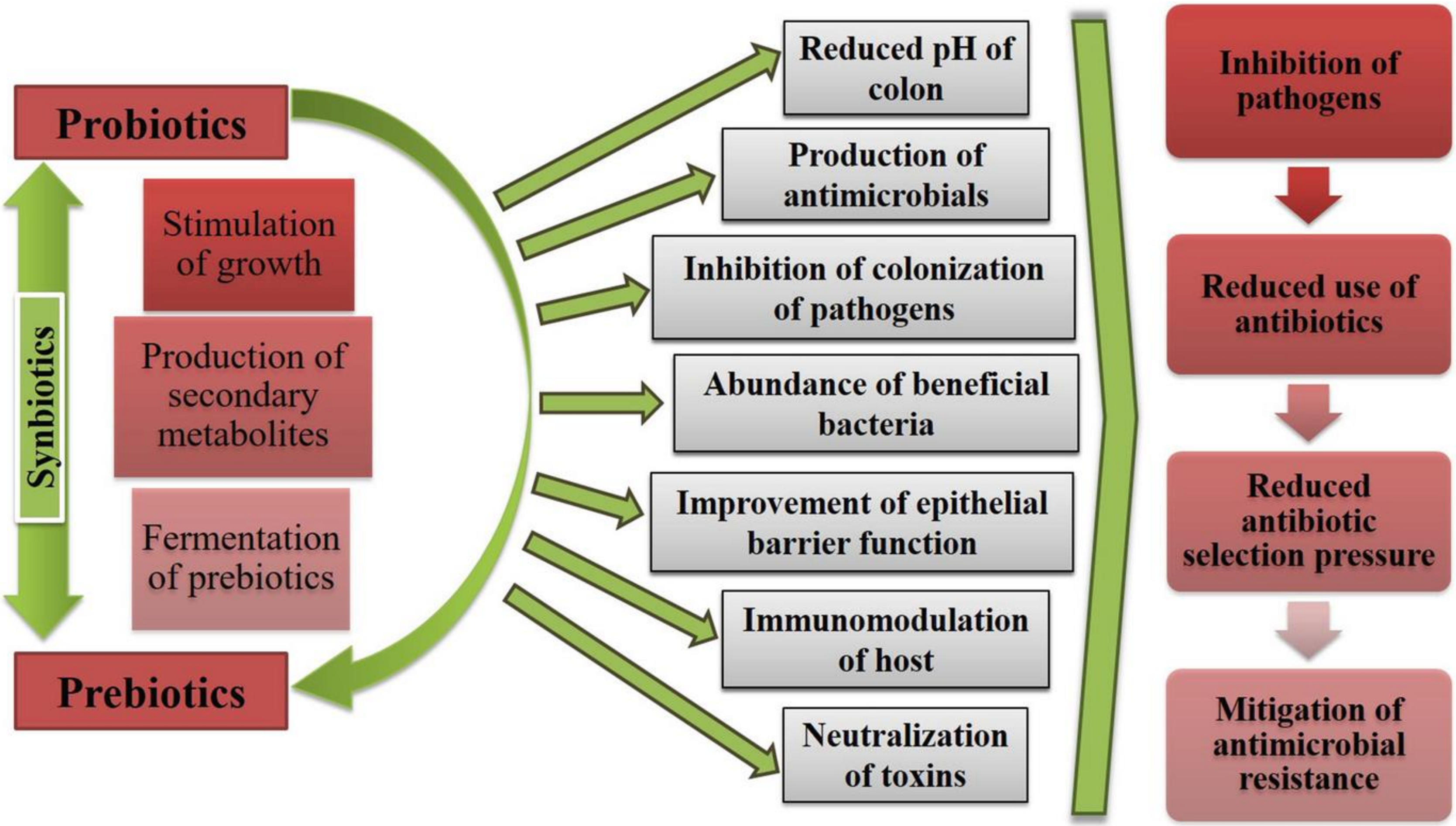
DISEASE MANAGEMENT MEASURES

- **Biofloc- the buzz word today**
- **Probiotics , Bioremediators, Bioaugmentors**
- **Immunostimulants**
- **Bacteriophages or phages**
- **Herbal preparations**
- **Feed and pond water management most crucial**
- **Genetic selection - PL quality, Brood stock quality**

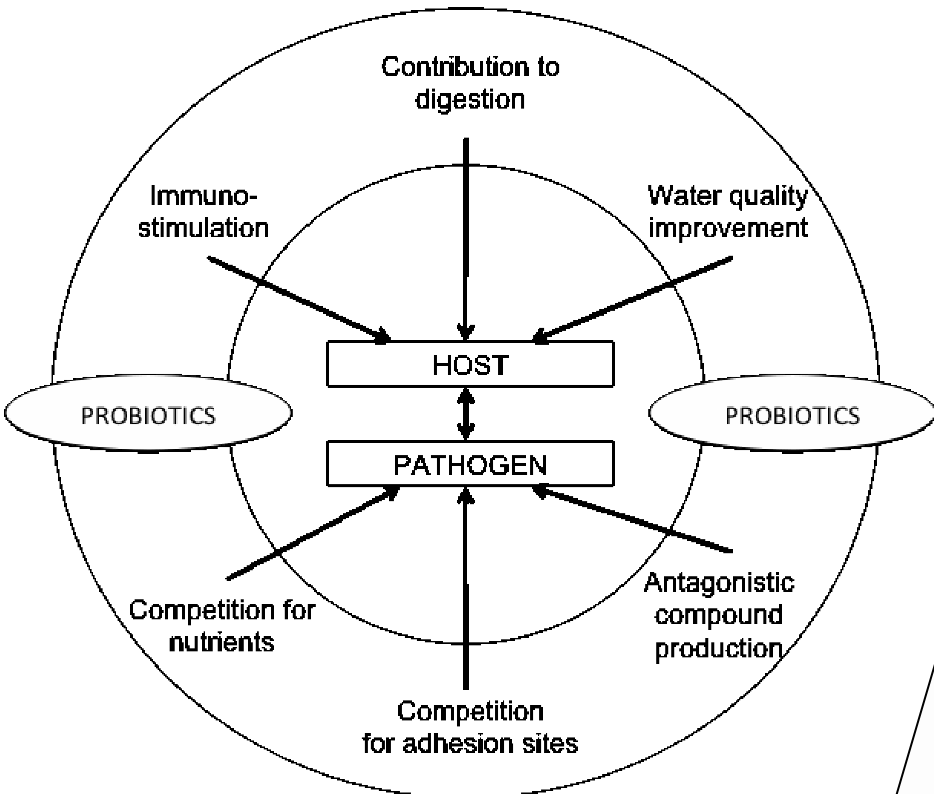
Microbiota Modulation



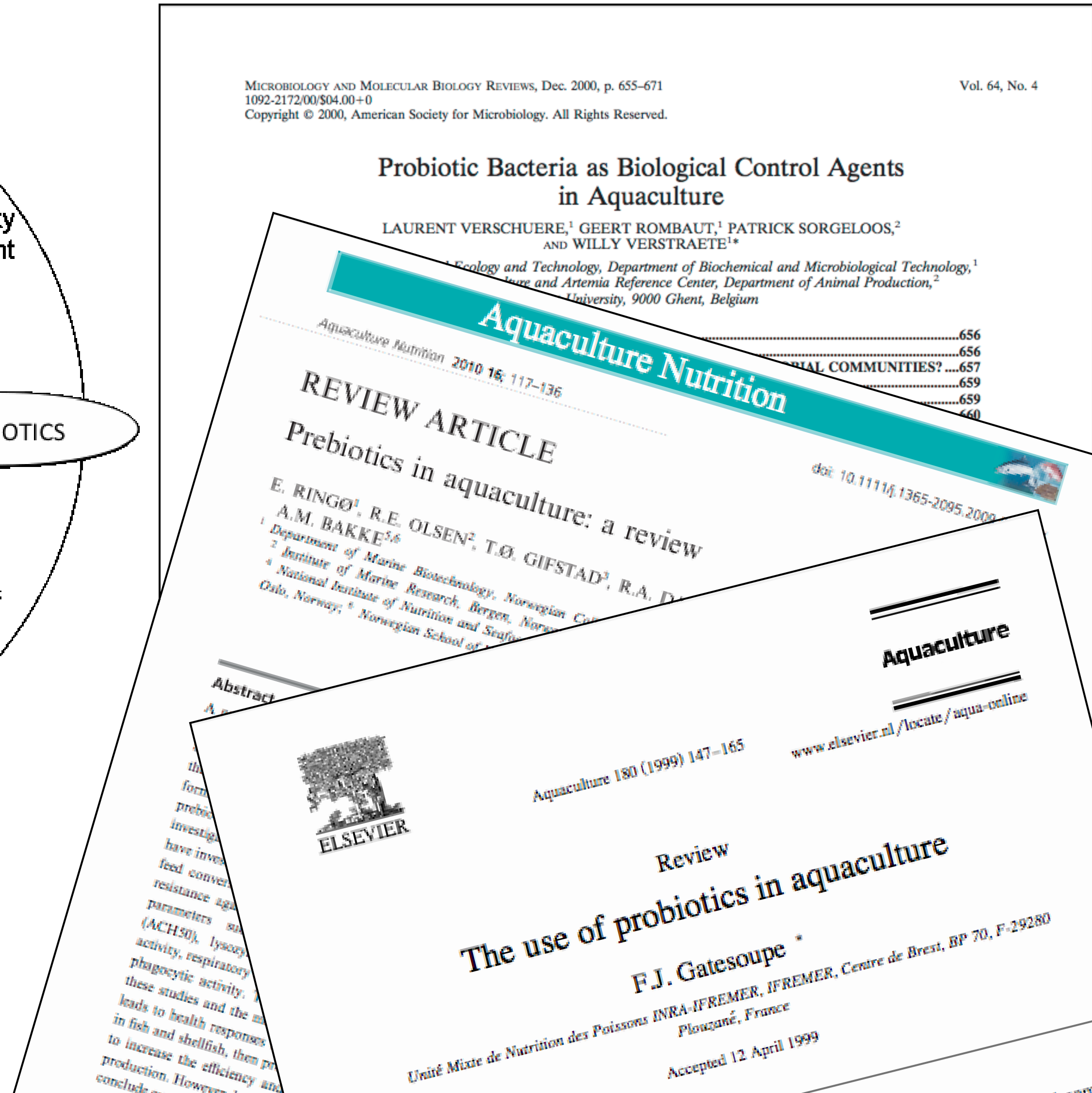
Mode of Action



Probiotics in aquaculture



De Schryver et al., 2012 (In: Infectious disease in aquaculture - prevention and control. Woodhead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Probiotic Bacteria as Biological Control Agents in Aquaculture

LAURENT VERSCHUERE,[1] GEERT ROMBAUT,[1] PATRICK SORGELOOS,[2] AND WILLY VERSTRAETE[1]*

Aquaculture Nutrition

Aquaculture Nutrition 2010 16; 117–136

REVIEW ARTICLE

Prebiotics in aquaculture: a review

E. RINGØ[1], R.E. OLSEN[2], T.Ø. GIFSTAD[3], R.A. D...
A.M. BAKKE[5,6]

Aquaculture

Aquaculture 180 (1999) 147–165

www.elsevier.nl/locate/aqua-online

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Review

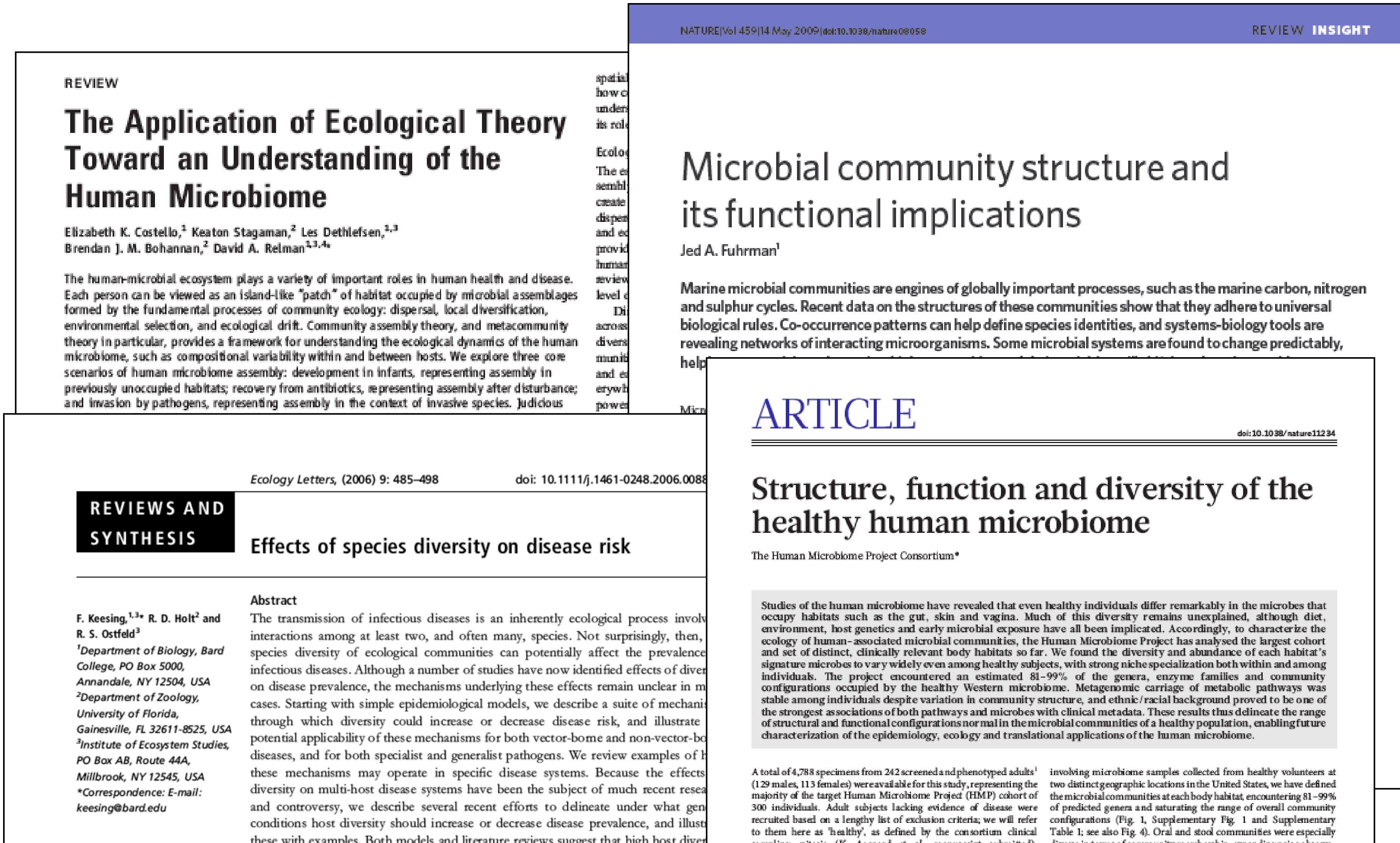
The use of probiotics in aquaculture

F.J. Gatesoupe *

Unité Mixte de Nutrition des Poissons INRA-IFREMER, IFREMER, Centre de Brest, BP 70, F-29280 Plouzané, France

Accepted 12 April 1999

Ecological theory PROMOTE useful MICROBES rather than KILL them.
Join them rather than beat them – Peter Schryver


REVIEW

The Application of Ecological Theory Toward an Understanding of the Human Microbiome

Elizabeth K. Costello,[1] Keaton Stagaman,[2] Les Dethlefsen,[1,3] Brendan J. M. Bohannan,[2] David A. Relman[1,3,4*]

The human-microbial ecosystem plays a variety of important roles in human health and disease. Each person can be viewed as an island-like "patch" of habitat occupied by microbial assemblages formed by the fundamental processes of community ecology: dispersal, local diversification, environmental selection, and ecological drift. Community assembly theory, and metacommunity theory in particular, provides a framework for understanding the ecological dynamics of the human microbiome, such as compositional variability within and between hosts. We explore three core scenarios of human microbiome assembly: development in infants, representing assembly in previously unoccupied habitats; recovery from antibiotics, representing assembly after disturbance; and invasion by pathogens, representing assembly in the context of invasive species. Judicious

NATURE|Vol 459|14 May 2009|doi:10.1038/nature08058 REVIEW INSIGHT

Microbial community structure and its functional implications

Jed A. Fuhrman[1]

Marine microbial communities are engines of globally important processes, such as the marine carbon, nitrogen and sulphur cycles. Recent data on the structures of these communities show that they adhere to universal biological rules. Co-occurrence patterns can help define species identities, and systems-biology tools are revealing networks of interacting microorganisms. Some microbial systems are found to change predictably,

Ecology Letters, (2006) 9: 485–498 doi: 10.1111/j.1461-0248.2006.0088

REVIEWS AND SYNTHESIS

Effects of species diversity on disease risk

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Abstract

The transmission of infectious diseases is an inherently ecological process invol interactions among at least two, and often many, species. Not surprisingly, then, species diversity of ecological communities can potentially affect the prevalence infectious diseases. Although a number of studies have now identified effects of diver on disease prevalence, the mechanisms underlying these effects remain unclear in m cases. Starting with simple epidemiological models, we describe a suite of mechani through which diversity could increase or decrease disease risk, and illustrate potential applicability of these mechanisms for both vector-borne and non-vector-bo diseases, and for both specialist and generalist pathogens. We review examples of h these mechanisms may operate in specific disease systems. Because the effects diversity on multi-host disease systems have been the subject of much recent resea and controversy, we describe several recent efforts to delineate under what gen conditions host diversity should increase or decrease disease prevalence, and illust

ARTICLE

doi:10.1038/nature11234

Structure, function and diversity of the healthy human microbiome

The Human Microbiome Project Consortium*

Studies of the human microbiome have revealed that even healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin and vagina. Much of this diversity remains unexplained, although diet, environment, host genetics and early microbial exposure have all been implicated. Accordingly, to characterize the ecology of human-associated microbial communities, the Human Microbiome Project has analysed the largest cohort and set of distinct, clinically relevant body habitats so far. We found the diversity and abundance of each habitat's signature microbes to vary widely even among healthy subjects, with strong niche specialization both within and among individuals. The project encountered an estimated 81–99% of the genera, enzyme families and community configurations occupied by the healthy Western microbiome. Metagenomic carriage of metabolic pathways was stable among individuals despite variation in community structure, and ethnic/racial background proved to be one of the strongest associations of both pathways and microbes with clinical metadata. These results thus delineate the range of structural and functional configurations normal in the microbial communities of a healthy population, enabling future characterization of the epidemiology, ecology and translational applications of the human microbiome.

A total of 4,788 specimens from 242 screened and phenotyped adults[1] (129 males, 113 females) were available for this study, representing the majority of the target Human Microbiome Project (HMP) cohort of 300 individuals. Adult subjects lacking evidence of disease were recruited based on a lengthy list of exclusion criteria; we will refer to them here as 'healthy', as defined by the consortium clinical

involving microbiome samples collected from healthy volunteers at two distinct geographic locations in the United States, we have defined the microbial communities at each body habitat, encountering 81–99% of predicted genera and saturating the range of overall community configurations (Fig. 1, Supplementary Fig. 1 and Supplementary Table 1; see also Fig. 4). Oral and stool communities were especially

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Aquaculture 128 (1994) 203–209

Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection

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Accepted 21 August 1994

Abstract

The cause of mass mortality in *Penaeus monodon* larvae in a hatchery was investigated. Antibiotic-resistant *Vibrio harveyi* could be isolated from all the infected larvae. These bacteria were absent in healthy eggs and nauplii. Although the intake seawater had *V. harveyi*, these strains were sensitive to antibiotics. The results suggest that antibiotic-resistant *V. harveyi* had been colonising larval tanks. The isolates from moribund larvae showed much lower LD_{50} values than isolates from natural seawater, thus indicating their higher virulence.

Keywords: Antibiotic-resistant bacteria; *Vibrio harveyi*; *Penaeus monodon*; Diseases and their control — crustaceans

Table 3
Antibiotic sensitivity of some *V. harveyi* isolates

Antibiotics	Sensitivity of isolates from:				
	SW1	SW1	PL_2 T_{12}	PL_3 T_8	L_2 T_8
Co-trimoxazole	S	S	R	R	R
Erythromycin	R	R	R	R	R
Streptomycin	S	S	R	R	R
Oxytetracycline	S	S	S	S	S
Neomycin	S	S	S	S	S
Chloramphenicol	S	S	R	R	R
Gentamicin	S	S	S	S	S

R = resistant; S = sensitive.

Table 4
Survival of *V. harveyi* isolates from larval tanks in seawater containing various antibiotics

Antibacterial agent	Survival at concentration (μg/ml)							
	10	25	50	75	100	500	750	1000
Chloramphenicol	+	+	+	+	+	+	+	+
Erythromycin	+	+	+	+	+	+	+	+
Neomycin	+	+	+	+	+	+	+	+
Oxytetracycline	+	+	+	+	+	+	+	+
Furazolidone	+	+	+	+	+	±	±	±
Nifurpirinol	+	+	+	+	+	+	+	+

Table 5
Mortalities of *P. monodon* postlarvae in the presence of various bacterial isolates

Isolate	Mortality in tanks containing bacteria/ml					
	10^2	10^3	10^4	10^5	10^6	Nil
V. harveyi from larval tank	2/50[a]	16/50	35/50	50/50	50/50	1/50
V. harveyi from seawater	3/50	5/50	12/50	22/50	40/50	2/50
Non-luminous bacterial isolates						
PL_1 *Pseudomonas*	2/50	3/50	8/50	10/50	20/50	2/50
PL_2 *Pseudomonas*	3/50	2/50	6/50	12/50	22/50	3/50
PL_3 *Vibrio* sp.	4/50	3/50	5/50	14/50	18/50	1/50

[a] Number of larvae dead/number tested.

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Aquaculture 140 (1996) 241–245

Aquaculture

Biofilm formation by *Vibrio harveyi* on surfaces

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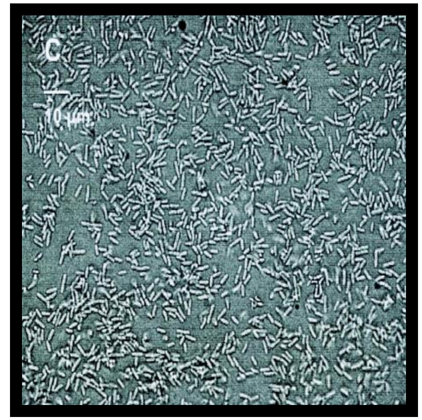
Accepted 26 September 1995



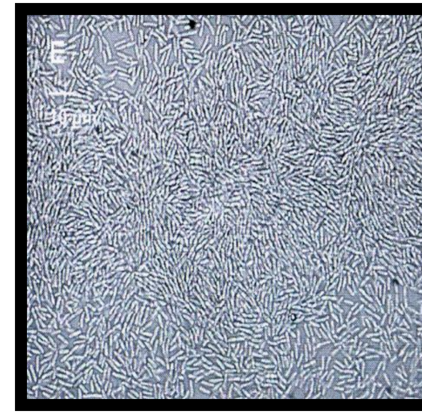


Table 2
Biofilm formation by *V. harveyi* on different surfaces and their sensitivity to chlorine (CFU cm^{-2})

Type of substrate	Control	Levels of chlorine and exposure time		
		20 ppm 10 min	100 ppm 10 min	200 ppm 10 min
Cement slab	8.49×10^{6}	6.62×10^{5}	5.67×10^{4}	4.36×10^{3}
Plastic (HDPE)	5.34×10^{7}	2.44×10^{5}	3.40×10^{3}	
Steel coupon	2.44×10^{6}	3.88×10^{3}	–	

Table 3
Biofilm formation by *V. harveyi* (CFU cm^{-2}) in the presence of the antibiotics tetracycline and chloramphenicol (both added at 50 ppm)

Type of substrate	Control	Tetracycline	Chloramphenicol
Plastic	5.34×10^{7}	5.59×10^{7}	3.08×10^{6}
Cement slab	1.23×10^{7}	1.17×10^{7}	1.14×10^{7}
Steel coupon	2.44×10^{6}	7.18×10^{6}	1.08×10^{7}

Bacterial replacement therapy:

- In the natural environment, shrimp eggs and larvae survive and develop in an environment that is rich in bacterial populations

How do they survive there ?

-eggs are colonised by commensal flora that are antagonistic to pathogens

What are probiotics?

- **Live microbial feed supplement that improves the health of the animals**
- **Also act as biocontrol agents: Pathogen control in the environment**
- **Also as bioremediator : sludge and waste degradation treatment**

This alteration in definition is because :

- Microbes added to the water body during their intrusion through the intestinal tract may survive there
- The gastrointestinal biota of fish/ shellfish is a reflection of the water biota as a large volume of water passes through/ filtered by them

Most probiotic preparations belong to:

- **Bacillus spp.**
- **Family vibrionaceae**
- **The pseudomonads**
- **Lactic acid bacteria**
- **Yeasts**
- **Nitrosomonas**
- **Nitrobacter**
- **Sulphide oxidisers**

SUCCESSFUL PROBIOTIC :

- Colonise the gut
- Antagonistic activity to pathogens
- Afford resistance to disease causing agents
- Competitieve exclusion of the pathogen by competing for nutrients or adhesion sites
- Immunostimulatory activity

How do probiotics work?

Enzymatic secretions

Competition for nutrients and space

Degradation of organic matter

Vibrios in the environment

Human pathogenic Vibrios

V. cholerae
V. parahaemolyticus
V. vulnificus

Pathogens of aquatic animals

Vibrio harveyi
Vibrio anguillarum
Alivibrio salmonicida
Vibrio penaecida
Vibrio vulnificus
Vibrio owensii

Vibrio harveyi **clade includes eleven species:**
V. harveyi, V. alginolyticus, V.parahaemolyticus, V. campbellii, V. rotiferianus, V. mytili, V. natriegens, V. azureus, V. sagamiensis, V. owensii, V. jasicida

- **Some pathogenic bacteria occur naturally in coastal waters worldwide**

- **A few cause disease outbreaks, depending on specific environmental conditions.**
  A good example : *Vibrio parahaemolyticus*
- **Autochthonous to estuarine, marine, and coastal environments.**

- **Occupies a variety of niches**

- **Can exist in a free-swimming state or sessile, attached to inert and animate surfaces such as suspended particulate matter, zooplankton, fish, and shellfish**

- **Distribution of *V. parahaemolyticus* - related to water temperature**

- **Rarely isolated from seawater until the temperature rises to 15ºC and higher**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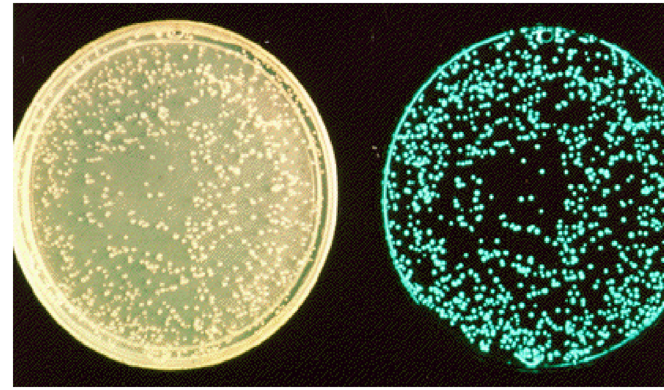
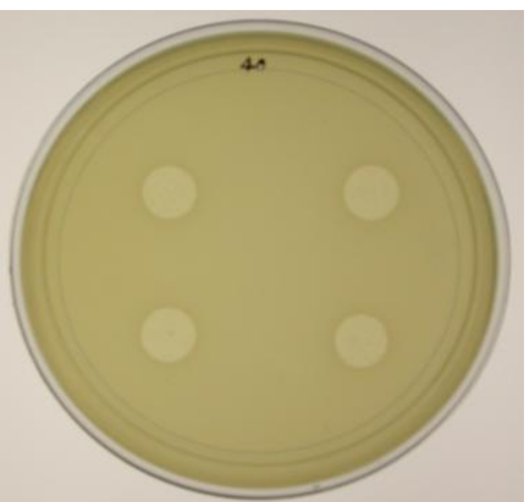
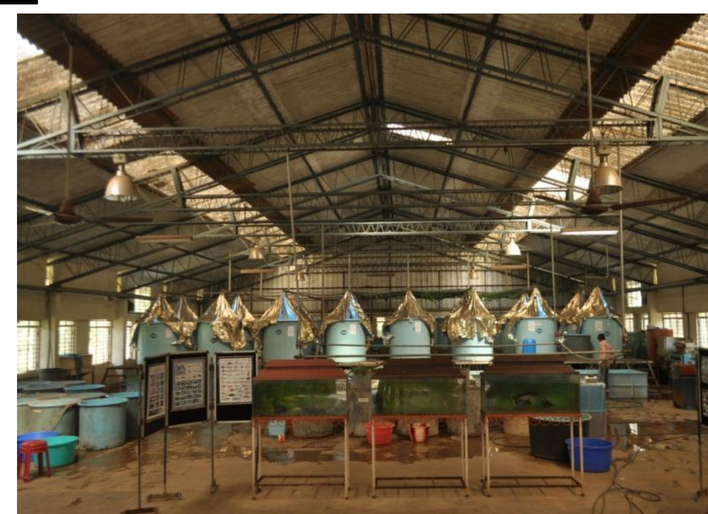






Luminous Bacterial Disease- Harveyi clade

- Problem in shrimp hatcheries & farms
- Causative agent : *Vibrios*
- Autochthonous flora of coastal waters
- Association with crustaceans
- Animals show luminescence
- Bacteria also show luminescence

Other diseases caused by bacteria of *Harveyi* clade - AHPND

New shrimp disease – a global threat

Early Mortality Syndrome (EMS) - old name

Acute Hepatopancreatic Necrosis Syndrome

Identified as a member of *Harveyi* clade related to *V. parahaemolyticus*

Phage therapy – the novel approach

What are phages ?

- viruses that infect bacteria

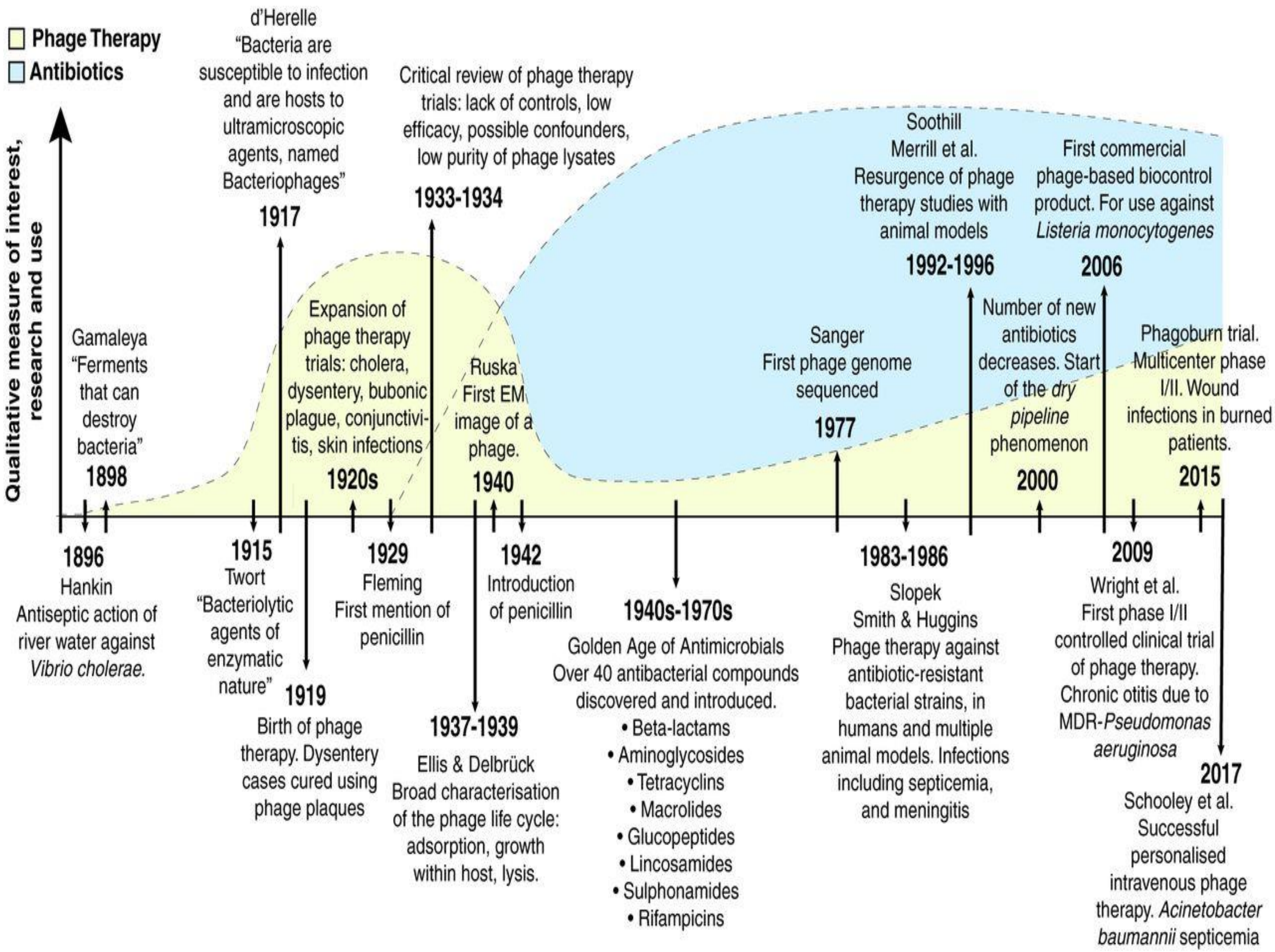
- have lytic and lysogenic life cycle

- lytic phages are good candidates for antibacterial therapy

- highly specific to one (rarely another) bacterial species

- nontoxic to animals and plants

EMERGENCE OF PATHOGENIC BACTERIA RESISTANT TO MOST OF THE ANTIMICROBIAL AGENTS HAS BECOME A CRITICAL PROBLEM



Phage Therapy
Antibiotics
Qualitative measure of interest, research and use
1896
Hankin
Antiseptic action of river water against *Vibrio cholerae.*
1898
Gamaleya
"Ferments that can destroy bacteria"
1915
Twort
"Bacteriolytic agents of enzymatic nature"
1917
d'Herelle
"Bacteria are susceptible to infection and are hosts to ultramicroscopic agents, named Bacteriophages"
1919
Birth of phage therapy. Dysentery cases cured using phage plaques
1920s
Expansion of phage therapy trials: cholera, dysentery, bubonic plague, conjunctivitis, skin infections
1929
Fleming
First mention of penicillin
1933-1934
Critical review of phage therapy trials: lack of controls, low efficacy, possible confounders, low purity of phage lysates
1937-1939
Ellis & Delbrück
Broad characterisation of the phage life cycle: adsorption, growth within host, lysis.
1940
Ruska
First EM image of a phage.
1942
Introduction of penicillin
1940s-1970s
Golden Age of Antimicrobials
Over 40 antibacterial compounds discovered and introduced.
• Beta-lactams
• Aminoglycosides
• Tetracyclins
• Macrolides
• Glucopeptides
• Lincosamides
• Sulphonamides
• Rifampicins
1977
Sanger
First phage genome sequenced
1983-1986
Slopek
Smith & Huggins
Phage therapy against antibiotic-resistant bacterial strains, in humans and multiple animal models. Infections including septicemia, and meningitis
1992-1996
Soothill
Merrill et al.
Resurgence of phage therapy studies with animal models
2000
Number of new antibiotics decreases. Start of the *dry pipeline* phenomenon
2006
First commercial phage-based biocontrol product. For use against *Listeria monocytogenes*
2009
Wright et al.
First phase I/II controlled clinical trial of phage therapy.
Chronic otitis due to MDR-*Pseudomonas aeruginosa*
2015
Phagoburn trial.
Multicenter phase I/II. Wound infections in burned patients.
2017
Schooley et al.
Successful personalised intravenous phage therapy. *Acinetobacter baumannii* septicemia

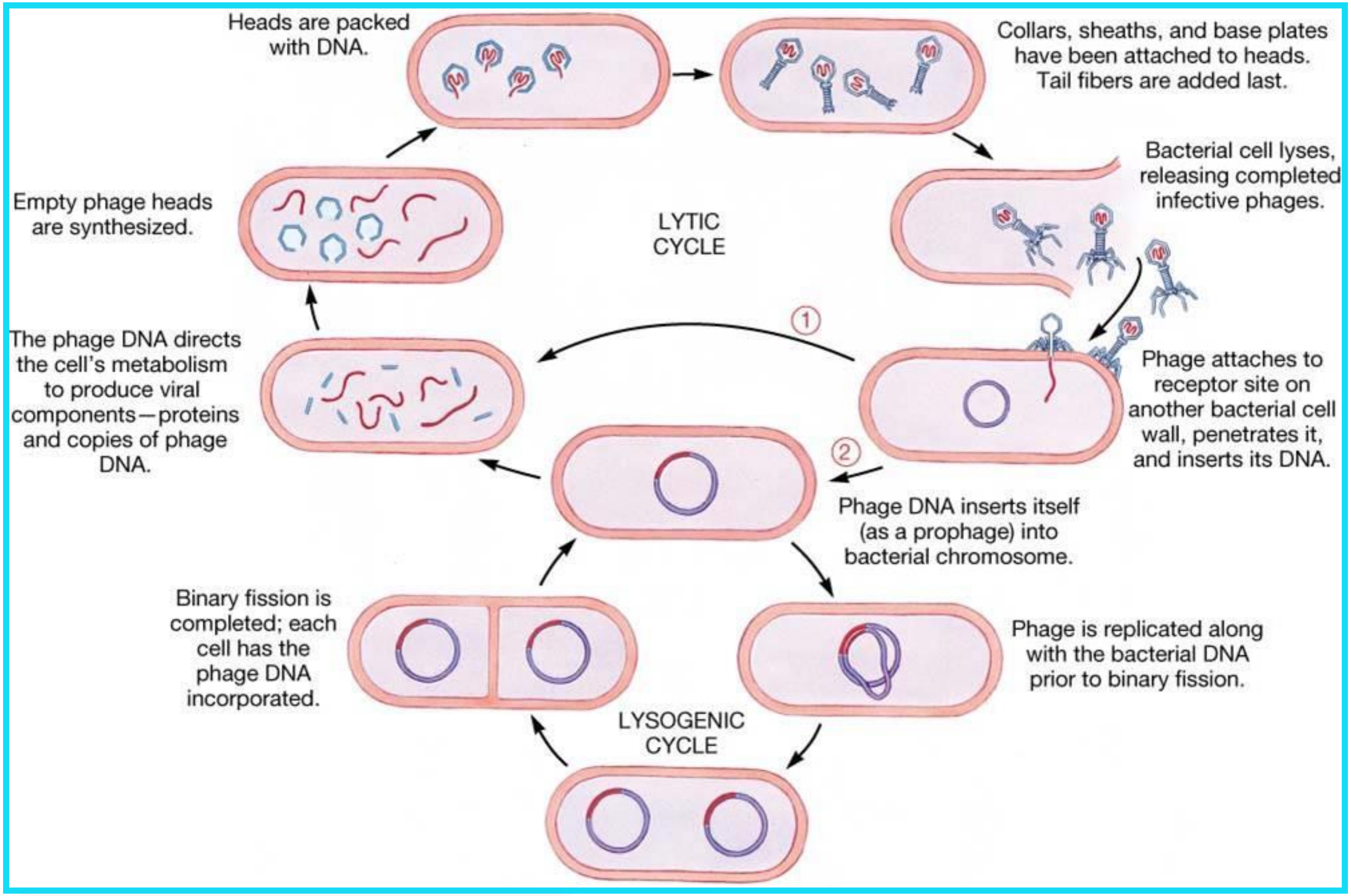


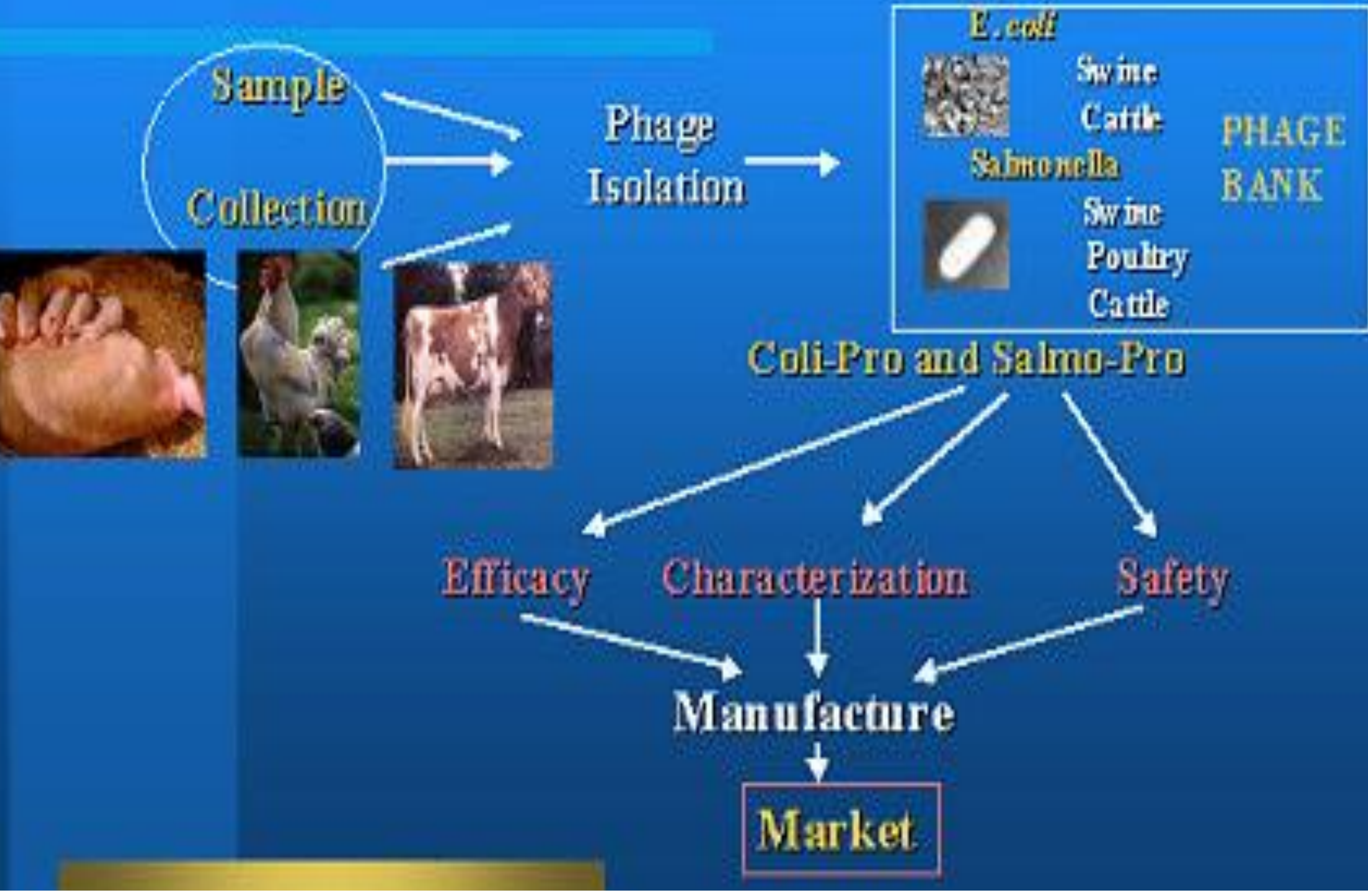
Fig.- Bacteriophage life cycle

Source:http://faculty.irsc.edu/FACULTY/TFischer/images/bacteriophage life cycle.jpg

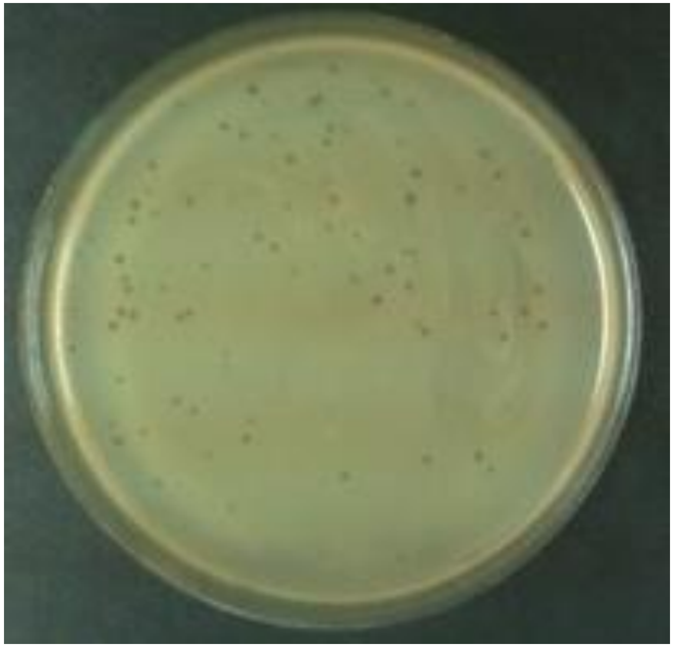
Attributes of phages that supports its therapeutic respons

The issue	Limitations of antibiotics	Advantages of phages
Fate of drug molecule	Metabolic destruction of molecule as it works	Exponential growth
Concentration of the drug	High conc is required	All or none effect
Resistance by bacteria	Antibiotics become obsolete over time	Co-evolve to overcome bacterial mutation
Spread of bacterial resistance	Broad spectrum	Host specific, do not cross species boundaries

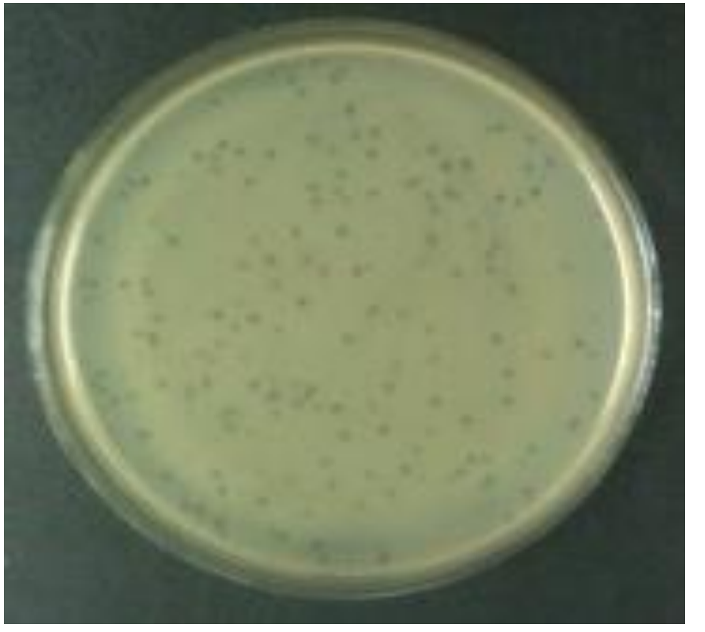
Phage Therapy Program



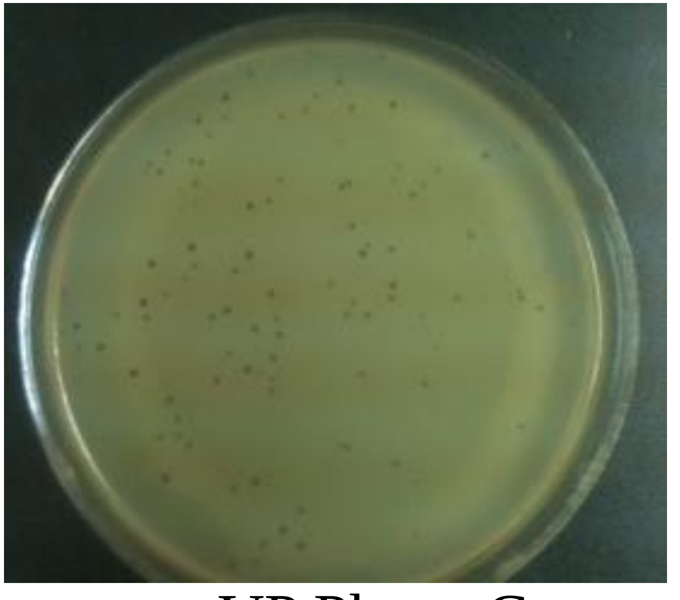
Plaque Assay



VP Phage A



VP Phage B



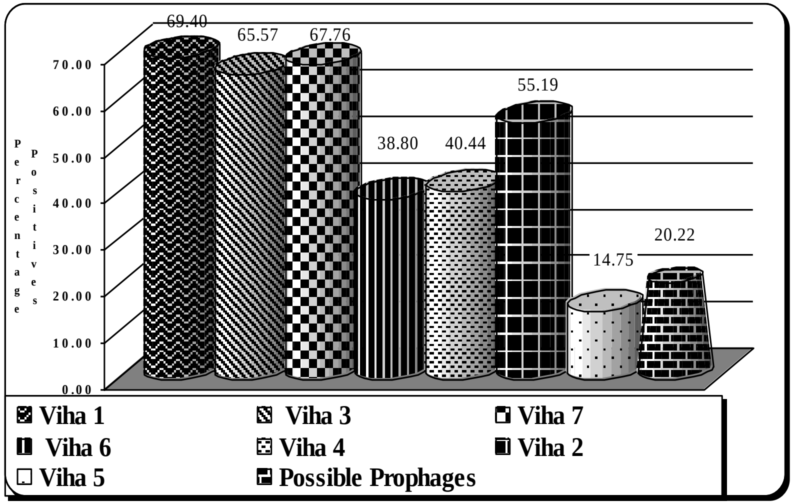
VP Phage C

Use of phages to control aquatic diseases is promising. Why ?

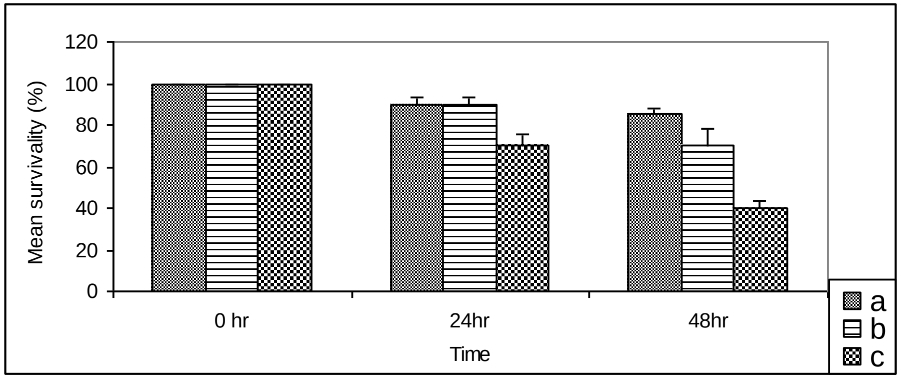
- Both bacteria and phages are in suspension similar to the lab conditions
- Therapeutic phage can have intimate contact with the pathogens of fish, crustacea and molluscs

- Advantages of Phage as a Biocontrol Agent
- Normal inhabitant of marine environment
- Specific
- Once host population disappears, phages also disappear
- Harmless to other normal flora, do not affect useful bacteria associated with larvae, animals or pond
- Therefore, an ecofriendly management measure

Lytic spectrum of *V. harveyi* phages



Mean survival of *Penaeus monodon* larvae and standard error for 3 replicate tanks for 48 hr after being challenged with strains of pathogenic *Vibrio harveyi* and treated with bacteriophage



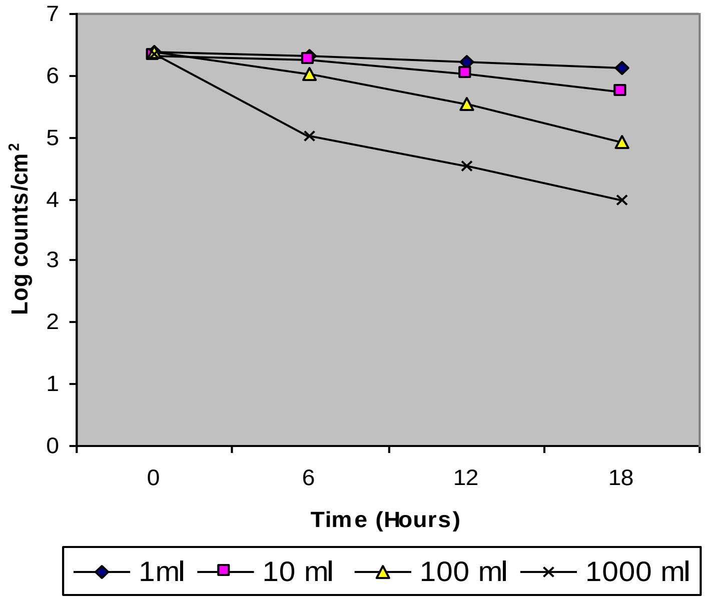
a- treated with two dosage of 100 µl phage for every 24 hr
b- treated with one dosage of 100 µl phage
c- control

***Shivu et al.*, 2007; Environmental Microbiology**

Effect of *Vibrio harveyi* bacteriophage on biofilm

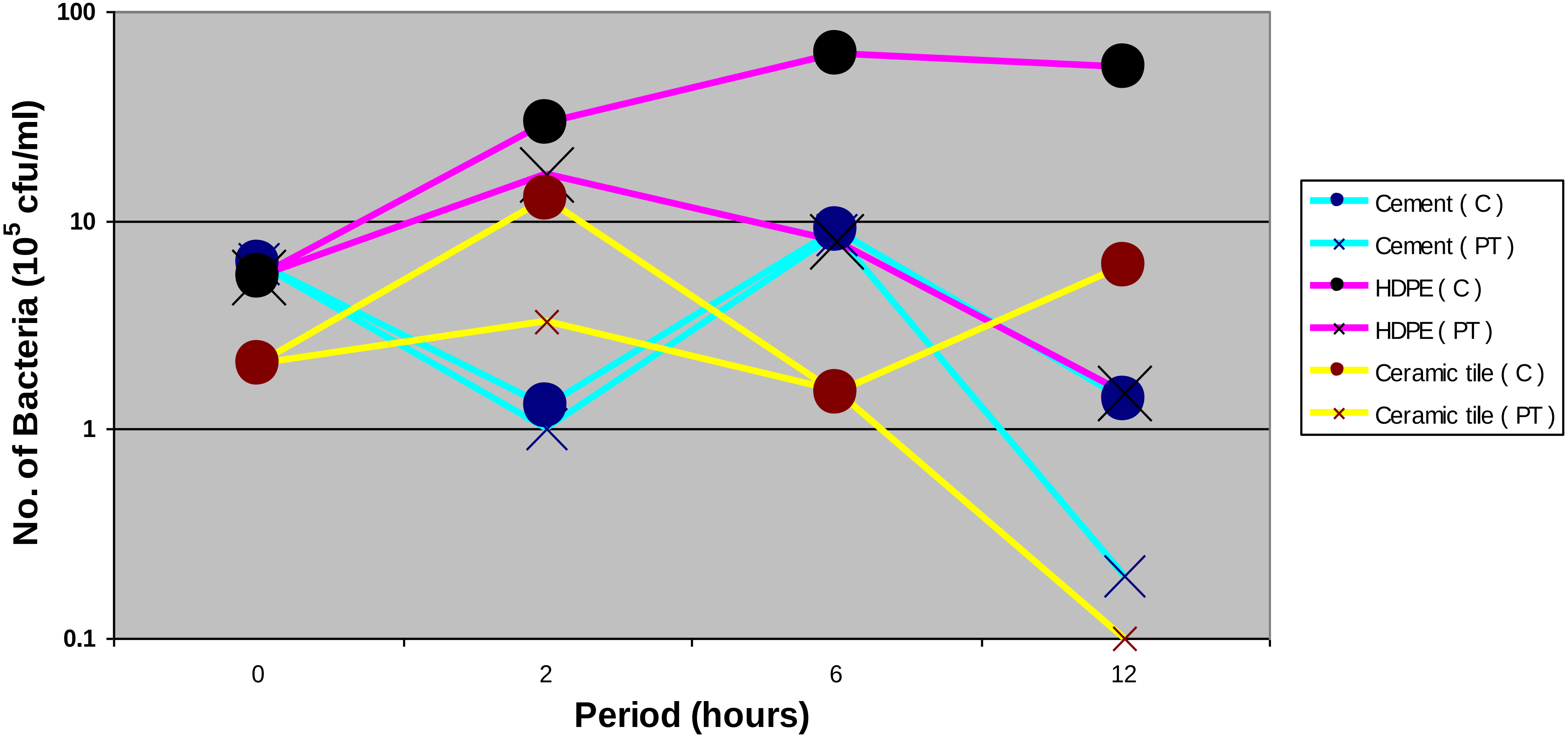
Vol of phage Time (hr)	1μl	10μl	100μl	1000μl	Control
0	2.36×10^6	2.16×10^6	2.43×10^6	2.19×10^6	2.81×10^6
6	2.06×10^6	1.75×10^6	1.06×10^6	1.06×10^5	2.87×10^6
12	1.76×10^6	1.03×10^6	3.9×10^5	3.5×10^4	2.78×10^6
18	1.38×10^6	5.5×10^5	8.3×10^4	9.4×10^3	2.74×10^6

Effect of *Vibrio harveyi* bacteriophage on biofilm

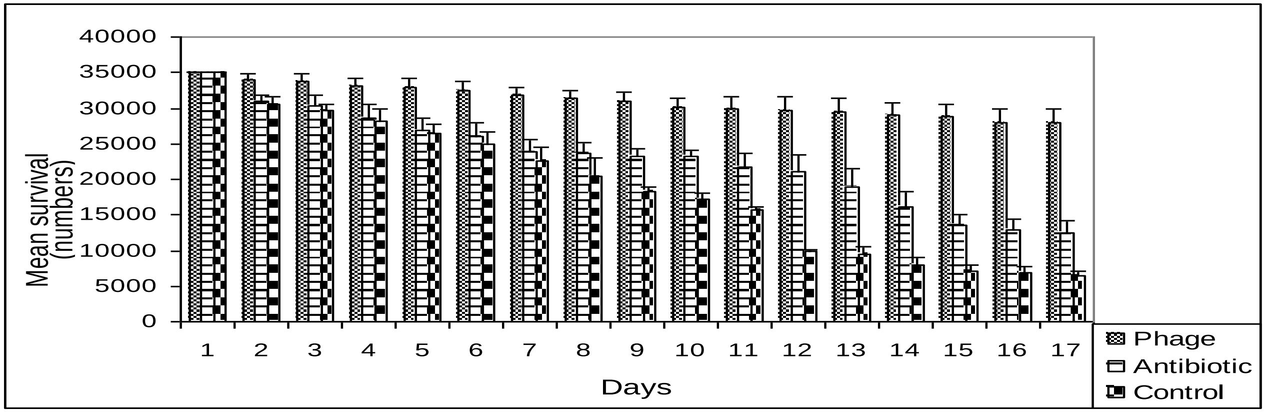


Karunasagar *et al*., 2007; Aquaculture

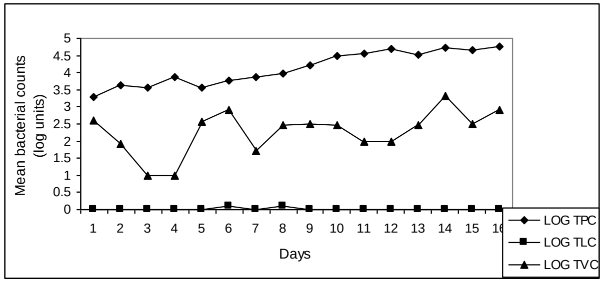
Effect of Phage Treatment on *V. harveyi* Biofilm Cells on Various Surfaces



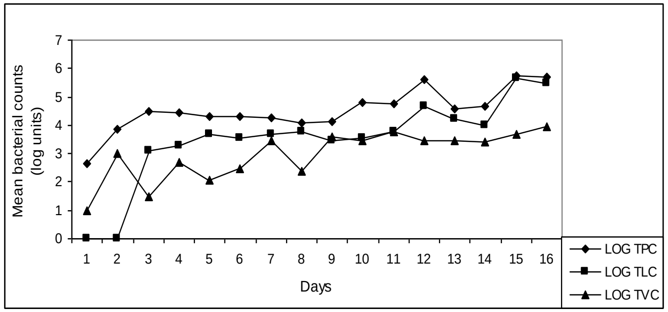
Mean survival of *Penaeus monodon* larvae and standard error for 3 replicate tanks of 35000 naupli larvae 17 reared for days (from zoea to post larvae) with 2 different treatments (Bacteriophage and antibiotic) and a control



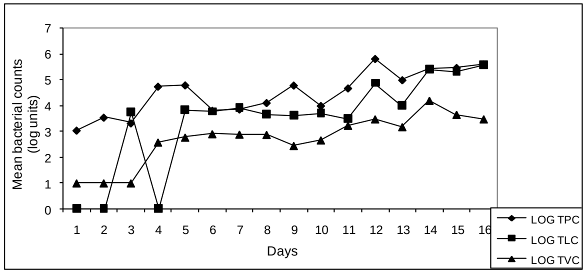
Mean bacterial counts of 3 replicate tanks treated with Bacteriophage



Mean bacterial counts of 3 replicate untreated tanks (control)



Mean bacterial counts of 3 replicate tanks treated with antibiotic



TPC- total plate count, TLC- Total luminous bacterial count , TVC- total Vibrio count

Vinod *et al*., 2006; Aquaculture

Issues in phage therapy

Standardization of the dose of phage to be applied under various environmental variables

• Salinity (ppt)	20, 25 and 30
• Temperature	20°C, 30°C and 37°C
• pH	6, 7 and 8
• Total disssolved solids	

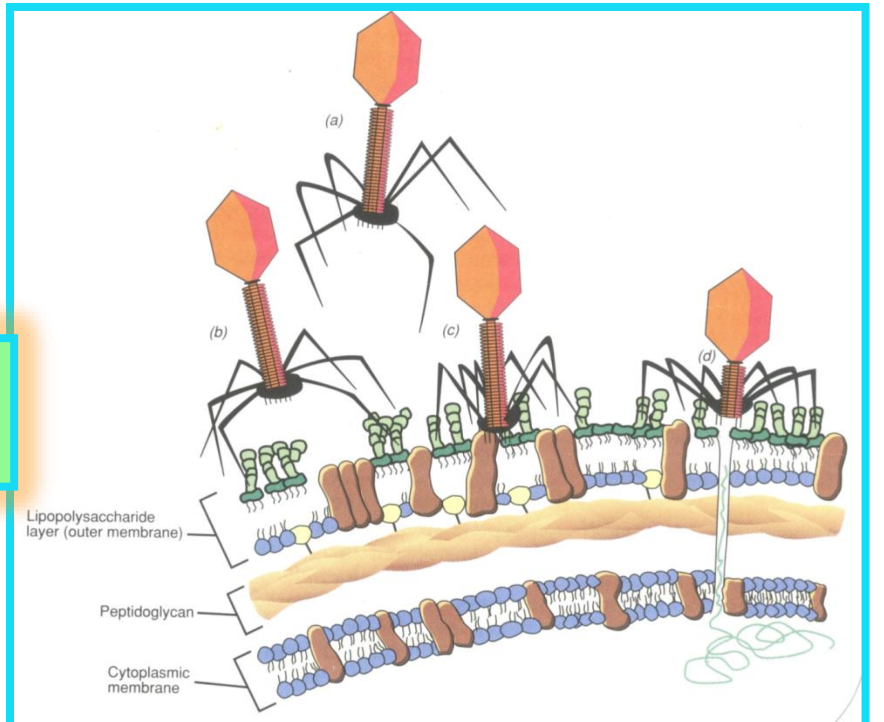
- **Phage therapy in Aquaculture –Lysozyme helps overcome phage resistance**

- **Role of lysozyme on phage activity**

  Lysozyme alone

  Phage alone

  Lysozyme and phage together



Attachment of bacteriophage particle to cell wall of bacteria

Madigan *et al.*, 1997

Madigan *et al.*, 1997

The penetration of phage DNA inside the bacteria is promoted by lysozyme produced by the phage

Tyagi *et al.*, 2007

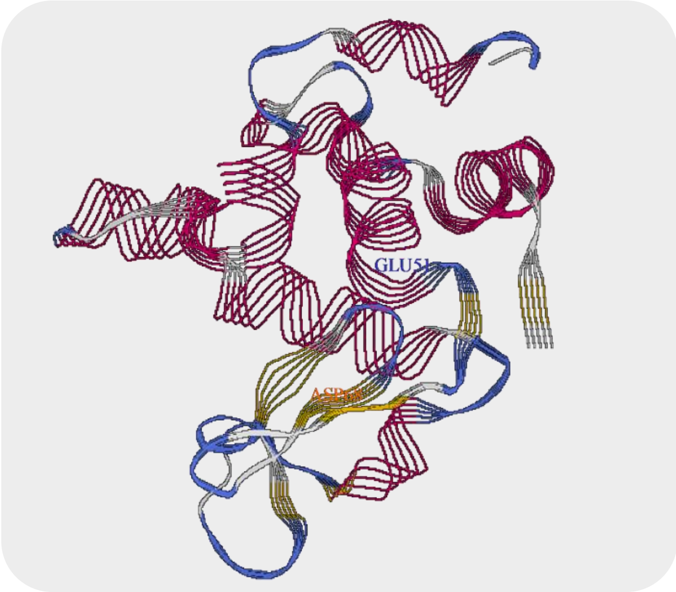
Recombinant lysozyme expressed from black tiger shrimp reduced *V. harveyi* in sea water by 3 log units in 1 hour

We surmised that phage penetration might increase in the presence of our recombinant shrimp lysozyme.

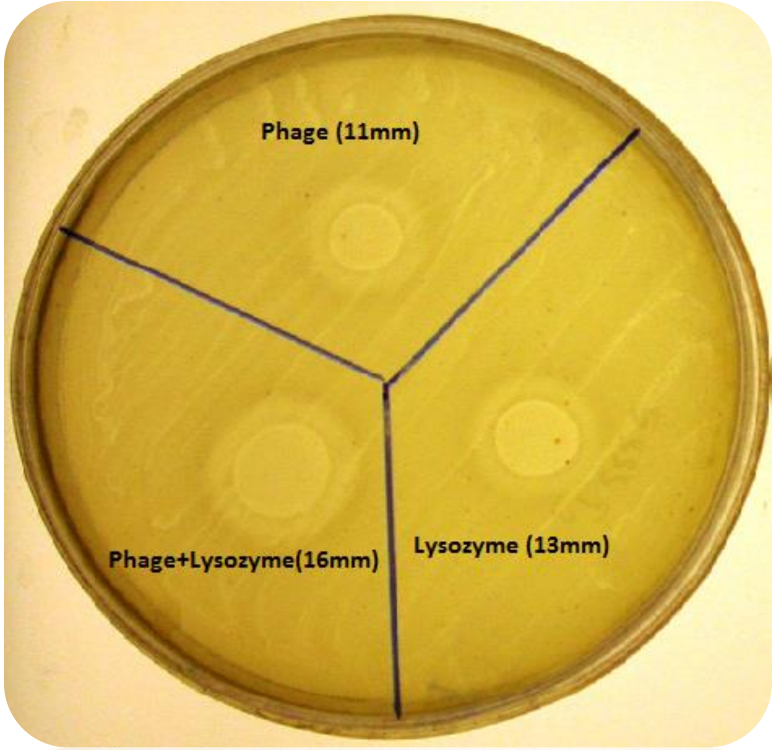
Expression of the recombinant shrimp lysozyme

Tyagi *et al.*, 2007

- Recombinant *E. coli* grown in 200 ml of LB broth until the OD_{600} was 0.5-0.7
- 1mM concentrations of IPTG added and incubated for 4 hr at 37˚C with constant agitation at 150 rpm
- Cells harvested by centrifugation at 11,000 × g for 5 min
- Polyacrylamide gel electrophoresis performed



3 D structure of shrimp lysozyme



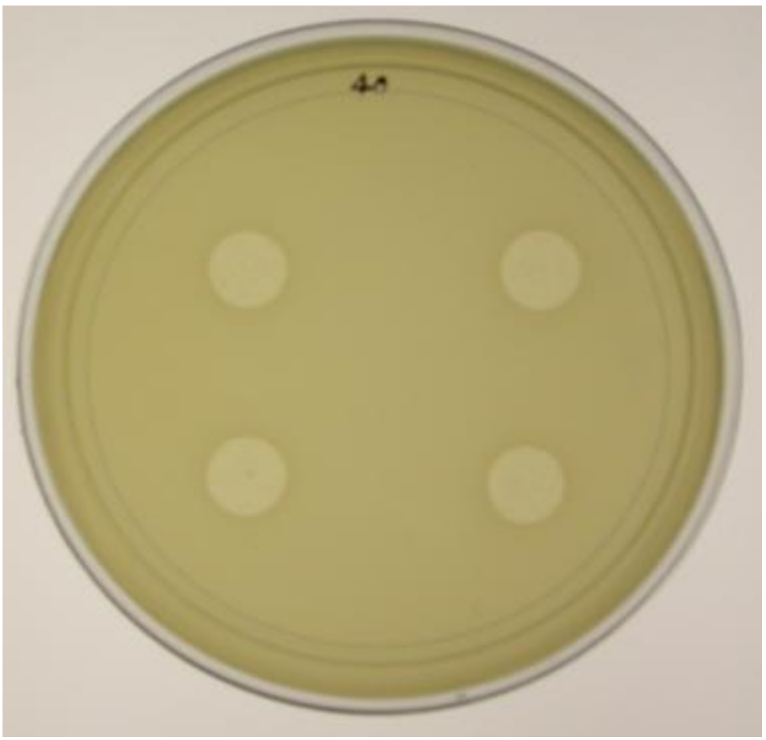
Zone of inhibition on Solid phase assay by phage alone, lysozyme alone and phage + lysozyme together.

Phage isolates with respective host bacteria and source

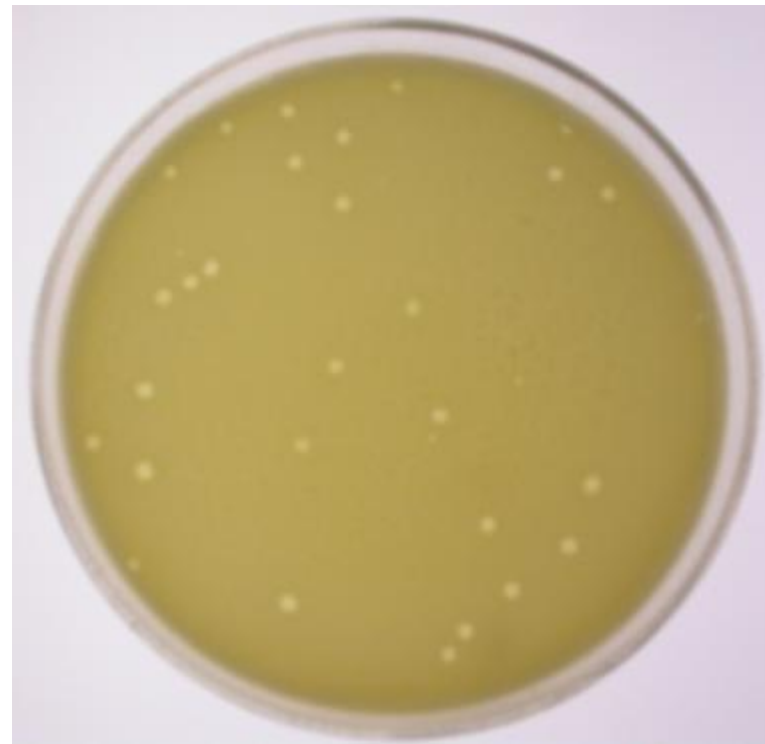
Phages	Host bacteria[a]	Source
Vf	*V. fischeri*	Shrimp farm water
Va	*V. alginolyticus*	Shrimp hatchery water
Vh	*V. harveyi*	Shrimp hatchery water
Vp	*V. parahaemolyticus*	Oysters
Vv	*V. vulnificus*	Oysters

[a]Bacterial isolates from our own culture collection

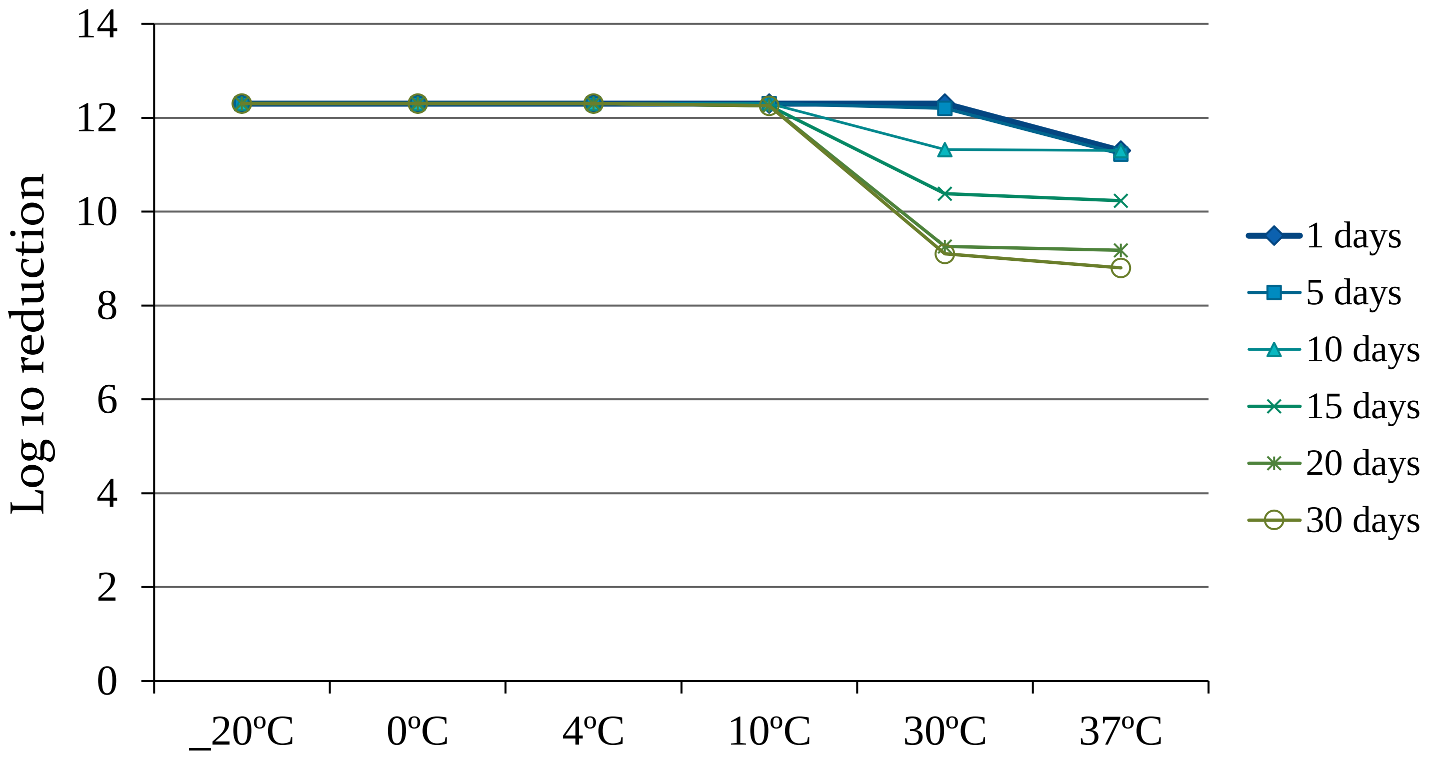
Zones of clearing due to phage isolate from *V. parahaemolyticus*



Plaques formed by *V. parahaemolyticus* phage on soft agar



Titre values of phage at different storage temperatures



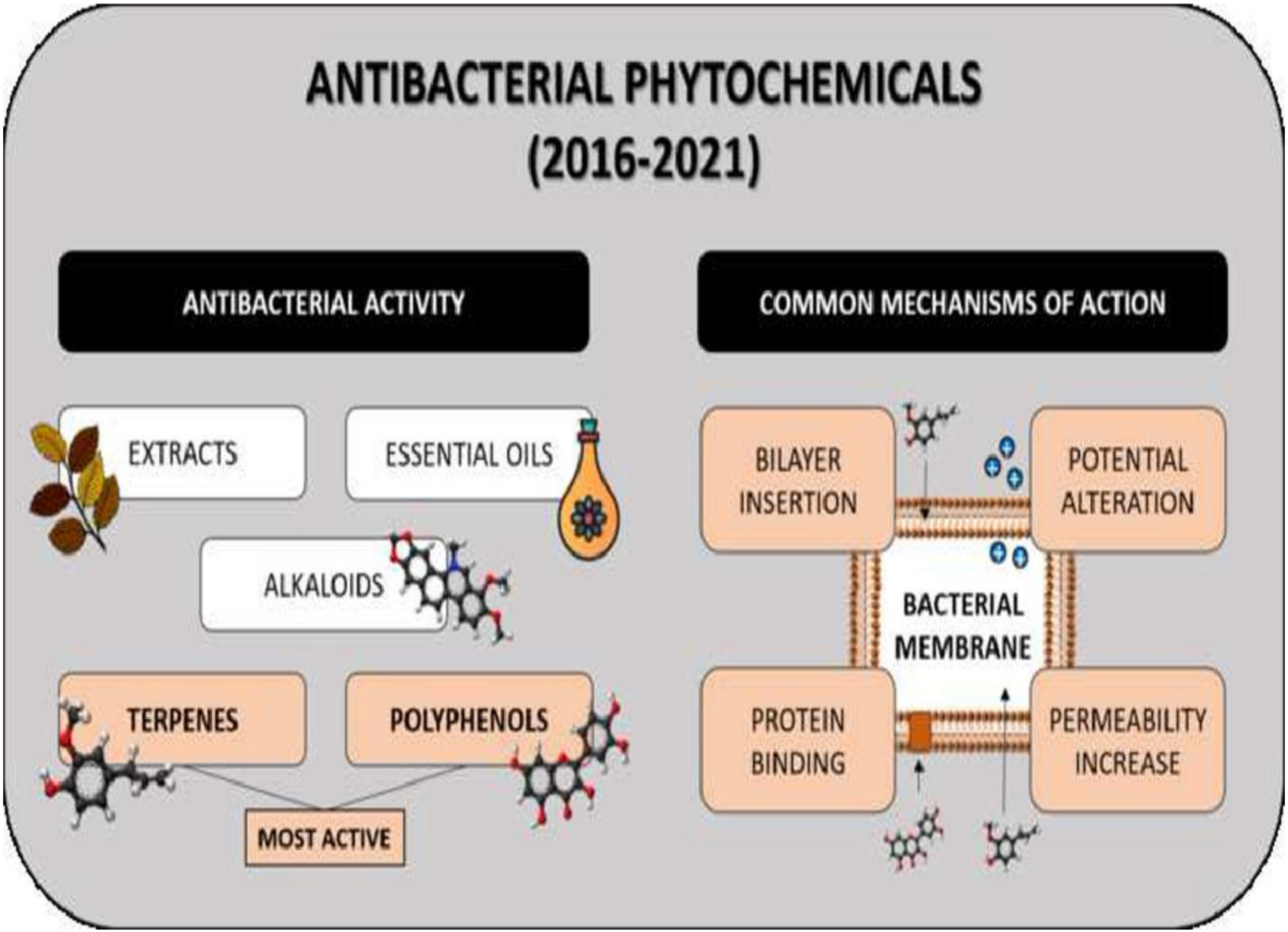
No change in titer of phage at low storage temperatures
Only at 30 and 37^{0}C , reduction in titer observed.

Results demonstrate promise for transport and field application

APPLICATION

- **As prophylactic to prevent build up of vibrio pathogens in hatcheries.**
- **To treat luminous bacterial disease in hatcheries and ponds.**
- **To treat broodstock, eggs, nauplii by dipping in phage**
- **To tackle biofilm formation by vibrios**

Phytochemicals as antimicrobials-mode of action and their effectiveness



Herbal applications in aquaculture

Name of Plant/product	Immunostimulatory activity on fish	Sources
Immunostimulant effect of Spirulina	Atlantic salmon, Channel catfish, Tilapia.	Gildberg et al., 1996 Duncan and Klesius, 1996; Park and Jeong,1996
Immunostimulant effect of *Ocimum sanctum* leaf extract *Azadirachta indica, Piper betle, Crossandra infundibuliformis* Sodium benzoate preserved extracts	Enhanced antibody response to *A. hydrophila*. Increase in antibody response and increase in neutrophil activity in *Oreochromis mossambicus* Enhanced neutrophil activity in Tilapia.	Dinakaran, 2001 Dinakaran, 2001 Venkatalakshmi and Dinakaran Michael, 2001
Acetone extract of *Phyllanthus niruri*, *Ocimum sanctum* and *Acalypha indica*	Enhanced the antiSRBC antibody response in Tilapia	Hemapriya, 1997

Herbal applications in aquaculture

Name of Plant/product	Immunostimulatory activity on fish	Sources
Catheranthus roseus , Calotropis gigantea* and *Datura stromoneum	Immunostimulatory effect on *Cyprinus carpio*	Kiran Kumar, 2001
Aqueous extract of neem	Enhance immune response of Balb-c mice to sheep red blood cells.	Nirjo and Kofi-Tsekpo, 1999
Leaf extract of neem	Higher IgM and IgG levels along with increased titer of antiovalbumin antibody.	Ray et al., 1996
Neem oil	Activate cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge.	Upadhyay et al., 1993
Achyranthes aspera	Stimulates immunity and increases resistance to infection in Indian major carps.	Chakrabarti, R. and Y.V. Rao (2006).

Herbal applications in aquaculture

Name of Plant/product	Immunostimulatory activity on fish	Sources
Holy basil, Tulsi *Ocimum sanctum*	Enhanced immunostimulatory action in Rohu fish *Labeo rohita*.	Das et al ., 2013
Chinese herbs (*Astragalus radix* and *Scutellaria radix*)	non-specific immune response of tilapia, *Oreochromis niloticus* (feeding period of 3 weeks)	Yin et al., 2006
aqueous extracts of mistletoe (*Viscum album*), nettle (*Urtica dioica*), and ginger (*Zingiber officinale*)	rainbow trout (*Oncorhynchus mykiss*)	Dügenci et al., 2003
water and hexane soluble fractions of the Indian medicinal plant, *Solanum trilobatum*	nonspecific immune mechanisms and disease resistance in *Oreochromis mossambicus*	Divyagnanesw ari et al., 2007
Aloe vera crude extract	Indian common carp (*Cyprinus carpio*)	Alishahi et al., 2010

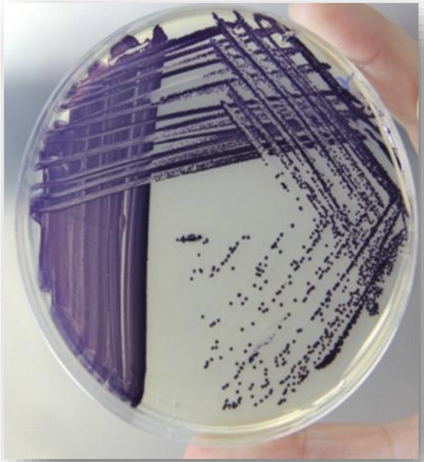
- **Novel antipathogenic compounds with anti-Quorum Sensing (anti-QS) property as an alternate to antibiotics to prevent aquatic diseases caused by antibiotic resistant bacterial pathogens.**

Quorum Sensing

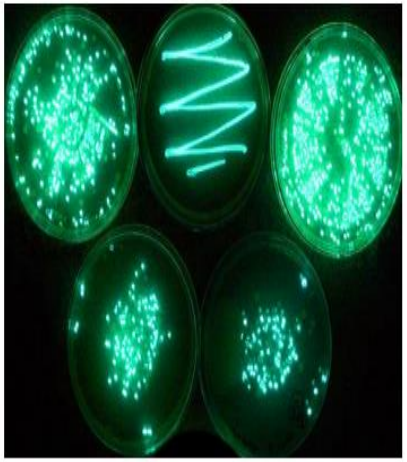
Cell density dependent signalling system in bacteria that controls the expression of various genes

Importance of studying QS system:

- **QS governs:**
  - Bioluminescence
  - Biofilm formation
  - Virulence factors production
  - Sporulation
  - Antibiotic production
  - Cell spreading
  - Competence
  - Production of exotoxins & lytic enzymes



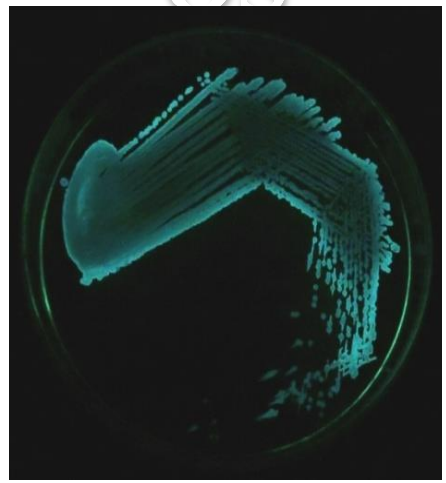
Chromobacterium violaceum



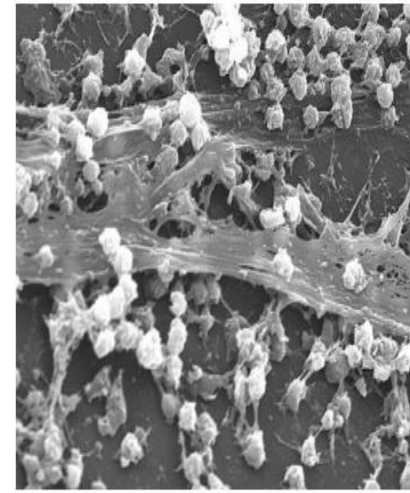
Vibrio harveyi

Factors controlled by QS

Virulence



Bioluminescence

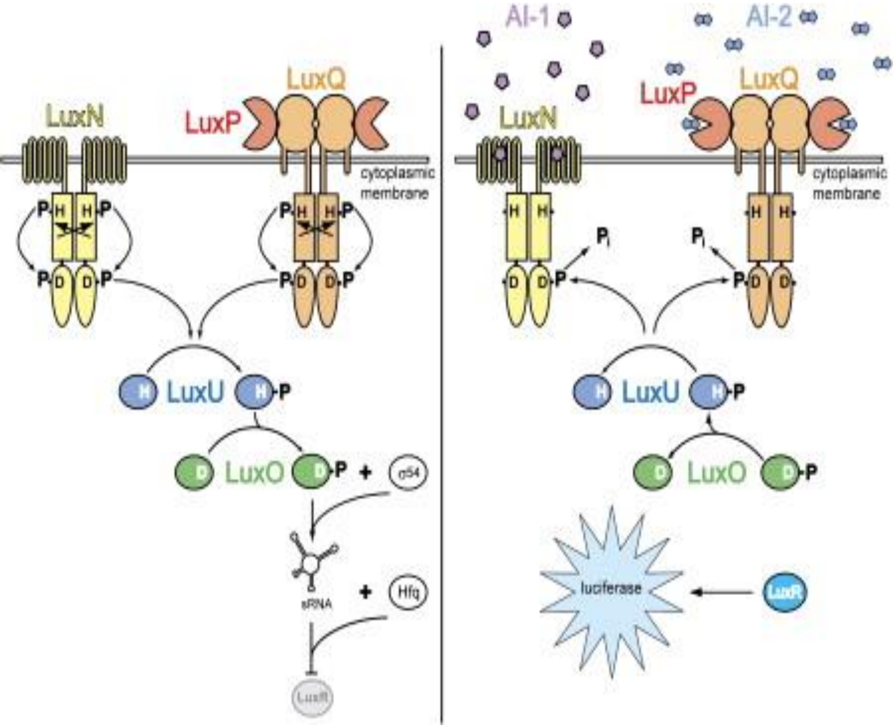


Biofilms

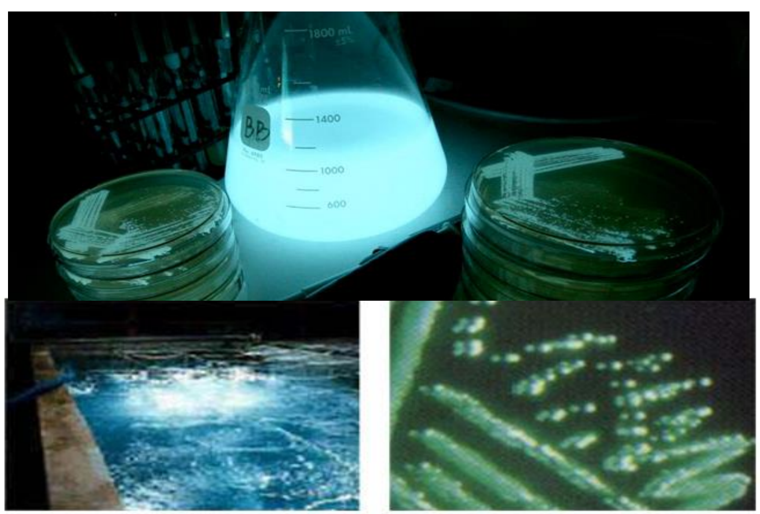
Motility

Pigment

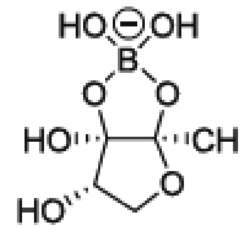
QS in *Vibrio harveyi*



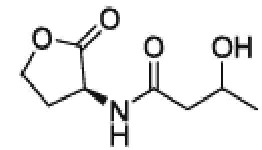
Bioluminescence in *V. harveyi*



Signalling molecules in *V. harveyi*



Furanosyl borate diester



3-hydroxy-C4-HSL

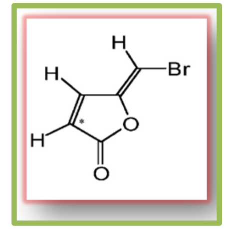
QS controlled phenomena in aquatic bacterial pathogens

- **Bioluminescence, protease and chitinase production in *Vibrio harveyi***
- **Exoproteases and exotoxin production in *Aeromonas hydrophila***
- **Serine metalloprotease and pigment production in *V. anguillarum***
- **Biofilm formation in *V. harveyi, V. anguillarum, V. vulnificus* and *A. hydrophila***

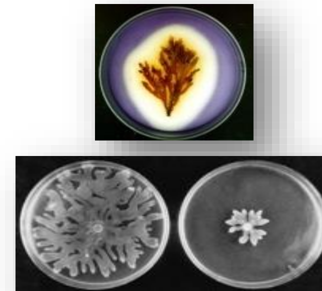
Anti-QS compounds from various natural resources

Marine micro algae

Delisea pulchra

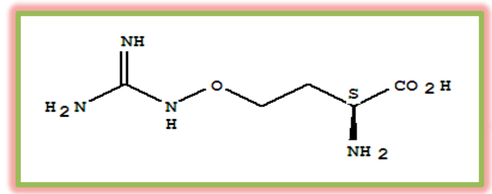


Brominated furanone

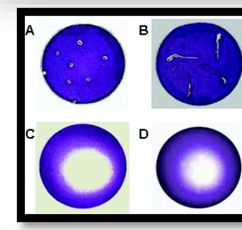


Medicinal plants

Medicago sativa

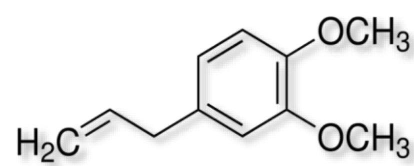


L-Canavanine



Edible spices

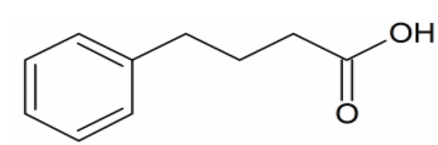
Cuminum cyminum



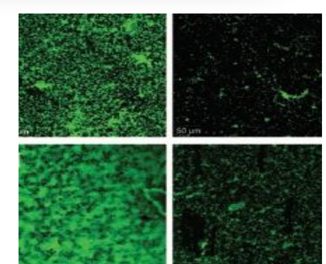
Methyl eugenol

Marine bacteria

Bacillus pumilus



4- Phenyl butanoic acid



Chemotherapy
International Journal of Experimental and Clinical Chemotherapy
Pharmacology

Evaluation of Anti-Quorum-Sensing Activity of Edible Plants and Fruits through Inhibition of the N-Acyl-Homoserine Lactone System in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*

K. Syed Musthafa, A. Veera Ravi, A. Annapoorani, I. Sybiya Vasantha Packiavathy, S. Karutha Pandian
pa University, Karaikudi, India

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journal homepage: www.elsevier.com/locate/bmcl

Inhibition of quorum sensing regulated biofilm formation in *Serratia marcescens* causing nosocomial infections

Dhamodharan Bakkiyaraj, Chandran Sivasankar, Shunmugiah Karutha Pandian *
Department of Biotechnology, Alagappa University, Karaikudi 630 003, India

J. Biosci. 36(1), March 2011, 55–67

Antipathogenic potential of marine *Bacillus* sp. SS4 on *N*-acyl-homoserine-lactone-mediated virulence factors production in *Pseudomonas aeruginosa* (PAO1)

K Syed Musthafa, V Saroja, S Karutha Pandian and A Veera Ravi*
Department of Biotechnology, Alagappa University, Karaikudi 630 003, India
**Corresponding author (Fax, +91-4565-225202; Email, aveeraravi@rediffmail.com)*

Ann Microbiol (2012) 62:443–447
DOI 10.1007/s13213-011-0262-1
SHORT COMMUNICATIONS

Inhibition of quorum-sensing-dependent phenotypic expression in *Serratia marcescens* by marine sediment *Bacillus* spp. SS4

Syed Musthafa Khadar ·
Karutha Pandian Shunmugiah · Veera Ravi Arumugam

Indian J Microbiol
DOI 10.1007/s12088-012-0272-0
ORIGINAL ARTICLE

Inhibition of Quorum Sensing Mediated Virulence Factors Production in Urinary Pathogen *Serratia marcescens* PS1 by Marine Sponges

Angusamy Annapoorani · Abdul Karim Kamil Abdul Jabbar ·
Syed Khadar Syed Musthafa · Shunmugiah Karutha Pandian ·
Arumugam Veera Ravi

Food Research International 45 (2012) 85–92
Contents lists available at SciVerse ScienceDirect
Food Research International
journal homepage: www.elsevier.com/locate/foodres

Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens

Issac Abraham Sybiya Vasantha Packiavathy, Palani Agilandeswari, Khadar Syed Musthafa, Shunmugiah Karutha Pandian, Arumugam Veera Ravi *
Department of Biotechnology, Alagappa University, Karaikudi 630 003, Tamil Nadu, India

ELSEVIER
Archives of Medical Research 42 (2011) 658–668
Archives of Medical Research
ORIGINAL ARTICLE

Antiquorum Sensing and Antibiofilm Potential of *Capparis spinosa*

Sybiya Vasantha Packiavathy Issac Abraham,[a] Agilandeswari Palani,[a] Babu Rajendran Ramaswamy,[b] Karutha Pandian Shunmugiah,[a] and Veera Ravi Arumugam[a]
[a]Department of Biotechnology, Alagappa University, Karaikudi, Tamil Nadu, India
[b]Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Received for publication July 26, 2011; accepted December 8, 2011 (ARCMED-D-11-00362).

Journal of Basic Microbiology 2012, *52*, 1–8

Research Paper

2,5-Piperazinedione inhibits quorum sensing-dependent factor production in *Pseudomonas aeruginosa* PAO1

Khadar Syed Musthafa, Krishnaswamy Balamurugan, Shunmugiah Karutha Pandian and Arumugam Veera Ravi

Department of Biotechnology, Alagappa University, Karaikudi, Tamil Nadu, India

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Department of Biotechnology, Alagappa University, Karaikudi 630 003, India

ORIGINAL ARTICLE

Screening and evaluation of probiotics as a biocontrol agent against pathogenic *Vibrios* in marine aquaculture

A.V. Ravi[1], K.S. Musthafa[1], G. Jegathammbal[1], K. Kathiresan[2] and S.K. Pandian[1]

1 Department of Biotechnology, Alagappa University, Karaikudi, Tamil Nadu, India

2 CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

Biofouling

Vol. 27, No. 5, May 2011, 519–528

Taylor & Francis
Taylor & Francis Group

A novel compound from the marine bacterium *Bacillus pumilus* S6-15 inhibits biofilm formation in Gram-positive and Gram-negative species

Chari Nithya, Muthu Gokila Devi and Shunmugiah Karutha Pandian*

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I Microbiol

.1007/s12088-012-0272-0

GINAL ARTICLE

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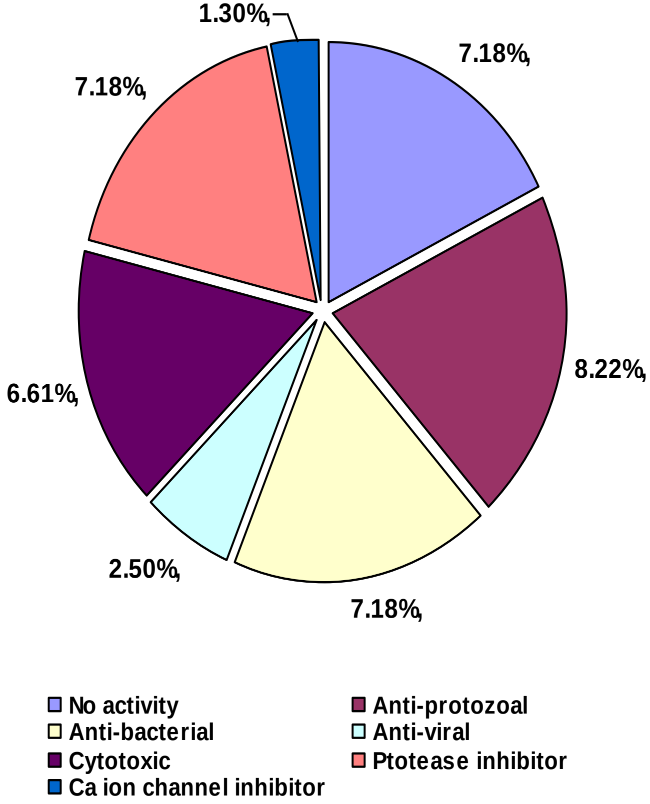
Angusamy Annapoorani · Abdul Karim Kamil Abdul Jabbar · Syed Khadar Syed Musthafa · Shunmugiah Karutha Pandian · Arumugam Veera Ravi

Cyanobacteria

- Cyanobacteria (also known as blue–green algae) are highly diverse prokaryotic Gram negative organisms and well known for their potential bioactive compounds with various medicinal properties.



Anti-QS compounds from cyanobacteria

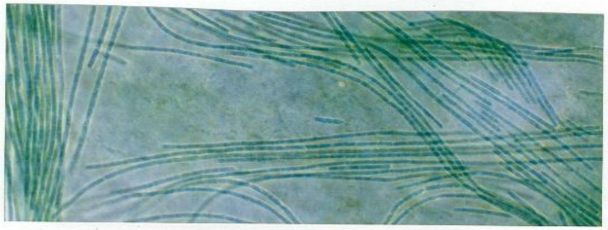
Tumonoic acid from marine cyanobacterium *Blennothrix cantharidosmum* against *V. harveyi* – Clark et al., (2008) J Nat Prod 71:1530–1537.

Malyngolide from the cyanobacterium *Lyngbya majuscula* against *P. aeruginosa* – Dobretsov et al., (2010) *Environ Microbiol Rep 6:*739–744.

Lyngbyoic acid from a marine cyanobacterium against *P. aeruginosa* – Kwan et al., (2011) Mol Biosyst. 7:1205-16.

Ref: Cyanobacteria: an emerging source for drug discovery, Singh et al., (2011), The Journal of Antibiotics 64, 401–412

Marine Cyanobacterial Morphological Diversity



Cyanobacteria	Compound/Activity	Reference
Microcystis aeruginosa	Microviridin Toxin BE-4, Siatoxin / Antibiotic, anticancer	Armente and Carmichael, 1996; Domingos *et al.* 1999; Shi *et al.*, 1999
Synechocystis trididemni	Didemnin / Anticancer, antiviral	Rinehart *et al.*, 1981; Chun *et al.*, 1986
Hyella caespitosa	Carazostatin / Antifungal	Cardellina *et al.*, 1979
Phromidium ectocarpii	Hierridin, 2, 4-dimethoxy-6-heptadecyl-phenol / Antiplasmodial, antibiotic	Murakami *et al.*, 1991
Lyngbya majuscula	Sulfolipid amide (bromo, chlor and pyrrole) fatty acid (chloro sulfo thiazoline) lipopeptides / Anti HIV, anticancer, Antifungal, antimicrobial	Gerwick *et al.*, 1994; Luesch et al. 2000; Mynderse *et al.*, 1988; Mitchell *et al.*, 2000; Milligan *et al.*, 2000
Scytonema pseudohofmanni	Scytophycine / Antifungal, Antiviral	Srivastava *et al.*, 1998, 1999
Hormothomnion enteromorphoides	Hormothomnin / Cytotoxic, antibiotic	Gerwick, 1990
Calothrix sp	Calothrixin / Antimalarial, anticancer	Issa, 1999

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Metal sensing-carbon dots loaded TiO_2-nanocomposite for photocatalytic bacterial deactivation and application in aquaculture

Rajaiah Alexpandi[1], Chandu V. V. Muralee Gopi[2], Ravindran Durgadevi[1], Hee-Je Kim[2], Shunmugiah Karutha Pandian[1] & Arumugam Veera Ravi[1]

THANK YOU