



Biotechnology And Biodiversity: Challenges and Opportunities

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- **India harbours >10% of global fish biodiversity**
- **ranks 3rd in the world fish production (6.4 million t in 2002, 0.60 million t in 1950)**
- **The fisheries sector contributes > 1% of the total national GDP and 5.3% of agricultural (GDP)**
- **The sector is growing at a rate of over 10% (2% in agriculture and animal husbandry)**
- **Major contributors to foreign exchange, +...Rs. 6000 crores**
- **Valuable protein, livelihood and employment to millions of people**

- *World human population projected to double between the years 1980 and 2025 to total of 8 billion*
- *The production of aquatic foods will have to increase from the 100 million metric tonnes to 165 million metric tonnes in the year 2025, to keep the present per capita availability*
- *The exploitation from natural waters (sea and other water bodies) has already been stagnating*
- *Only alternative, for meeting the global fish demand is **THROUGH AQUACULTURE** and Aquaculture Biotechnologies*

Man and Fish



- Application of modern biology
- Marine Biotechnology
- Marine Biodiversity

- Biotechnology is the KEY, for the intervention, in Fisheries and Aquaculture

- Understanding and preserving biodiversity was one of the most important global challenges for the past 20 years and will continue to be an important scientific issue into the new millennium.

- The global environment is experiencing rapid and accelerating changes, largely originating from human activity,
- they come from local requirements or from the more dispersed effects of global climate change.
- Widespread realization that biodiversity is strongly modified by these changes has generated plans to conserve and protect biodiversity in many parts of the world.

Our understanding of marine biodiversity is weak.

we do not have enough scientific information to design programmes for conservation and the sustainable use of coastal resources. Some of the unique features of marine systems are:

The physical environment in the oceans is three dimensional, land is only two-dimensional.

The main marine primary producers are very small and usually mobile, whereas on land primary producers are large and stationary.

Higher level carnivores often play key roles in structuring marine biodiversity and overexploitation and overfishing results in severe cascading effects on biodiversity and on ecosystem functions.

Marine systems are more open and dispersal of species occurs over much larger geographical ranges.

Life in the sea is much older, the diversity at higher taxonomic levels is much higher in the sea (we have 14 totally marine animal phyla, whereas only one phylum is unique to land)

The sum total of genetic resources in the sea is much more diverse and on average, genetic diversity within a species (i.e. below the species level) is higher in marine than in terrestrial species.

Exploitation of marine biodiversity is less regulated, still in the hunting-gathering mode but advanced harvesting technology is threatening many marine species with extinction

- **Marine organisms are the major, sustaining components of ecosystem processes and are responsible for biogeochemical reactions that drive our climate changes.**
- **Many marine organisms are poorly described and little is known of broad spatial and temporal scale trends in their abundance and distribution.**
- **With new molecular and analytical techniques we can advance our knowledge of marine biodiversity at the species level to understand how marine biodiversity supports ecosystem structure, dynamics and resilience.**
- **We can then interpret environmental, ecological and evolutionary processes controlling and structuring marine ecosystem biodiversity.**
- **With better analytical methods available, we can augment our understanding of biodiversity and ecosystem dynamics.**
- **Using novel molecular tools, researchers in marine ecosystems were able to provide better, faster and more accurate estimates of marine biodiversity in the community.**

Attention to genetic diversity and biodiversity in aquaculture development and aquatic resource management are therefore, crucial elements for sustainable environments.

Introduction of new species/strains can affect biodiversity via impacts on the native gene pool.

New species/strains can hybridize with native stocks, and hence alter the natural genetic architecture.

This may be expressed as a loss of valuable genetic material such as locally adapted genes or gene complexes or homogenization of previously structured populations via flooding with exogenous genes.

One example of such impacts is the outcome of hybridisation between the Thai walking catfish, *Clarias macrocephalus* and the African catfish *C. gariepinus*

- Application of Molecular Taxonomy:
- Resolving taxonomic uncertainties, and phylogenetic relationships, especially for those species or populations that are endangered and/or commercially important
- Documenting patterns of natural genetic diversity and identifying management units
- Assessing genetic impacts of cultured stocks on indigenous stocks
- populations may diverge genetically without any changes appearing in their external morphology.

Molecular Taxonomy: Molecular markers

Used in stock assessment, aqua farming & conservation of Biodiversity”

Isozymes, the molecular genetic markers used in early studies, evolve so slowly that closely related populations appear identical.

This fact has undoubtedly propagated the early ideas of the absence of genetic diversity in marine biota.

The use of high resolution DNA fingerprinting techniques *sensu lato* circumvents these problems and has thus opened areas previously considered intractable.

- **Morphologic identification of fish eggs and larvae from field collections are cumbersome and imperfect**
- **Molecular taxonomy for larval identification, a promising tool**
- **Molecular marker-based prediction system of bivalve spatfall and larval abundance**

- Molecular tools in general offer the possibility to estimate biodiversity at all levels, e.g., kingdom/class/family/species level, in a comparatively small environmental sample. In some cases even a few milliliters of seawater may be enough.
- Moreover, some of the techniques are very sensitive, e.g., offer the possibility to detect single cells in a sample. One may wish to detect as many species as possible in a given sample.
- The establishment of an rRNA clone library with subsequent sequencing of as many clones as possible can uncover the biodiversity in the sample in great detail.
- General assessment of comparative biodiversity in a larger number of samples can be achieved with fingerprinting methods based on restriction fragment length polymorphisms (RFLPs), RAPDs, Microsatellites
- Presence or absence of a known species can be monitored with species-specific probes using chemiluminescent detection with dot blot techniques or, more sophisticated, with fluorescent *in-situ* hybridization (FISH) .
- Distinction of individuals at the family or even species level can be obtained using highly variable molecular markers such as ITS sequences (inter-transcribed spacer) or microsatellites.

Advantages of Molecular techniques over traditional methods:

1. Only very small samples (in the range of milliliters up to a liter) are required for most analyses.
2. Sensitivity of many methods is very high, e.g., enabling the researcher to detect even single specific cells among thousands of others.
3. Dead or non-culturable cells can be analysed.
4. Species-specific data (such as sequences) can be obtained without the need to culture or even isolate a species.

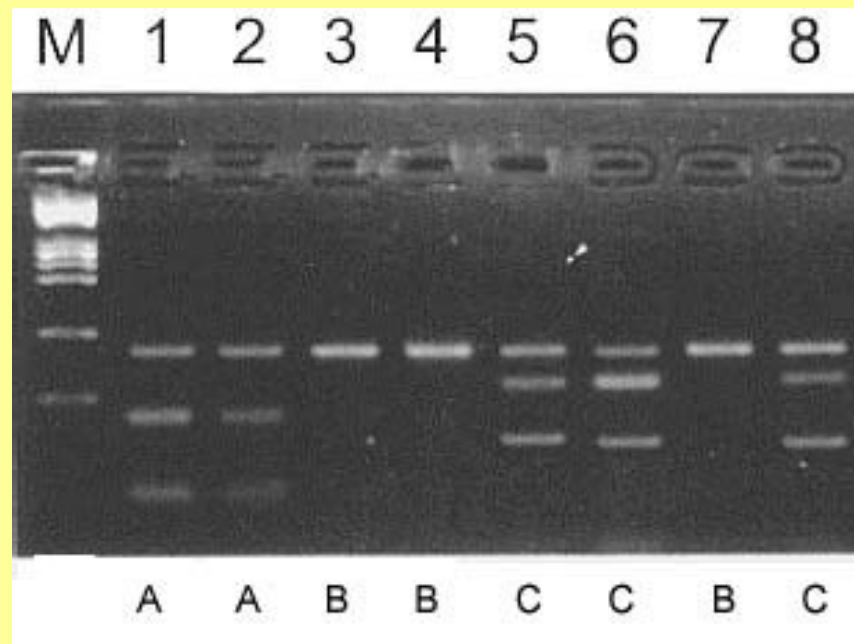
- Before development of molecular technologies, studies of external morphological phenotypes was in vogue.
- **RFLPs were the first DNA markers to be used by population biologists (Parker et al. 1998).**
- **The technique involves cutting a DNA strand at specific nucleotide sequences using a restriction endonuclease and thereby producing a pool of different sized DNA fragments.**
- **RFLP variation can be visualized directly by staining with ethidium bromide following electrophoresis of the DNA in an agarose gel.**
- **This can be done for small molecules, such as the entire mitochondrial DNA, which produce a manageable number of fragments with many restriction enzymes**

- **RAPD** markers are produced by PCR using short oligonucleotide primers of random sequences.
- Different RAPD patterns arise when genomic regions vary according to the presence/absence of complementary primer annealing sites.
- The primers are typically 10 bp long (Williams et al. 1990) and no specific knowledge of a particular DNA sequence is required.
- Primers suggests that the technique will be useful for a variety of questions, including individual identification, pedigree analysis, strain identification, and phylogenetic analysis.

- The **AFLP** protocol involves the following steps:
 1. DNA digestion with two different restriction enzymes (typically EcoR I and Mse I),
 2. Ligation of double-stranded adapters to the ends of the restriction fragments,
 3. Optional DNA pre-amplification of ligated product directed by primers complementary to adapter and restriction site sequences,
 4. DNA amplification of subsets of restriction fragments using selective AFLP primers and labeling of amplified products,
 5. Separation of fragments via electrophoresis

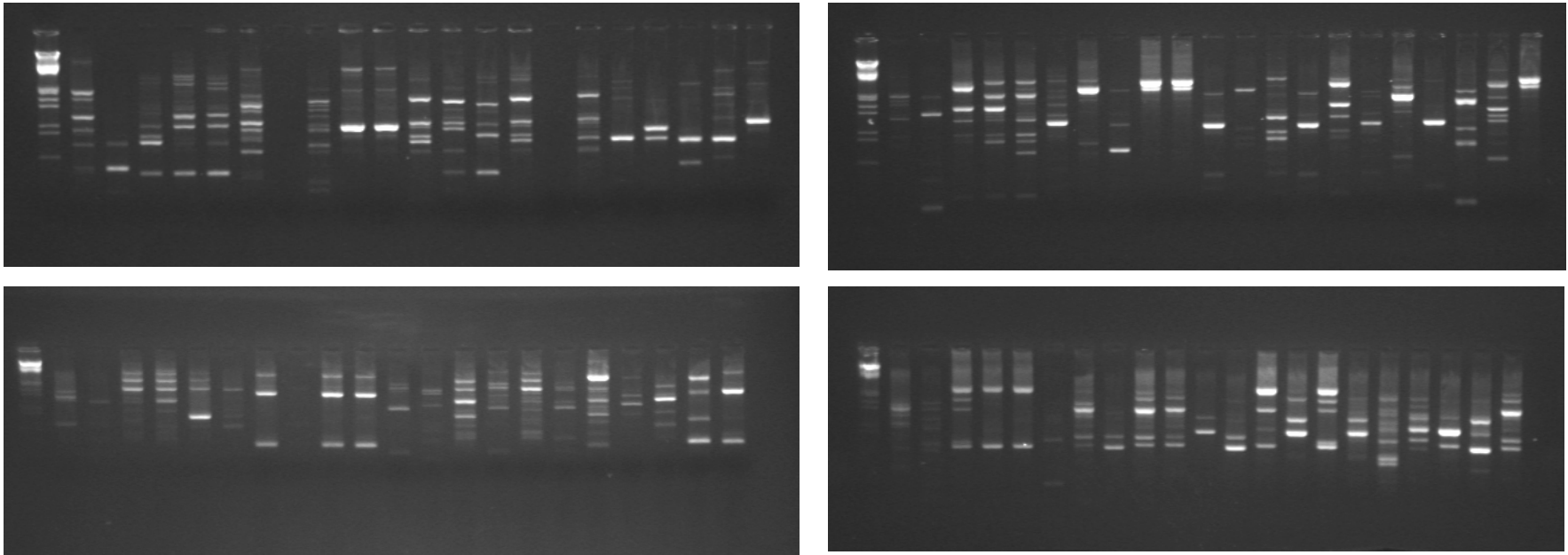
- Microsatellite loci can be identified by screening genomic libraries with probes made up of tandemly repeated oligonucleotides and then sequenced to identify conserved flanking regions for primer design.
- Loci identified in this way are analysed by amplifying the target region using PCR, followed by electrophoresis

An Example of RFLP



DNA(RAPD) fingerprinting-Molecular systematics is not an alternative for classical Taxonomy, but a novel complimenting tool.

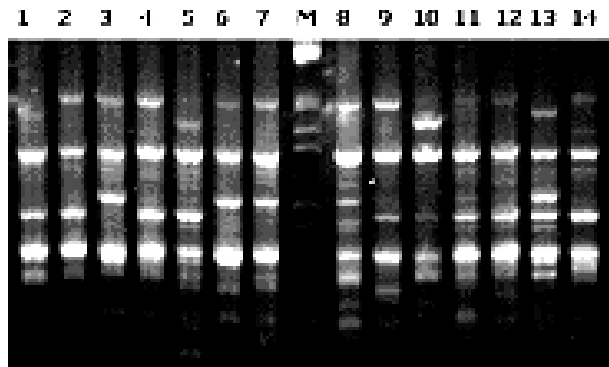
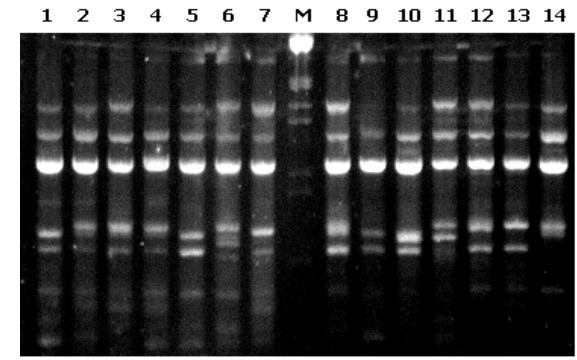
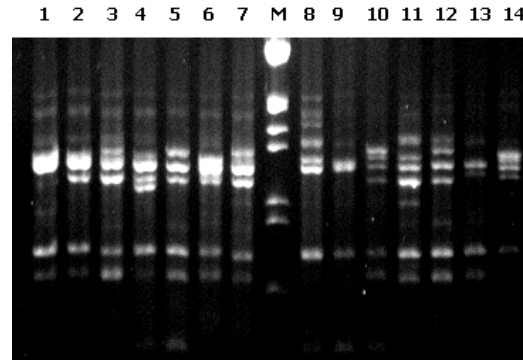
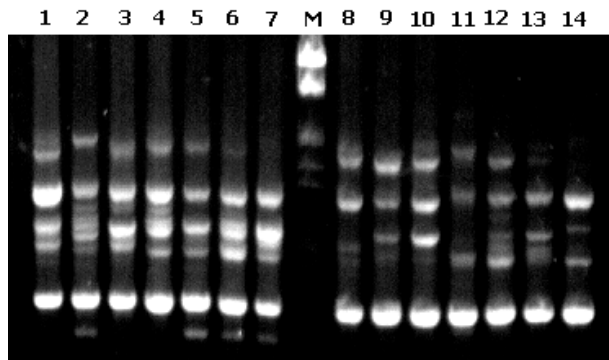
Finfishes



OPD 5, 11, 16, 20

***Bivalves* : Genetic analysis (RAPD)**





Primers: OPA-07, OPAA-12, OPAC-14 & OPB-08



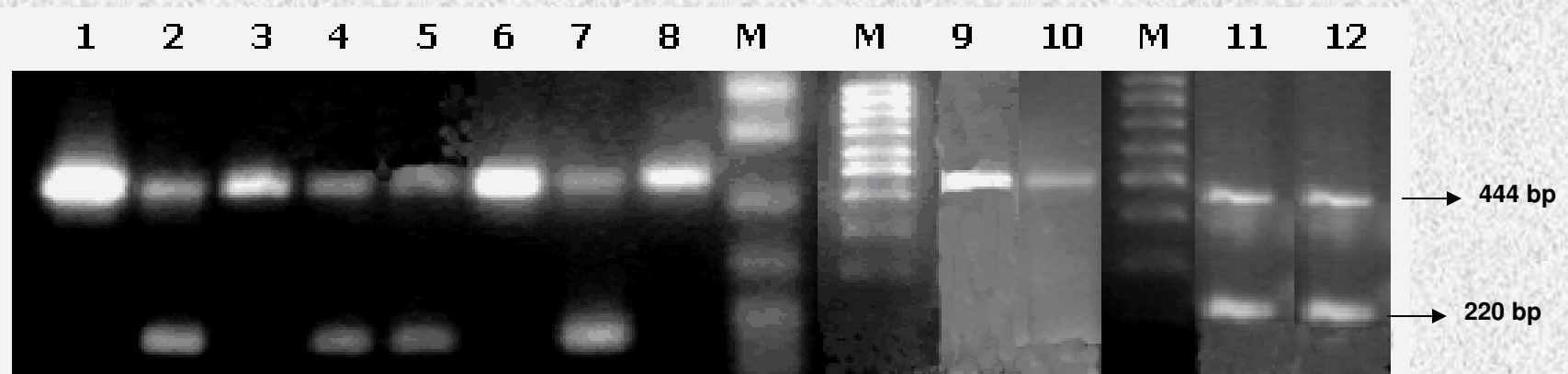
Further studies with mtDNA and microsatellites envisaged

For Conservation...

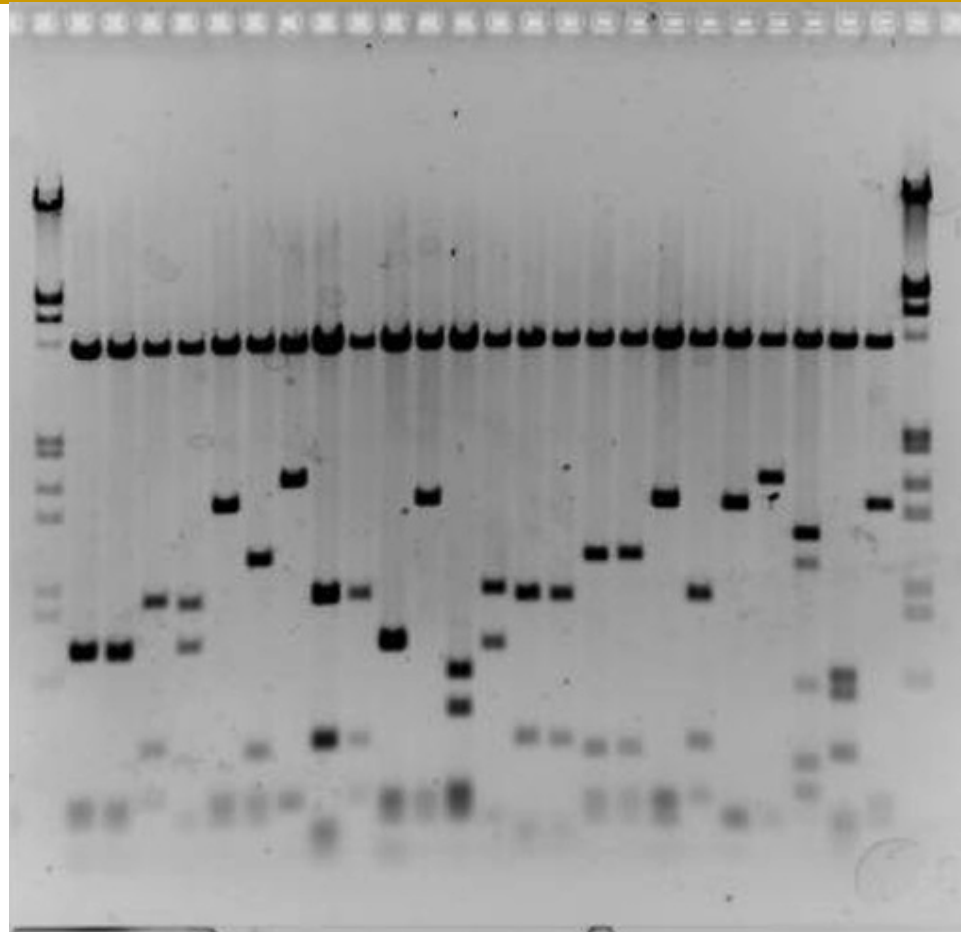
Sex-specific molecular markers

-  **Unambiguous identification of female and male brood fishes for captive breeding programmes**
-  **Estimation of sex ratio of fish at early stages of maturity**
-  **The markers could serve as the starting points for the identification of genes involved with the regulation of sex determination as well as early gonad differentiation**
-  **Molecular identification of sex could be useful for rapid testing of possible environmental and chemical effects on the reproduction of cultured species**

DNA (PCR) based gender identification in marine mammals (8 species)



1, Finless porpoise Female; 2, Finless porpoise Male; 3, Spinner dolphin Male; 4, Spinner dolphin Female; 5, Bottlenose dolphin Male; 6, Bottlenose dolphin Female; 7, Indopacific humpbacked dolphin Male; 8, Indopacific humpbacked dolphin Female; 9, Risso's dolphin Female; 10, Dugong Female; 11, Blue whale Male; 12, Bryde's whale Male; M, DNA size markers



Biodiversity of marine Picoplankton. DNA was isolated from an environmental sample, i.e., 3 μm filtered sea water. A clone library of PCR-amplified SSU rRNA was established and plasmids were analysed by restriction enzyme digestion. The figure shows the typical variability of such clone libraries.

(Lanes 1 & 26 = size markers, lanes 2-25 = 24 individual clones digested with restriction enzymes.)

Molecular Methods-certain biases:

- **The harvesting of cells through filtration or centrifugation may be harmful for fragile organisms, which thus may escape the analysis.**
 - **For many techniques the lysis of organisms with subsequent isolation of DNA is a prerequisite. Both steps may not be equally effective in all organisms.**
 - **In PCR-based approaches biases are evident concerning the choice of (universal) primers, PCR conditions (e.g., the amount of DNA or primers used, the annealing temp., cycle number etc.), machines or enzymes used etc.**
 - **The copy number of genes of interest (mostly ribosomal RNA genes) differ greatly among various organisms.**
 - **If cloning steps are involved, then the choice of vectors, enzymes or bacterial strains may be relevant.**
 - **Hybridization experiments are susceptible to hybridization conditions (temperature, salt concentration, time) or base composition and subsequent detection of fluorescence may be hampered by auto-fluorescence.**
 - **All these are important when absolute quantification of results is desired.**
 - **In general, the same caution need to be taken when interpreting the results of molecular methods, as for all other methods. Results are not more reliable because they come from a “molecular” approach rather than a “classical” one.**
-

Indian Aquaculture-Mariculture

STRENGTH

Coastline- 8118 km, EEZ- 2.02 million sq.km

Water bodies suitable for aquaculture

Reservoirs- 3 million ha

Swamps, Ponds, lakes n paddy fields-2.5 million ha

Brackishwater-1.9 million ha

Coastal seas for cage culture, seaweed culture.

Tropical Climate, Species diversity and Cheap labour

WEAKNESS

Unregulated development

DISEASE PROBLEMS

Lack of scientific approaches

Diseases in
Ocean
Ecosystems
and their
Dynamics in
relation to
Climate
Change.

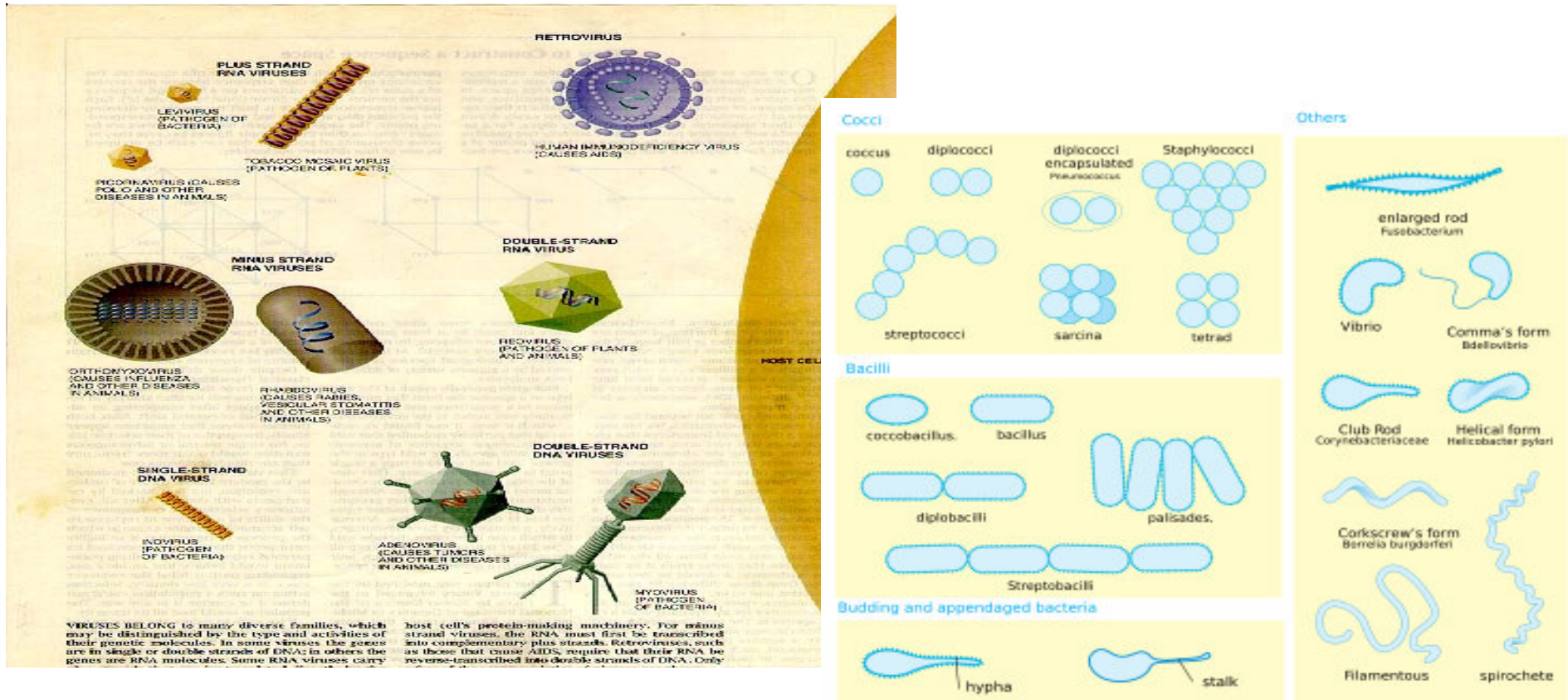


Emerging diseases

- Fifty years ago, many experts believed that the war against infectious diseases had largely been won
- But during the last 30 years, when humans altered and polluted the ecosystem, numerous viruses (for example, HIV, Ebola, avian respiratory viruses-bird flu) have jumped from their long-term animal hosts to people, probably with a less immune status
- Often succumb to virulent 'emerging diseases'
- Also, old enemies such as dengue has re-emerged to cause human epidemics

Although the importance of diseases and disease causing pathogens in terrestrial ecosystems has long been recognized, their role in most marine communities is comparatively unknown

This paucity of information is surprising, given that the sea is a 'microbial soup' supporting an immeasurable abundance and diversity of potential parasites-pathogens



Disease causing organisms can have significant impacts on marine species and communities, as demonstrated by series of disease outbreaks that have even caused mass mortalities over a wide range of marine taxa

For some important marine taxa, diseases and their impacts appear to have increased over the past 30 years. These include turtles, corals, molluscs, urchins and marine mammals.



Molecular Diagnostics

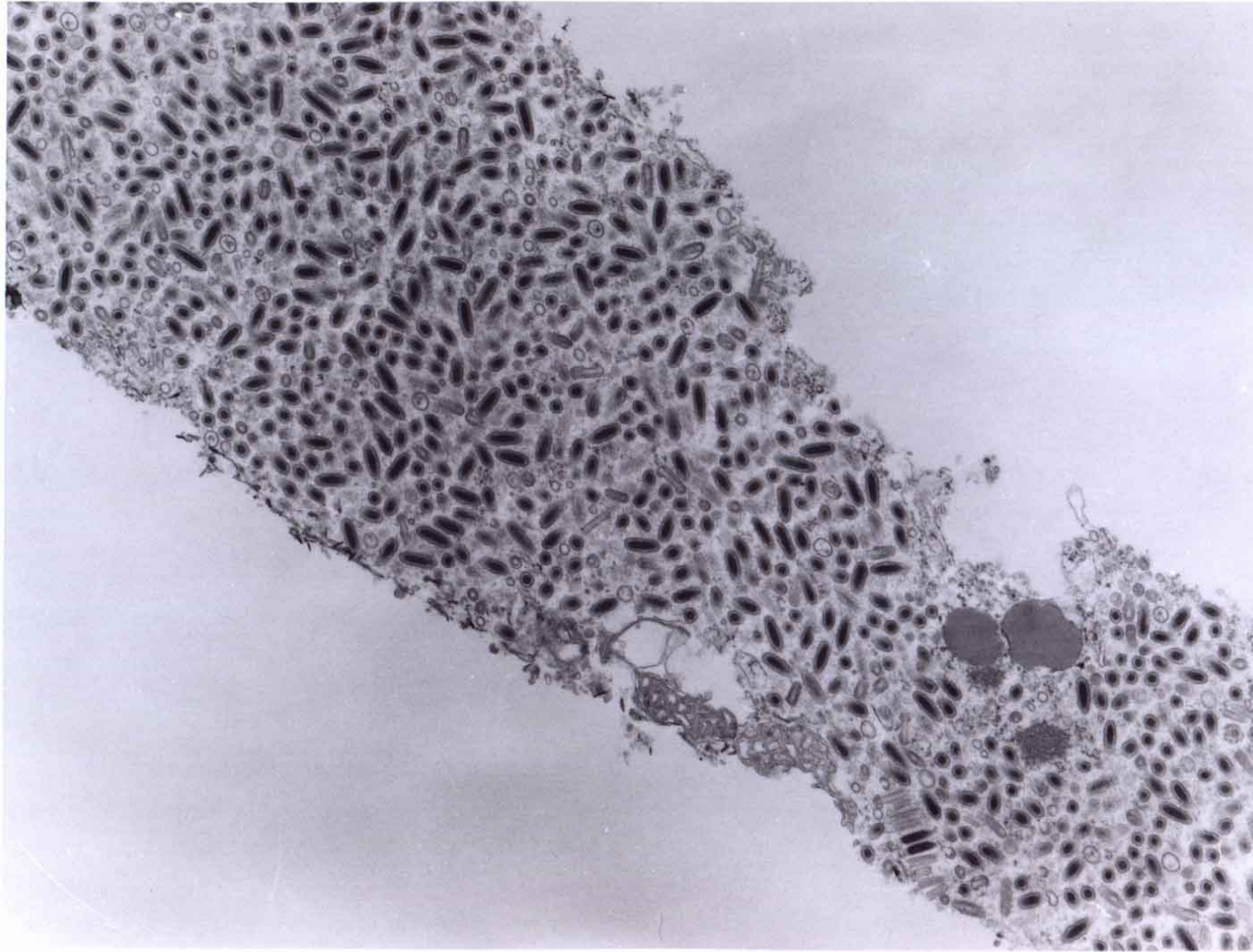
- Short life span hence rapid accurate diagnostics
- Viral pathogens of shrimp and prawns
- PCR based diagnostics
- CIBA has developed Molecular diagnostic kits for white spot and white muscle viral disease which are commercialized



WSSV: change in viral virulence or viral accommodation in the host shrimp?

- **WSSV the most lethal animal virus**
 - **Affects all stages of farmed and wild penaeids**
- 100% mortality within 2-10 days of post infection**
- Collapse of the shrimp farming industry across the world**
- **Annual loss of ~Rs 300 crores (@80 Million US \$) in India,**
 - **Accumulated Loss during the last one decade is about 1 Billion US \$)**

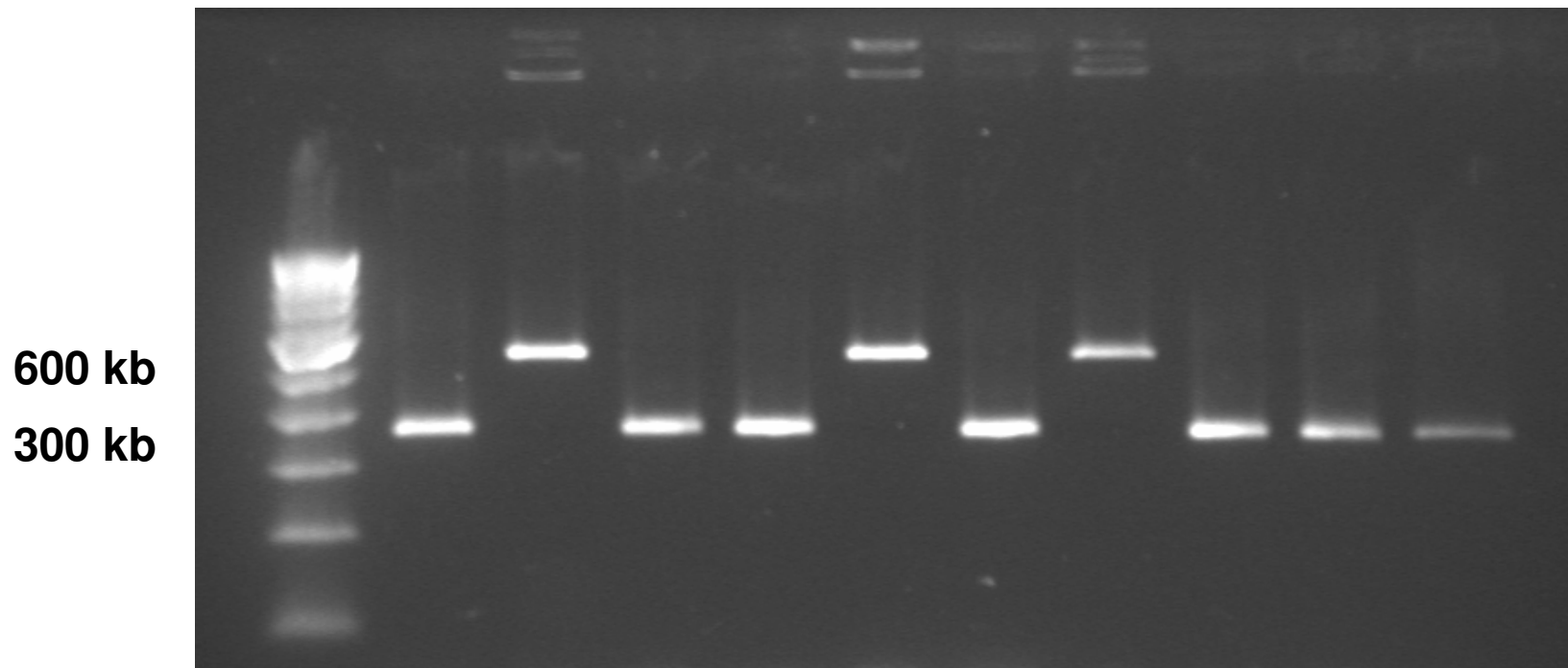
**Electron micrograph of
White Spot Syndrome Virus
in infected nuclei in epidermal tissue**



WSSV Sequence

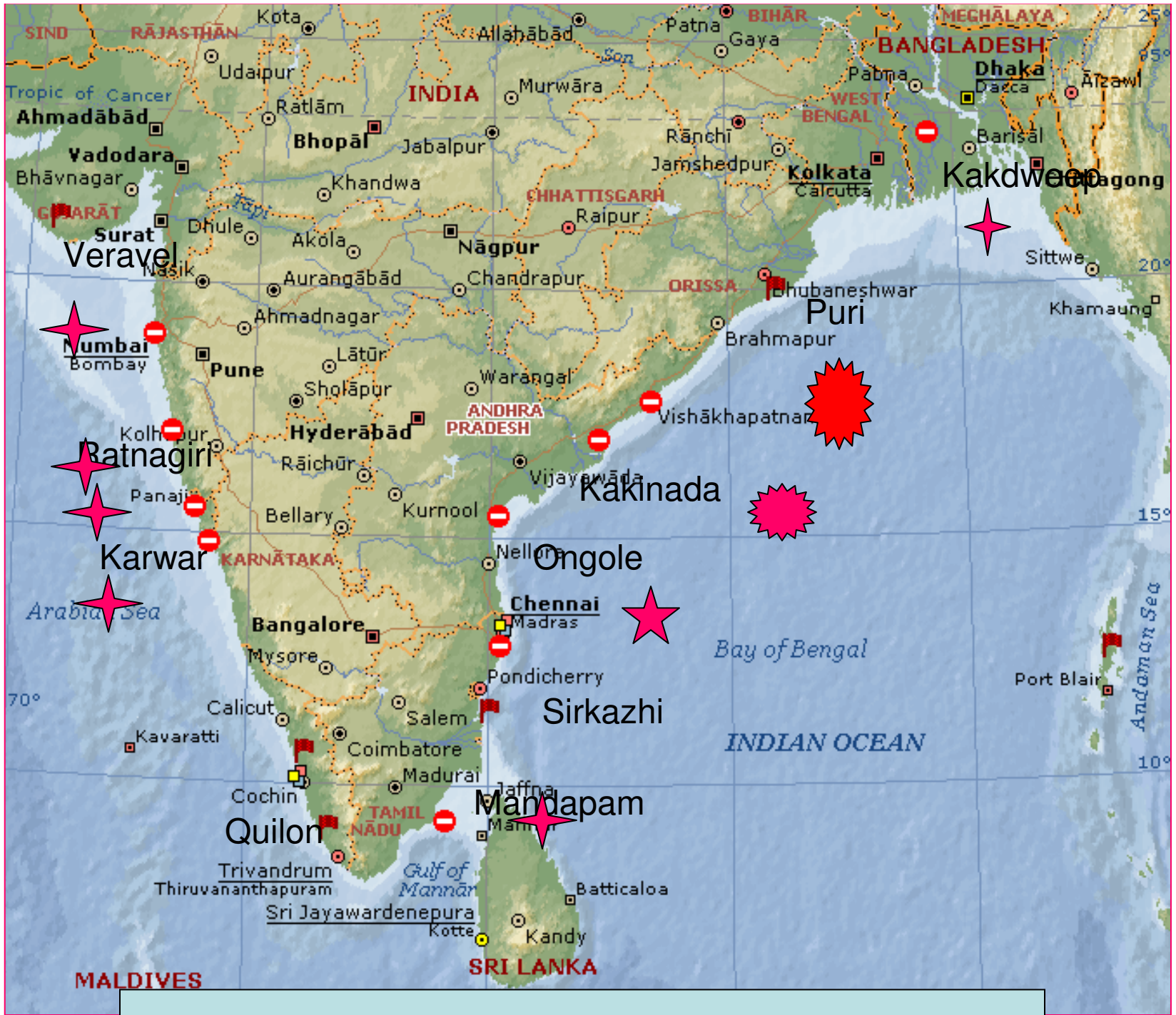
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GAGGTGATAATTACGTTGGGTCGGCTAGCGATTCTAGAGTATTGTC CAAGACT
CTTAGTACGCGACTTTTAACTTTTATGTTCATGCTGATCATGCCGCCTATCA
CTTTAATCAGTTTATAAAAACGGGTGATGCGGGCTATGATCATGAAGATATAA
GGACTAGAAAGATGCCGTTGAAACCAAGAGAGGTTAAATACTGTCACGATCTA
ATCTCTTCTATACCCAATAAACACTTGACTGACTATTTAAAGTCCCATGATGT
ACTTAGGATTGAAGACGTTGCAGTTTCAAACAGTCATTGTTCTAA TTTGGAA
GTATATTTACTCATAATTCCGATTCAAGTGATAACGAATGAATATTATAAAGT
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AATGTGAAAAGAAGGAGAGTTTAAACAGTGGAAGATATAATTGAGGATATATAT
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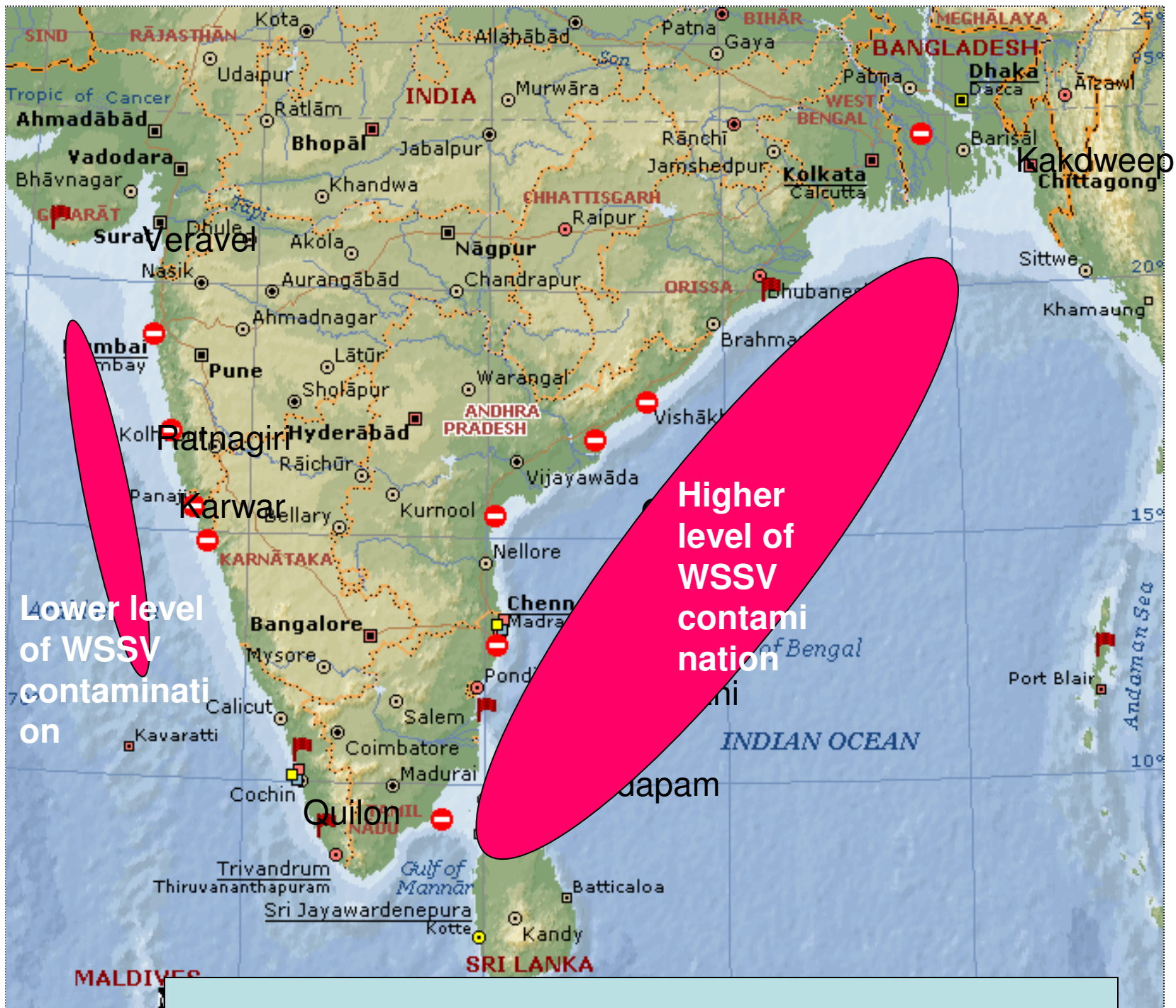
WSSV-nested PCR



CIBA-ICAR *nested PCR* KIT for WSSV (Commercialized with Genei Bangalore)







Mariculture

Global mariculture production in 2002

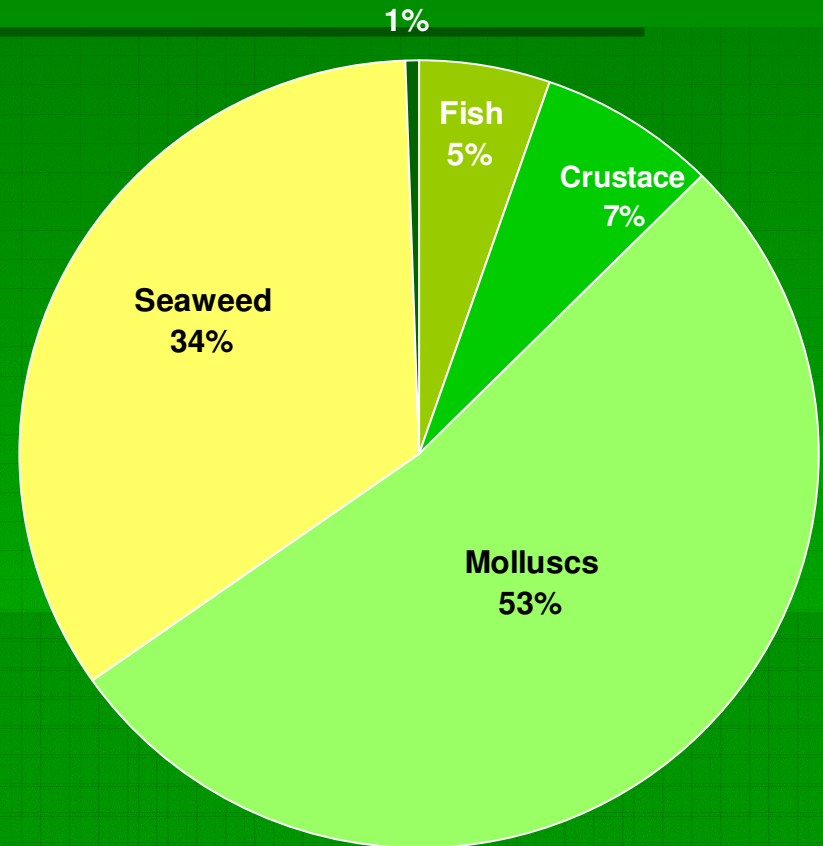
26.08 million tonnes valued at US\$ 25 billion

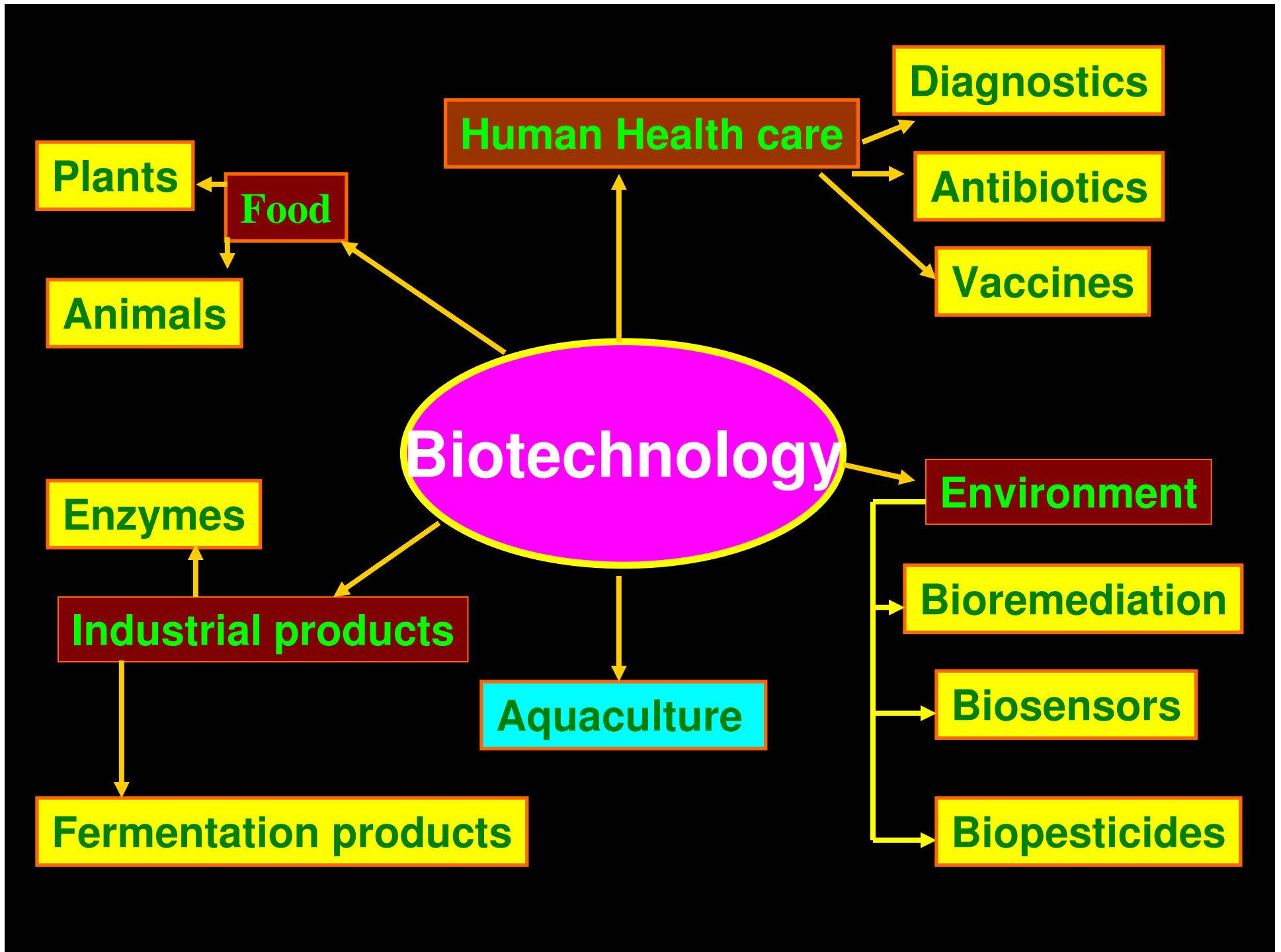
Most popular farming resources are oysters, clams, scallops and mussels

Seaweed farming popular in Asia

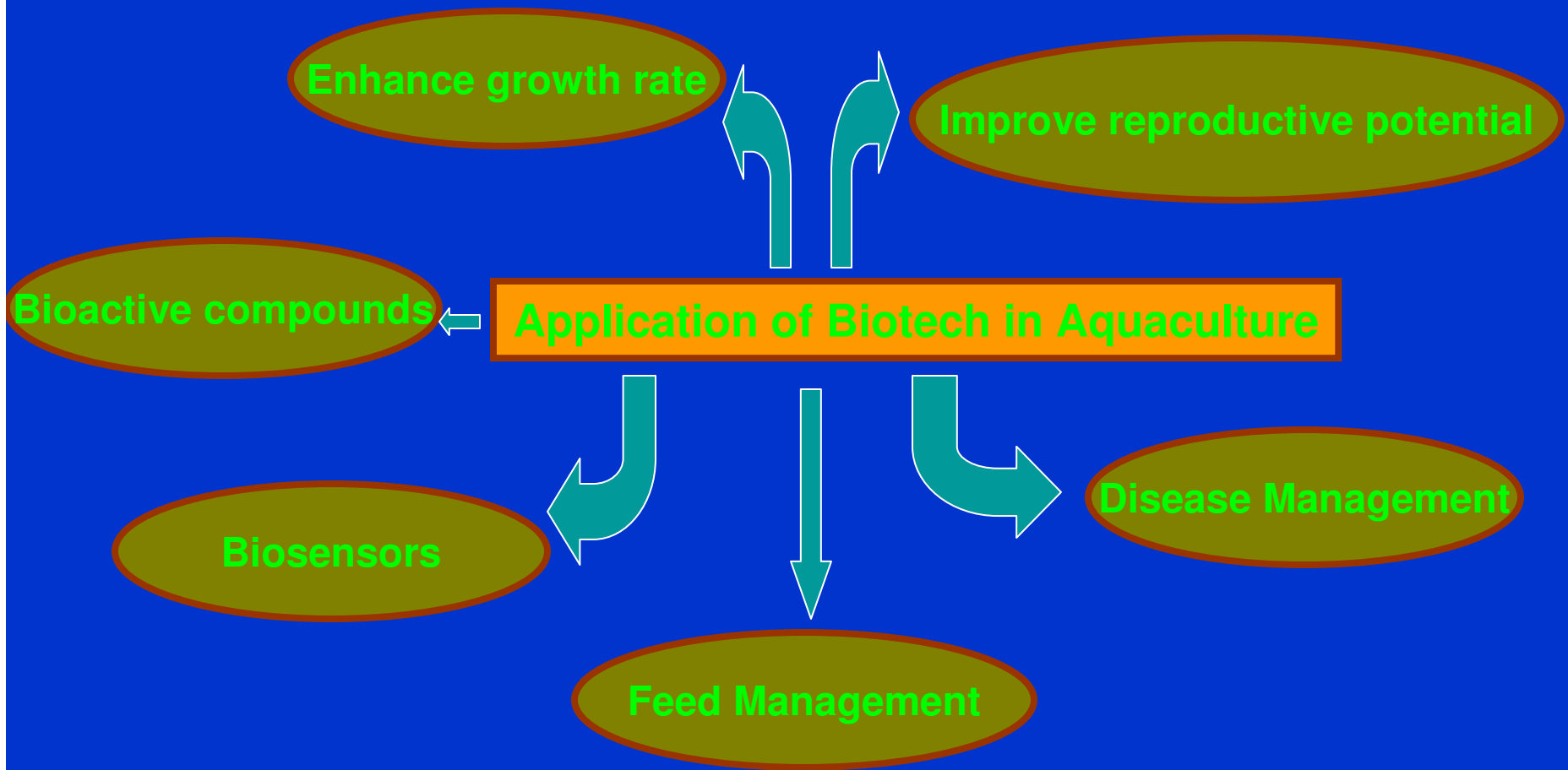
Finfish farming is capital intensive, feed requirement high; 5 kg of wild fish is required to produce 1 kg of farmed carnivorous fish.

Emergence of sea cucumber farming, abalone farming and other minor invertebrates like corals





Biotechnology in Aquaculture/ Mariculture



Marine biotechnology

- Marine biotechnology is in its infancy
- Biotechnology is a cocktail of biology and engineering principles
- It is a modern science of our time
- It has evolved into a powerful tool
- It is influenced by modern developments



Prospects of marine biotechnology

- It deals with freshwater, brackish water & marine ecosystems
- Aquatic biotechnology is the apt terminology
- Throws new insight into aquatic biology
- Aims at providing food security, nutritional security & novel business opportunities
- Employment, and gender equity too



Aquaculture/Mariculture Biotechnology

Opportunities and Challenges

- Reproduction
- Genetics
- Nutrition
- Health Management – **Disease Problems**
- Bioprospecting

Fish Breeding

- Hormonal manipulations
- Cryopreservation

Aqua-- biotechnology

- Aquaculture for food: shellfish and finfish culture
- Single cell proteins (*Spirulina*)
- Drugs and chemicals - vitamin A (*Dunaliella*)
- *Artemia* culture -larval feed
- Ornaments- pearl culture
- Ornamental fishes- with colour genes
- **Green fluorescent protein from Jelly fish**





-Genomics

-Human Genome Project

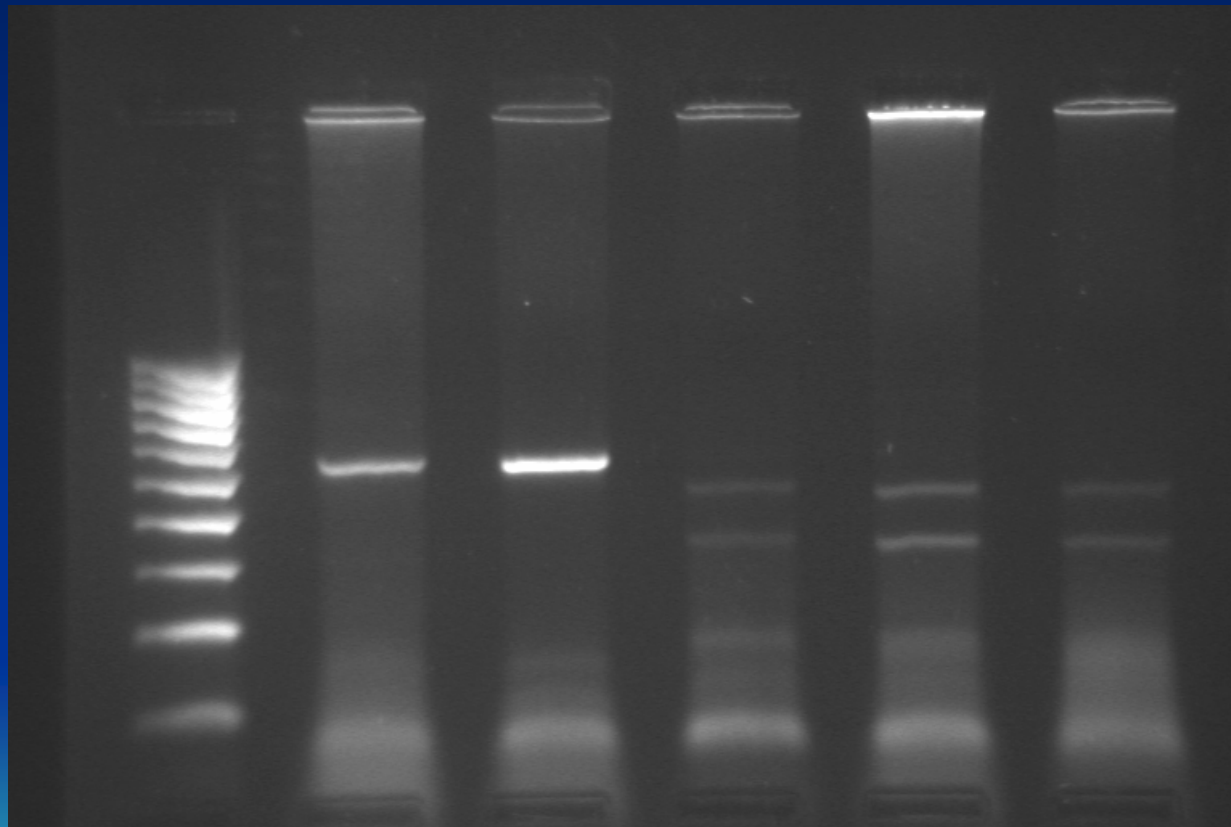
- **Genomic mapping and sequencing, has steadily extended its dominion in all areas of applied biology**
- **While understanding biological systems with 100s, 1000s to 100,000s of genes, require organizing the parts by their properties.**

Gene mining

- Aquatic animals harbour novel genes of economic importance
- Antifreeze genes, GFP genes
- Antiviral genes, penaeidins, salt tolerant genes bioluminescent genes
- They are used for health management and in molecular biology



Antiviral genes in *Penaeus monodon* and *Machrobrachium rosenbergii*



Lanes 2 & 3 *p. monodon*, lanes 4,5 &6 *M.rosenbergii*

Safeguarding against bio-piracy

- ‘Diversity’ thy name is India
- Microbial diversity
- *V. harveyi*, *Pseudomonas*, Mangroves, corals etc.
- Requires molecular cataloguing
- DNA finger prints, RAPD, RFLP, AFLP etc.
- Helps in safeguarding from bio-piracy



Fish Transgenesis

- Integration into a living organism of a foreign gene that confers upon the organism a new property that it will transmit to its descendants
- Transgenic fish have been produced that exhibit accelerated growth rates, increased disease resistance, altered body shape and composition, altered coloration, expression of anti-freeze proteins and potential sterility
- **GENE MINING**

Transgenics

- Introduction of novel genes into an organism
- It is successful in plants and in animals
- Fish has certain advantages
- No ethical considerations
- Large number of eggs, external fertilizations
- Easy screening, short life span, genetic plasticity
- Flavour, palatability and as bioreactor



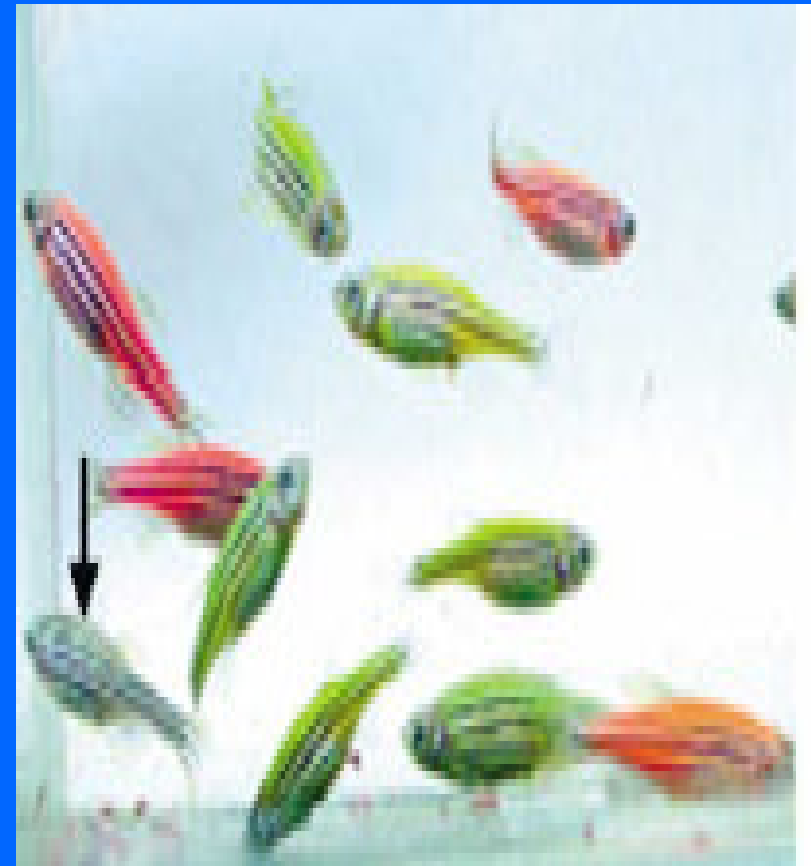
Example of transgenic species

- rainbow trout (*Salmo gairdneri*)
- goldfish (*Carassius auratus*)
- northern pike (*Esox lucius*)
- walleye (*Stizostedium vitreum vitreum*)
- transgenic loach,
- Carp
- northern pike
- Zebra fish (Glofish)
- Medaka
- Tilapia
- Brine shrimp, Sea urchin, Abalone, sea weed.....

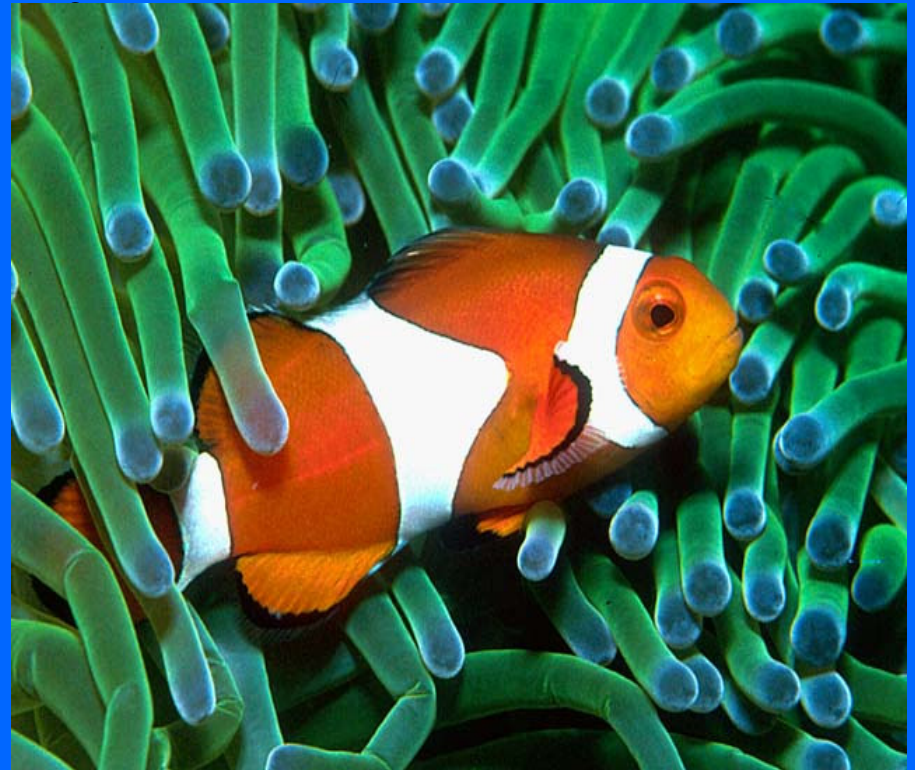
Atlantic salmon expressing GH transgene



GloFish



Potential candidates for transgenesis



Bioremediation

- Soil and water pollution
- Pollution accumulators
- Probiotics , immunostimulants
- DNA vaccines
- Therapeutic RNAi



Microbes from aquatic ecosystem

- Good guys and bad guys
- Industrially useful microbes:
 - *Saccharomyces*, *Streptomyces*
- Bioactive: agarase, antimicrobials
- Mol biol tools: Restriction Enzymes
- Source of drugs and antiviral compounds
- It is a source of great wealth
- Duty to safeguard and pass it on to the generations to come



It is recorded that only 7% of the oceans and 1% of the oceans' floor has been sampled till date and over 93% of the ocean still remain unexplored and thus, the current state of knowledge regarding its biodiversity and distribution is extremely poor.

Among the 1.7 million species catalogued today, about 2,50,000 are from the marine environment. So far only about 1,80,000 species of marine algae, animals, bacteria, fungi and viruses have been identified and characterized and more are yet to be discovered.

Estimates of the number of described species and possible undescribed species of microorganisms

Group	Described Species	Estimated species	%known
Bacteria	4000	3,000,000	0.1
Fungi	70,000	1,50,000	5.0
Viruses, Plasmids, Phages	5000	500,000	1.0

~only 3% of the worlds microorganisms have been described !!!!!

Marine Bioprospecting

‘Bioprospecting is the systematic search for and development of new sources of chemical compounds, genes, micro- and macro organisms, and other valuable products from the nature’

Why Marine Bioprospecting?

- Oceans harbour about 300,000 described plants & animals to date and already yielded about 12,000 novel chemicals from only a small portion of that diversity
- **Applications of marine biomolecules/metabolites: pharmaceuticals, enzymes, cryoprotectants, cosmaceuticals, agrichemicals, bioremediators, nutraceuticals etc.**
- **Wide range of useful organisms: Microbes, tunicates, sponges, soft corals, sea hares, nudibranchs, bryozoans, sea slugs and seaweeds/seagrass**

‘Estimates put worldwide sales of marine biotechnology-related products at over US\$100 billion’

Bio-business from sea

Sales of products developed through biotechnology were up 17% in 1998 to \$13 billion a figure with the potential to reach \$34 billion in 2008.

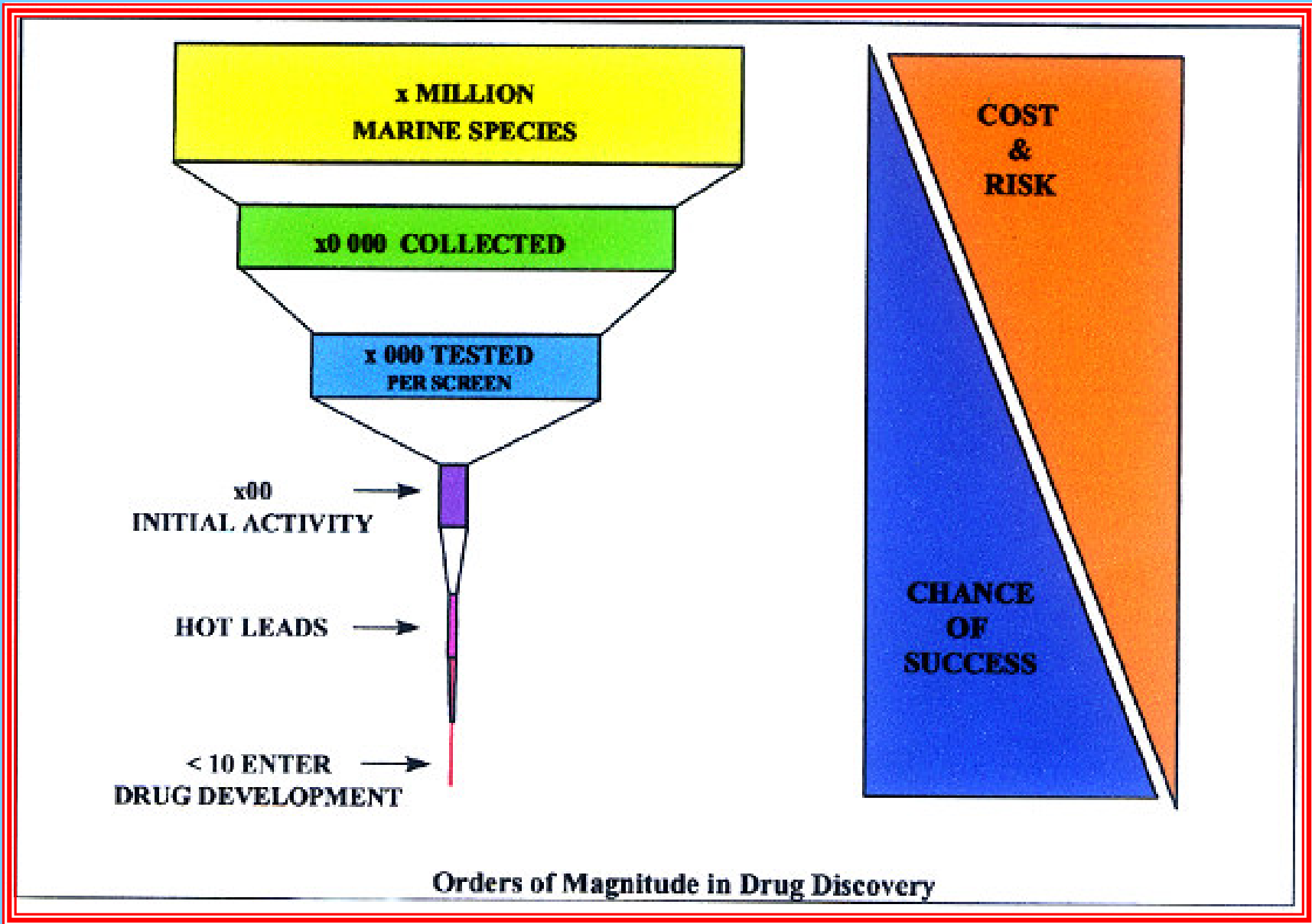
These developments have been largely based upon the molecular genetic characteristics of terrestrial organisms, even though more than 80 percent of all the Earth's phyla are found only in the sea.

In 1992, the U.S. invested \$40 million in marine biotechnology research leading to at least 190 U.S. patents and at least 30 preclinical trials targeting cancer, inflammation and AIDS. The market value of just five of these has been estimated to be \$2 billion.

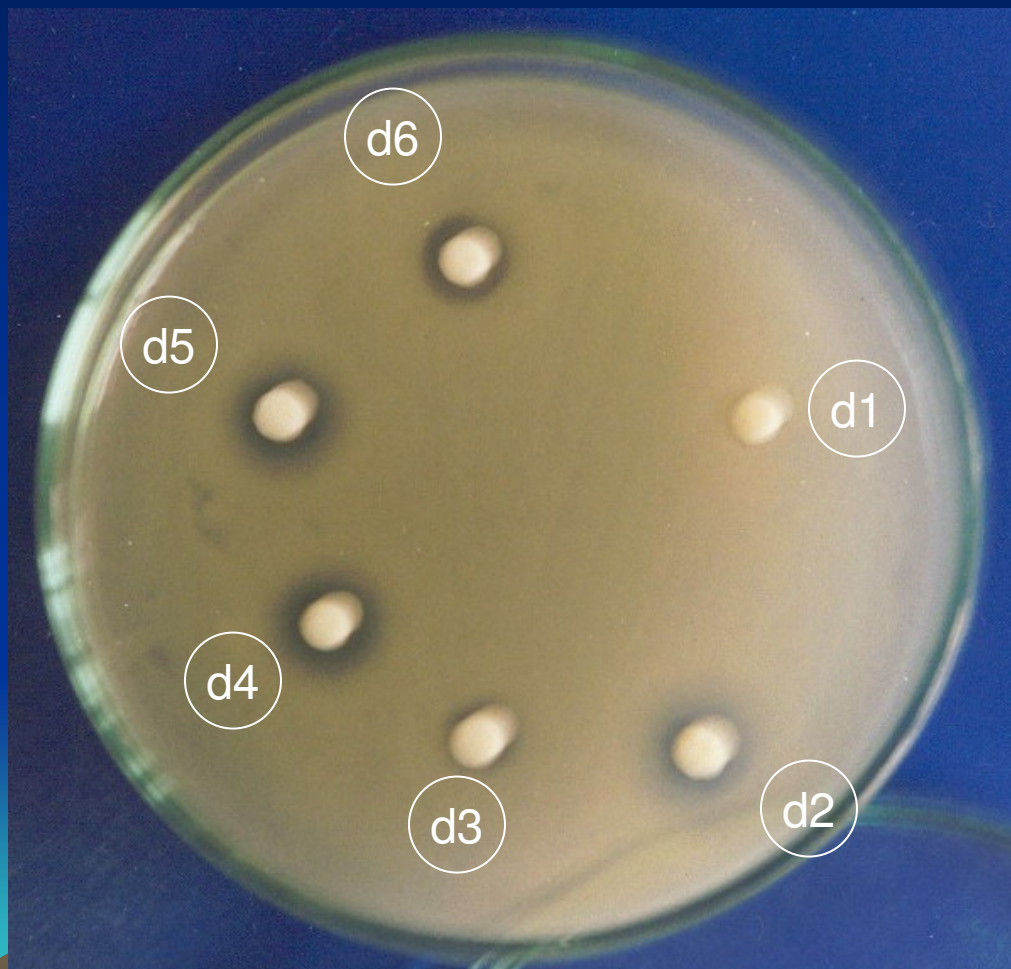
Glimpse of Global Research Outputs

Marine organism	Metabolite/Biomolecule activity & Novel genes	Reference
Microorganisms - cyanobacteria	Anti-tumour, antibiotic & anti HIV agents, carotenoids and phycobiliproteins	Carte 1996, Cardillina <i>et al.</i> 2004, Greer & Harvey 2004
Seaweeds	Anti-tumour, anti-inflammatory & anti bacterial agents	Donia and Hamann 2003, Haefner 2003 & Faulkner 2002
Sponges & Ascidians	Anti-tumour, anti-inflammatory & Anti asthma agents	Burres and Clement 1989, Petitt <i>et al.</i> 1993 & Fenical <i>et al.</i> 2002
Cnidarians	Prostaglandins & Palytoxins	Carte 1996
Molluscs	Neurotoxins, cytotoxins & antifungal agents	Pickrell 2003 and Rorsener & Scheuer 1986
Fishes & sea snakes	Antidotes, hormones, Fugu poison & Ciguatoxin	Oliviera <i>et al.</i> 2003
Atlantic salmon & Abalones	Growth hormone gene & antifreeze gene	Hew & Fletcher 2001
Sea grass & Mangroves	Genes encoding anti porter, proton pump & osmolytes	Fukuhara <i>et al.</i> 1996, Parani and Parida 1999 & Benito <i>et al.</i> 2002

Expensive and effort-intensive

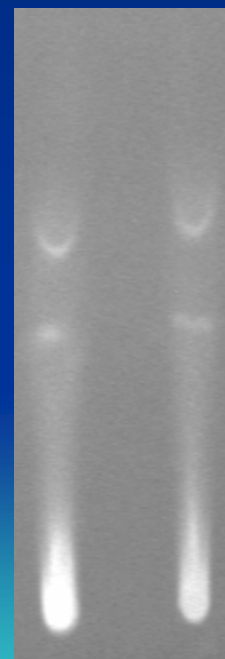


Preliminary characterization of the antibacterial compound from 99 H isolate:



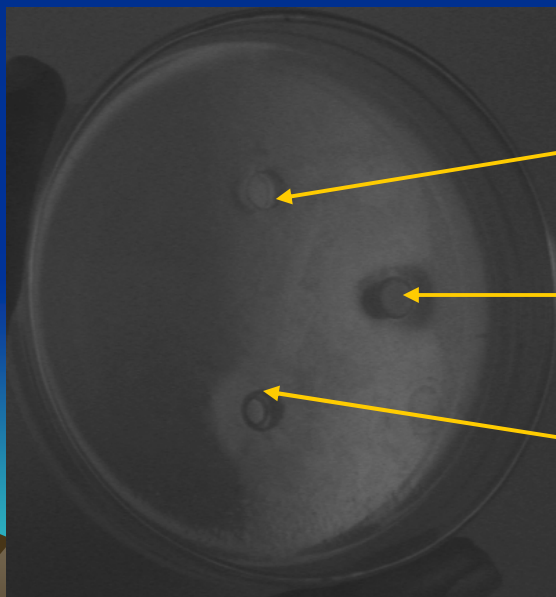
- Culture sup extracted with acetone
- Concentrated the extract by freeze drying
- 25 μ l spotted on TLC plate
- Mobile phase: Chloroform: methanol: water (1.2:0.6:0.08)

RF values:
Spot 1: 0.625
Spot 2: 0.55



Agarolytic bacteria

- Isolated from marine environment
- Shows high agarolytic activity
- Potential source for molecular grade agarase enzyme.



Control

**Activity of
purified fraction**

**Positive
Control**

- Biotechnology has the potential to replace *information technology* as the engine of economic development for India n Asia
- New jobs, Business opportunities
- Green revolution helped India to meet the challenges in food sector at that point of time..
- **Now A GENE REVOLUTION IS IN WAITING.....**

Potential stakeholders

Scientific and technological
Personnel from Universities
Research Institutions and
Centres Pursuing R&D in

- **Human Health**
- **Agriculture**
- **Veterinary**
- **Fisheries and
Aquaculture**

Biotechnology industry

- **Biopolymers**
- **Enzymes**
- **Pharmaceuticals**
- **Starter cultures**

Manufacturing industries

- **Fermentation**
- **Food and beverages**

This and the coming decades is
all set to see a
(AQUA)BIOTECHNOLOGY BOOM ...

- And our time will belong to those who have the power, energy and vision to convert their dreams into realities..



■ Thank you
all