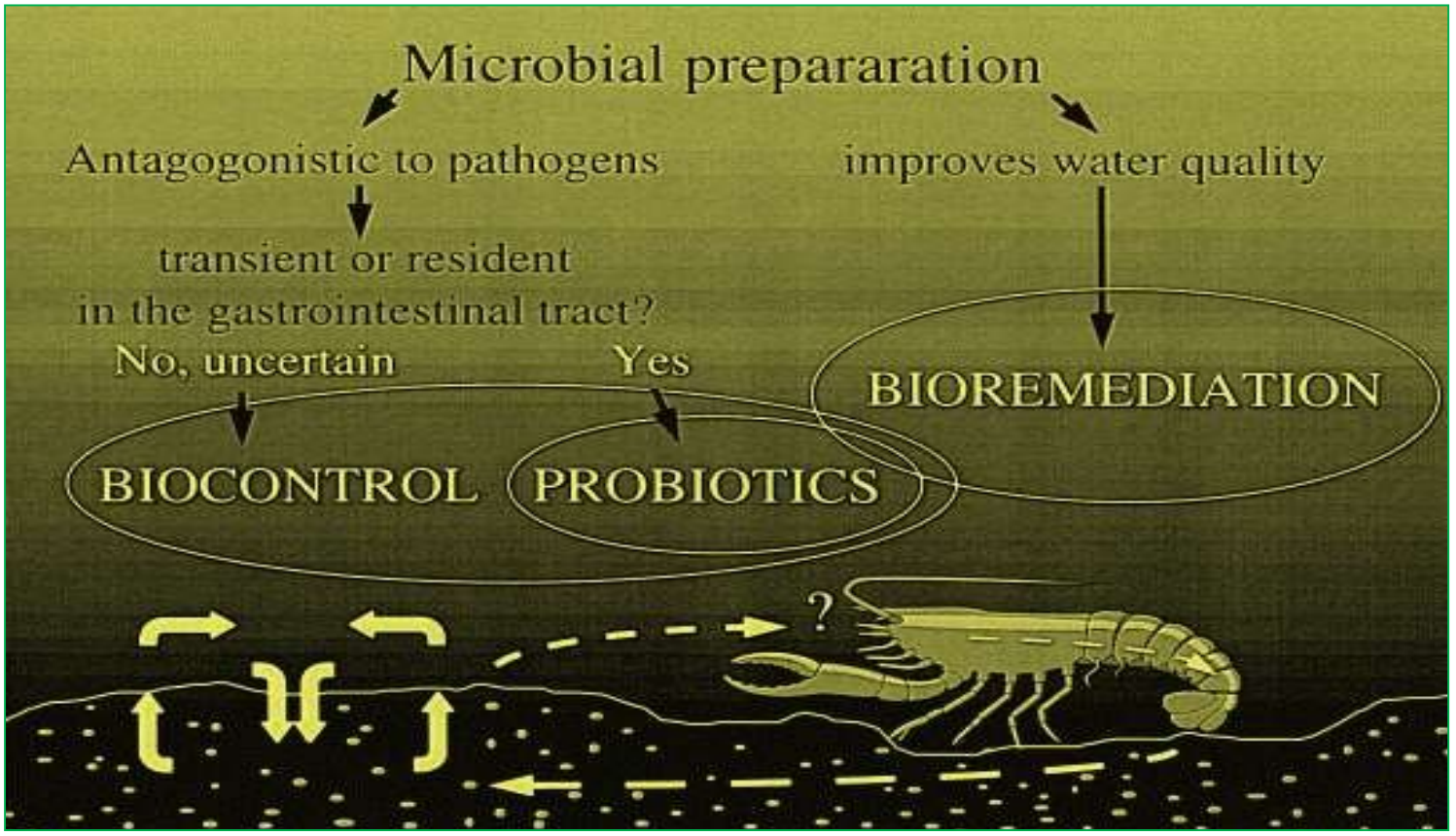
Probiotics In Aquaculture



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Prebiotics & Probiotics : Is there any difference ?

	Probiotics	Prebiotics
Nature of the Prep	Microorganism	Food supplement (eg: FOS)
Prime Fn	To kill harmful pathogen	To supply nutrition (Killing the pathogen is an additional effect)

Probiotics

- The term Probiotic is derived from the Greek words, Pro (favour) and Bios (life). So, in purely literary terms probiotics can be defined as organisms/ substances that favour life.
- At the start of the 20th century, Russian noble prize winner and father of modern immunology, Elie Metchnikoff, a scientist at the Pasteur institute, was the first conceptualize “probiotics”.
- Probiotic term coined in 1965 by Lilly and Stillwell.

Probiotics

- Live microbial feed supplements that have beneficial effects on the host by improving its intestinal microbial balance.
- A Probiotic is proven to survive the passage through the gastro-intestinal tract, intestinal tract(where it exerts its effects on digestive system) .

Prebiotics

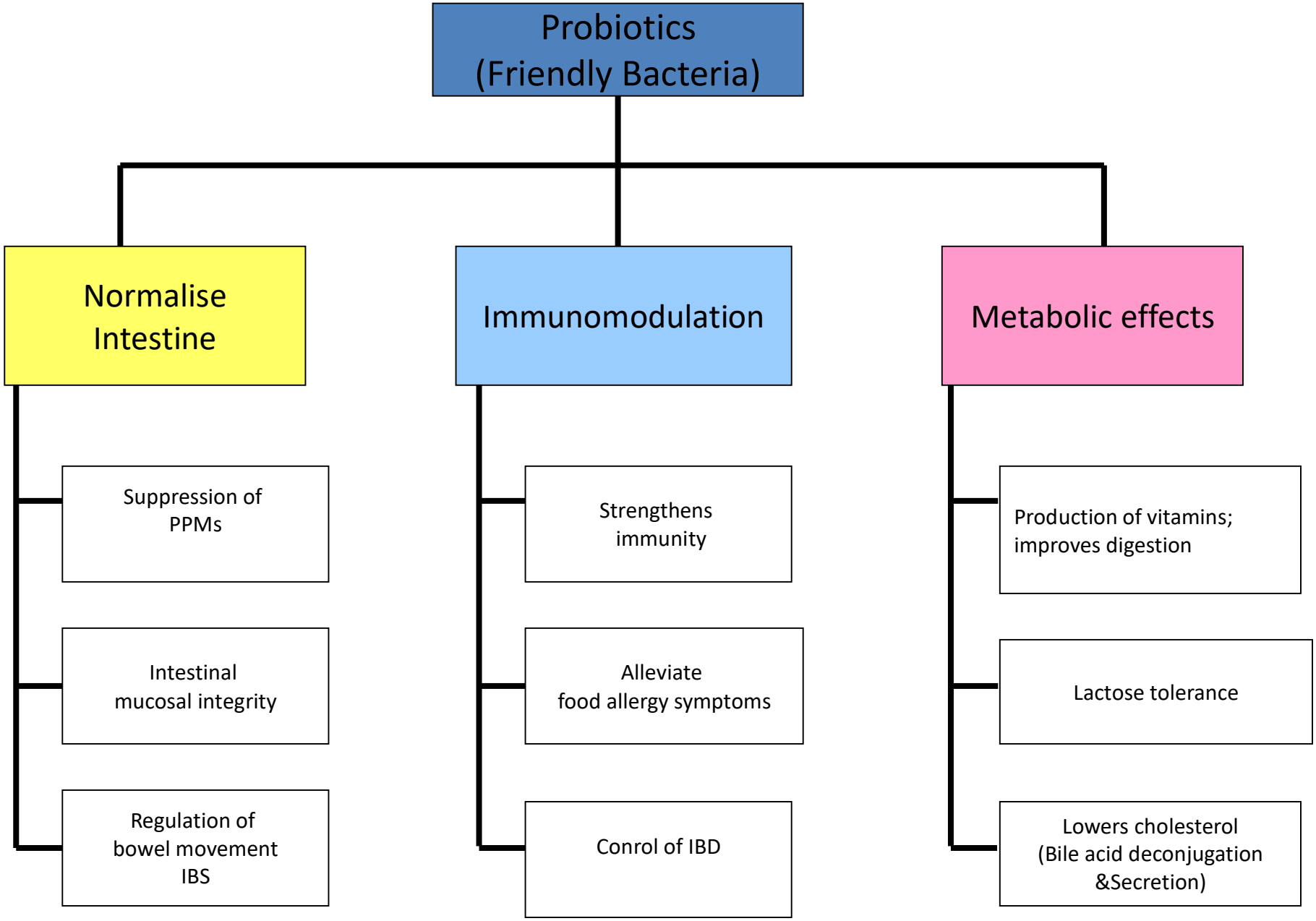
- A prebiotic is a nondigestible component which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of colonic bacteria, thereby improving the health of the host .
- Examples- insulin, garlic, onions, chicory root, Asparagus, whole wheat, rye, barley.

Characteristics of Prebiotics

- Should not be hydrolysed or absorbed in the upper part of G.I tract.
- Should be a selective substrate for one or a limited number of potentially bacterial commercial to the colon culture protagonist.
- Should be able to alter the colonic microflora towards a healthier composition or selectively stimulates the growth and or activity of intestinal bacteria associated with health and well being.
- Should help increase the absorption of certain minerals such as calcium and magnesium.
- Favourable effect on the immune system and provide improved resistance against infection.

Synbiotics

- PROBIOTICS + PREBIOTICS
- A probiotic organism in combination with its prebiotic food.
- Providing both the organism and substrate at the time of ingestion may offer improved chance of survival in GI tract.
- Foods containing the combination of probiotics and prebiotics are referred to as sybiotics.
- Improved survival in upper GIT and more efficient implantation.


Probiotics
(Friendly Bacteria)
Normalise
Intestine
Immunomodulation
Metabolic effects
Suppression of
PPMs
Intestinal
mucosal integrity
Regulation of
bowel movement
IBS
Strengthens
immunity
Alleviate
food allergy symptoms
Conrol of IBD
Production of vitamins;
improves digestion
Lactose tolerance
Lowers cholesterol
(Bile acid deconjugation
&Secretion)

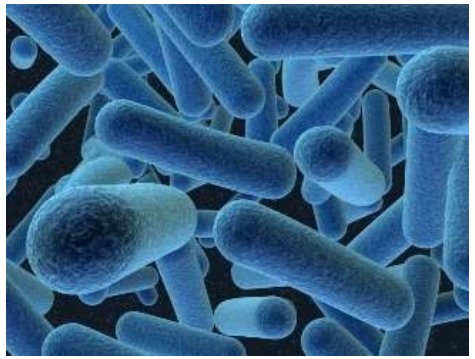
Ideal properties of a Probiotic

1. Be nonpathogenic and nontoxic to the host
2. Be antagonistic to pathogens
3. Exert a beneficial effect on the host
4. **Capable of surviving, colonizing and proliferating in the gut (should not be killed by gastric juice / bile acids)**
5. Able to inhabit in the S & L intestine
6. Must be of human origin
7. Contain a large number of viable cells and remain viable during storage and use

Characteristics of Effective Probiotics

- Able to survive the passage through the digestive system.
- Able to attach to the intestinal epithelia and colonise.
- Able to maintain good viability.
- Able to utilise the nutrients and substrates in a normal diet.
- non pathogenic and non toxic.
- Capable of exerting a benificial effect on the host.
- Stability of desired characteristics during processing, storage and transportation.
- Anti-inflammatory, antimutagenic, immunostimulatory.

Probiotic strains

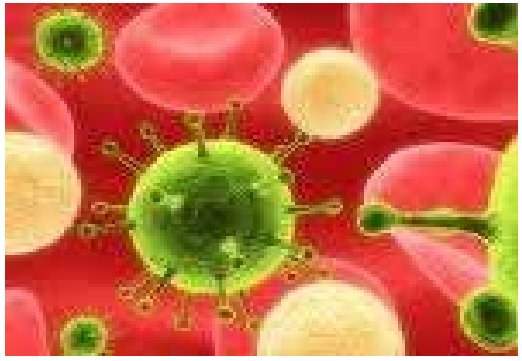


Lactobacillus species

- ***L. acidophilus***
- ***L. plantarum***
- ***L. casei subspecies rhamnosus***
- ***L. brevis***
- ***L. delbreuckii subspecies bulgaricus***

***Bifidobacterium* species**

- ***B. adolescentis***
- ***B. bifidum***
- ***B. longum***
- ***B. infantis***
- ***B. breve***



Others

- ***Streptococcus salivarius* ssp. *thermophilus***
- ***Lactococcus lactis* ssp. *lactis***
- ***Lactococcus lactis s* ssp. *cremoris***
- ***Enterococcus faecium***
- ***Leuconostoc mesenteroides* ssp. *dextranicum***
- ***Propionibacterium freudenreichii***
- ***Pediococcus acidilactici***
- ***Saccharomyces boulardii***

List of microorganism authorized as probiotics in feedstuffs under Council Directive 70/524/EEC

- *Bacillus cereus var. toyoi*
- *Bacillus licheniformis*
- *Bacillus subtilis*
- *Enterococcus faecium*
- *Lactobacillus casei*
- *Lactobacillus farciminis*
- *Lactobacillus plantarum*
- *Lactobacillus rhamnosus*
- *Pediococcus acidilactici*
- *Saccharomyces cerevisiae*
- *Streptococcus infantarius*

Mechanisms of action

Free amino acids

Organic acids

Neutralization of dietary carcinogens

β-Galactosidase activity

Probiotics

Oligosaccharides

Immunostimulatory

Cholesterol assimilation

Antioxidant

Short chain fatty acids

Bioactive peptides

Survival and adhesion competitions with pathogenic bacteria

Bacteriocin

Mechanism of Action

Inhibit Potentially Pathogenic Microorganisms (PPMs)

- **Reduction in Intestinal pH (through production of SCFAs)**
- **Production of bacteriocins**
- **Competitive blocking of adhesion sites**
- **Competition for nutrients**

MOA of Probiotics

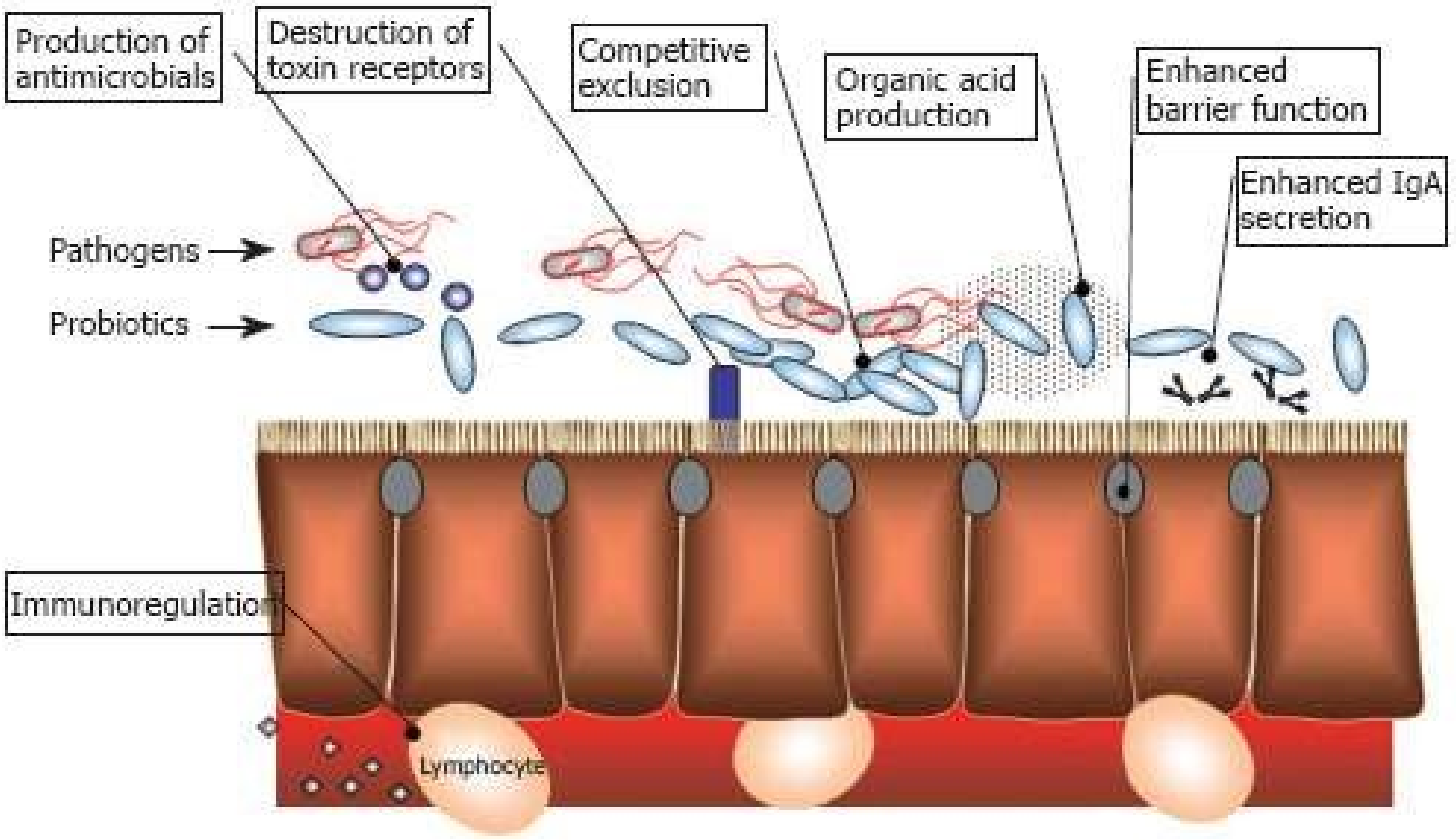


Figure 3 Mechanisms of probiotic activity.

modes of action of the probiotic bacteria in the aquaculture

- Production of Inhibitory Compounds:
  - Probiotic bacteria release a variety of chemical compounds that are inhibitory to both Gram-positive and Gram-negative bacteria. These include bacteriocins, siderophores,lysozymes, proteases, hydrogen peroxides etc.
- Competition for Adhesion Sites:
  - Probiotic organisms compete with the pathogens for the adhesion sites and food in the gut epithelial surface and finally prevent their colonization(Vanbelle et al, 1990). Adhesion capacity and growth on or in intestinal or external mucous has been demonstrated in vitro for fish pathogens like *Vibrio anguillarum* and *Aeromonas hydrophila* (Krovacek, 1987).

- Competition for Nutrients:
  - Probiotics utilizes nutrients otherwise consumed by pathogenic microbes. Competition for nutrients can play an important role in the composition of the microbiota of the intestinal tract or ambient environment of the cultured aquatic organisms(Ringo and Gatesoupe,1998). Hence successful application of the principle of competition to natural situation is not easy and this remains as a major task far microbial ecologists.
- Enhancement of Immune Response:
  - Probiotics stimulate the host's specific and non-specific immunity. Some of the bacteria like Lactic acid bacteria (LAB) increase the resistance to enteric infection(Holzapfet et al., 1998).

- Improvement of Water Quality:
  - It has been reported that gram-positive bacteria especially Bacillus sp., improve the water quality by converting the organic matter back to carbon dioxide in the environment.
- Interaction with Phytoplankton:
  - Probiotic bacteria have a significant algicidal effect on many species of microalgae, particularly of red tide plankton (Fukami et al., 1997). Bacteria antagonistic towards algae would be undesirable in green water larval rearing technique in hatchery where unicellular algae are cultured and added, but would be advantageous when undesired algae species developed in the culture pond.

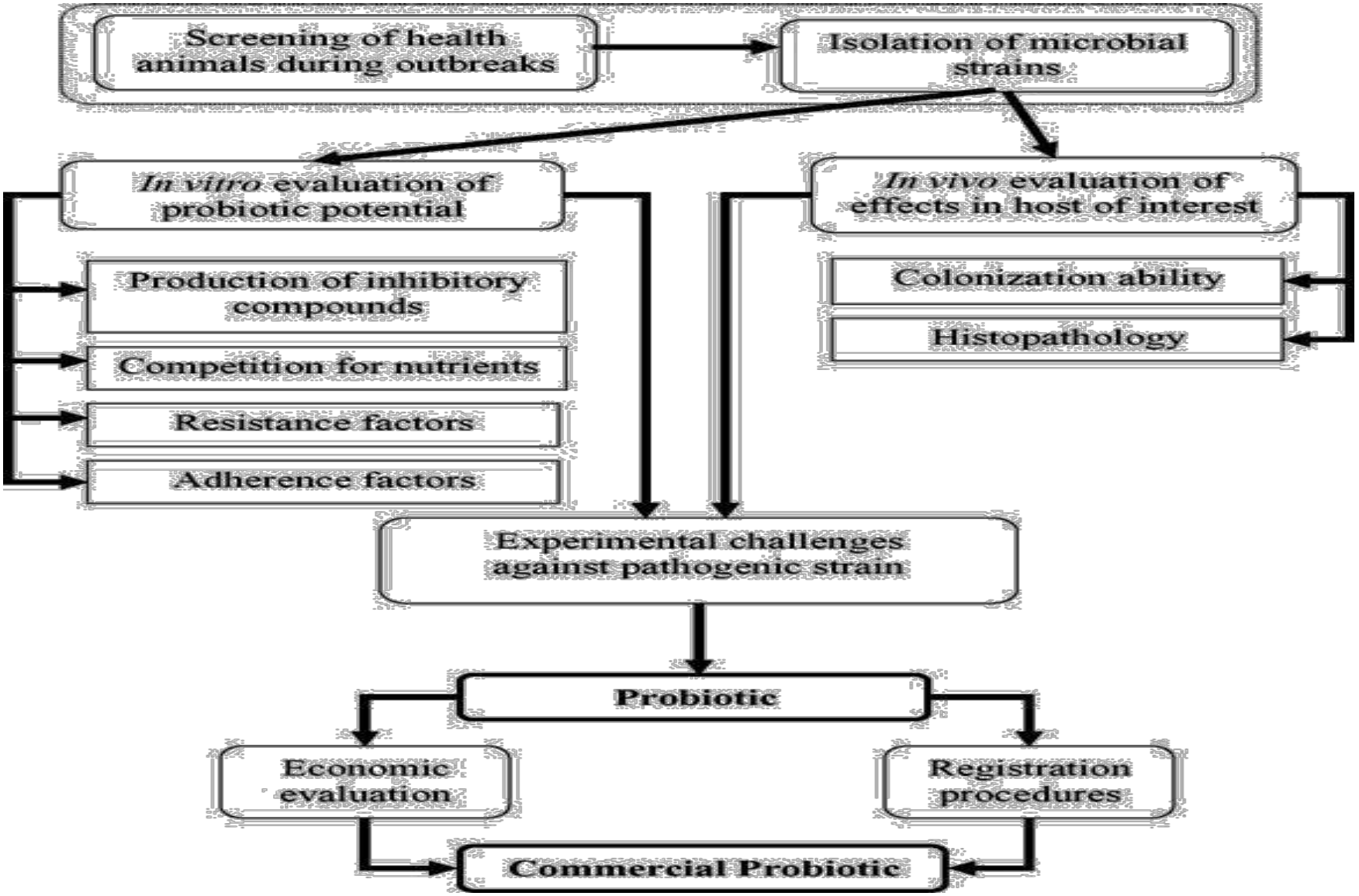
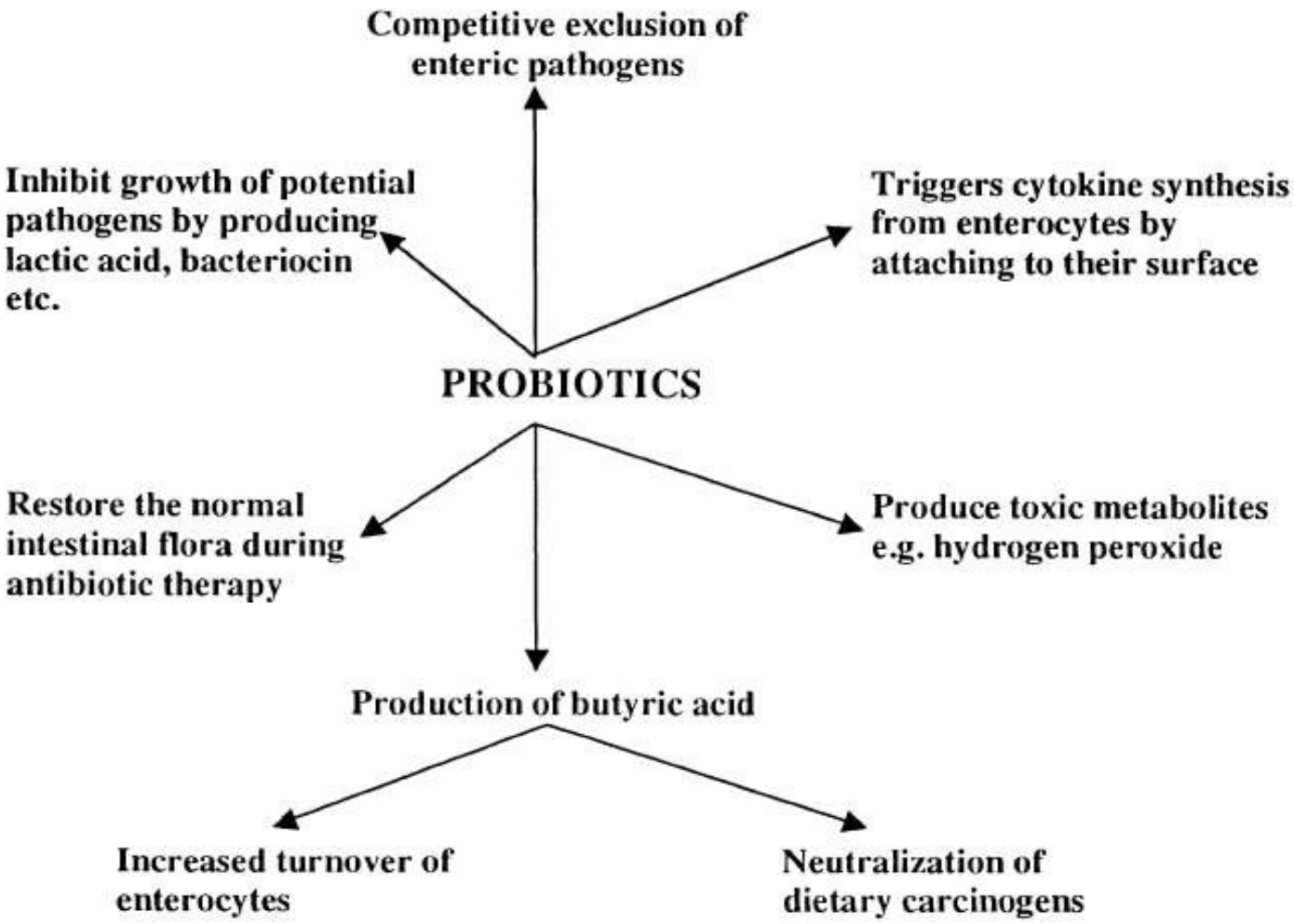


Fig. : Diagram for selection of probiotics as biocontrol agents in aquaculture.

MECHANISM OF ACTION OF PROBIOTICS



Probiotics should have the following attributes

1)GRAS (Generally Regarded As Safe) status.

2) Capacity to establish well and eventually colonize the gut environment(resistant to low pH and organic acids).

3) Non-pathogenic and non-toxic to the hosts.

4) Maintain a healthy gut during stress period;

5) Remaining viable at higher temperature and for longer duration.

6) It should not undergo any genetic modification due to change in the environmental parameters.

7) Its action should be multifarious i.e. improvement of nutritional status, feed conversion efficiency, growth and antagonizing effect over a large number of pathogens.

8) It should be cheap, efficient and easily available.

Methods to select probiotic bacteria for use in culture system

- Include the following steps:
- Collection of Background Information:
  - Before the start of research on development of probiotics, the activities about culture practices and economics of the development should be studied. A close knowledge of the rearing practices used in an aquaculture farm is necessary to determine whether a probiotic application would be feasible or not.
- Acquisition of Putative Probiotics:
  - The acquisition of a good pool of candidate probiotics is of major importance in this process. It is vital in this phase that the choice of the strain is made as a function of the possible role of the probiotics to be developed. There is no unequivocal indication that putative probiotics isolated from the host or from their ambient environment perform better than isolates completely alien to the cultured species or those that originate from a very different habitat.

- Screening of Putative Probiotics:
  - A common way to screen the candidate probiotics is to perform in vitro antagonism tests, in which pathogens are exposed to the candidate probiotics in liquid or solid medium. Candidate probiotics can be selected based on production of inhibitory compounds like bacteriocines, siderophores or when in competition for nutrients (Dopazo et al., 1988).
- Evaluation of Pathogenicity of Selected Strains:
  - Probiotics should not be pathogenic to the hosts and this should be confirmed prior to acceptance. Therefore, the host must be challenged under stressed and non-stressed conditions. This can be accomplished by adding probiotic to the culture water. When probiotics are selected for larval rearing by green water technique, their possible interaction with algae should be considered.
- In vivo Evaluation:
  - The effect of candidate probiotics should be tested in vivo as well. It involves introducing candidate species to the host under culture and then monitoring the growth, survival and physico-chemical parameters.

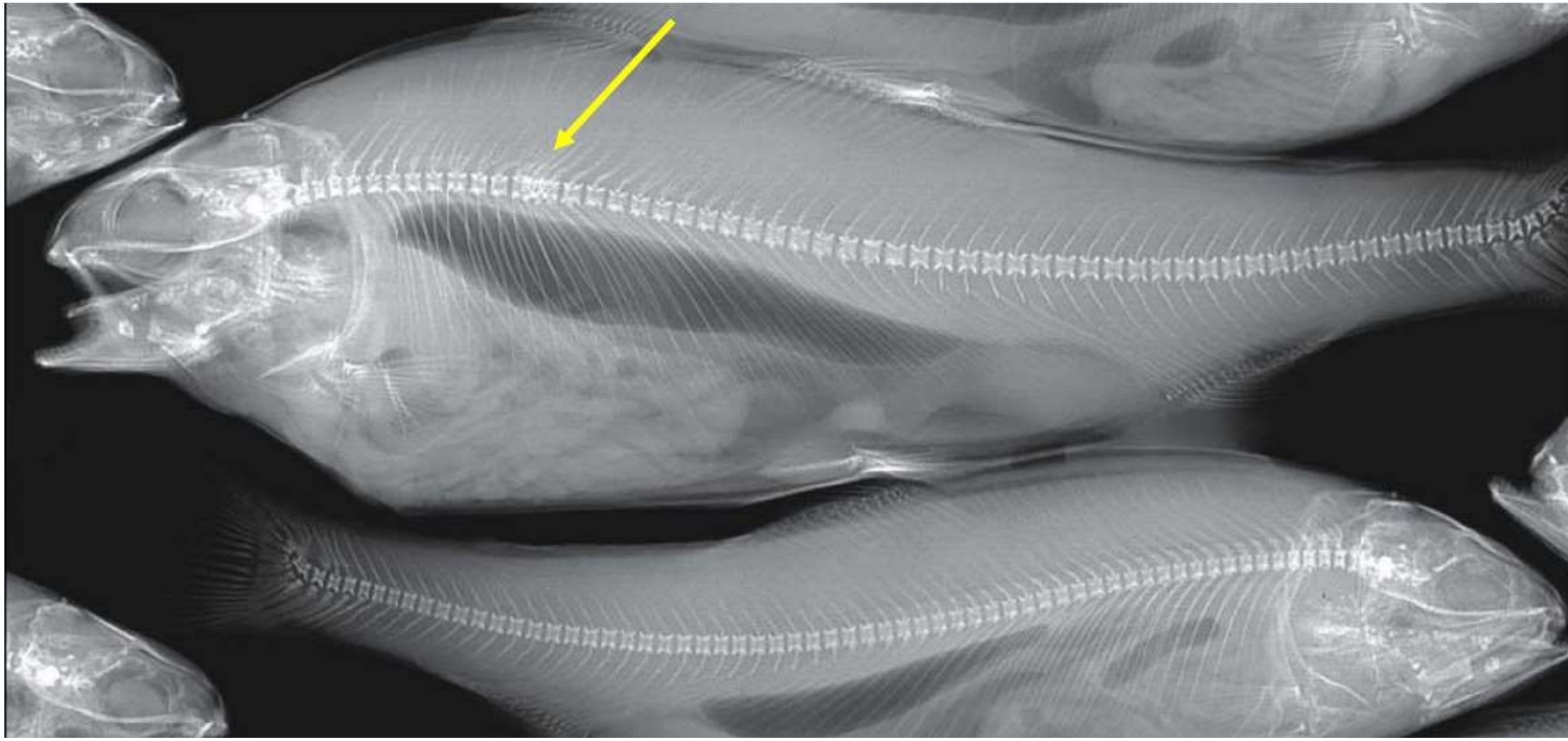
-using all isclates
-- target groups ide,ntificiltic.n

TARGET EVALUATION

Choose most promising ins / speciesl ccmblnatlcns for intervention studies

Aquaculture:
microbiota irregularity identification
as *basis for selecting & characterizing pnpbliotics*

Focus on str',ains, target DrC.DQrti4!S and end use.combinations



Probiotics may replace antibiotics in some cases. For instance, it was possible to reduce the incidence of vertebral compression in rainbow trout (arrow) by introducing in the diet either *florfenicol for the first ten days of feeding, or Pediococcus acidilactici* for five months post start feeding *(Aubin et al. 2005).* Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout *(Oncorhynchus mykiss*

Walbaum). Aquac. Res. 36, 758-767).

Probiotic strain	Source	Used on	Method of application	Reference
Streptococcus lactis and *Lactobacillus bulgaricus*	?	Turbot larvae *tScoplnhatmus lIlaximus)*	Enrichment of live food	Garda de la Banda et al. (1992)
Lactobacillus sp, and *Camobacterium* sp.	Roo fers *tBradiionus plicatilis)*	Turbot larvae	Enrichment of rotifers	Gatesoupe (1994)
Vibrio atginotyticus	Commercial shrimp hatchery	Atlantic salmon *iSalmo solar* L.)	Bathing in bacterial suspension	Austin er al. (1995)
Camobacterium divergens	lntestines of Atlantic salmon	Atlant ic cod fry	Addition to diet	Gildberg and Mikkelsen (1998)
Bacillus megaterium, B, subtilis. B. polymyxa. B. Iicheniformis	Commercial product (BiOSt3t)	Channel catfish	Addition to pond water	Queiroz and Boyd (1998)
Vibrio pelagius	Turbot larvae	Turbot	Addition to culture water	Ringa and Vadsteln (1998)
G-probiotic	Commercial product	*Oreochromis niloticus*	Addition to diet	Naik et al. (1999)
Pseudomonas fluorescens	Iced freshwater fish *(Lares lIiloricus)*	Rainbow trout *(01lco rhynchus lIIykiss)*	Addition to culture water	Gram et al, (J 999)
Camobacterium sp.	Intestines of Atlantic salmon	Atlantic salmon	Addition to diet	Robertson et al. (2000)
Lactobacillus r1101111105115 ATCC 53103	Culture collection	Rainbow trout	Addition to diet	Nikoskelainen et al. (2001)
Ael'OIIIOllaSiydrophila, Vibri()jllIVialis. Cornobacterium sp., *Micrococcus* II/TellS	Digestive tract of ra inbow trout	Rainbow trout	Addition to diet	Irianto and Austin (2002)
Enterococcus faecium SF68	Commercial product (Cernivet)	*Anguilla anguilla*	Addition to diet	Chang and Liu (2002)
L *r/UnlIIIOSIt3*CM I 136	Culture cclllection	Rainbow trout	Addition to diet	Panigrahi et at (2004)
Roseobacter sp. strain 27-4	Turbot larvae, *Tetraselmis* copepod-fcd larvae	Turbot larvae	Addition 10 culture water	Hjelm et al. (2004)
Bacillus circulans	Intestines of *Labeo rohita*	L *rohita*	Addition to diet	Ghosh et at (2004)

Probiotics considered as biological control agents in aquaculture of fishes

Probioticsr.ra.in	Source	Used on	Method of application	Reference
Crustaceaus				
Bacillus sp, SII	*Penaeus* nlQJ'tJdi'H,	p. nW'JnhdJJl1	Addition to diet	Rengpi p31 er aI. (1998)
BacUlussp.	Commercial product (OMS)	*P. monodon*	Addition to culture W31et	Moriarty (1m)
LacJobtu.ilblS spp.	Digestive tract of chicken	*P_ monodon*	Addi nOu Lo diet	Phi311phak ec aI. (1999)
SaccJUJIIJY')'C1!S cerevisiae .• S. *exiguus,*	Commercial product	*Penaeus* \wutanre;	Additiotl Lo diet	Scholz et al. (1999)
PhalJia rhodozyma \fibril) *hepasarius, vibrio* sp .. *Bacillus* sp.			Addition Lo diet	Balclzar (2003)
I-fbrio P62, *Vibrio* P63. *Bacillus* P64		*P. vannamei*	Addition to culture "Taler	Gulli"" et al, (2004)
PSeJuWntOnJ$ sp., *vibrio j'uvialis*			Addition to culture W3leJ'	Alavaad i . . aI. (2004)
Mollu$cs				
MrontOLUU media strain *Al99*	')	C *rassos:* nod *gigas*	Additioo [o culture water	Gibson et aI. (1998)
Roseobacrer <p. BSI07	Scallop larval cultures	*Pecten ,n.axinJUS*	i\.ddiUofi Lo culture water	Ruiz-I\)n.I" er aJ. (1999)
Alseromona l.aloplanktis		*Argop«:lcn purpuraius*	Addition to culture "Taler	Riquelme et al. (2000)
Live food				
Flavobacterium sp.	*Chaetoce ros gracilis* culwte	C. *gracilis. I. galvona, P. lutberi*	Additioo Lo culture W3leJ'	Sumiruo aod lliroyama (1997)
LAcJ()COCCJLt /acti.f AR21	Roofer culture	ROOfers	Additiotl Lo culture water	liat7.evili er al, (1998)
V. aIgillOlytiou C7b	Seawater	*C haesoceros muelleri*	i\.ddiUofi Lo culture water	Gomez-Gil et aJ. (2002)
Pl:dioc MC *us add ita eli* d	ComIuetCial product	*Artemia*	Addi 0011 Lo culture WaJet	Gatesou pe (2002)
Lacsobaciltus easel. L *brevis,* *L helvesicus.* IJu:IIX'()CCUS *lactic* spp. *tactis: Leuconostoc,* *1~1~senlell)ide$* spp. *meM'!nJeroidt!~*	Culture collecuon	*Artemia* nauplii	Addition to culture "Tater	Villamil et al. (2003)
Pediococcus acidiloaici				

Probiotics considered as biological control agents in aquaculture of crustaceans, molluscs, and live food

\Vater	Prole~in' probiotic range (Benefits)	Lower@ 100 em depth	Upper @ 150 em depth
Salinity	G-40 ppt	10 ppt	35 ppt
pH	6.5-9.0	7.5	8.5
Temperature	25°C-35°C	28°C	32°C
Alkalinity	>80 ppm		
Transparency	(Balances)	30cm	45 em
Colour	(Balances)	Light green	Brownish green
DO	(Improves)	> 3.5 ppm	
Total ammonia	(Reduces)	< 1.0 ppm	
Nitrate	(Reduces)	<0.2 ppm	
P as Orthophosphate	(Balances)	> 0.5 ppm	> 1.0 ppm
Total bacteria and *Vibrio* spp.	Onhibits)	10^{3}-1 $()_4$ CFU/ml	
TOlalluminous bacteria (pathogenic *nbrio)*	0nhibits)	$<10^{1}$	
Beneficial algae	60o/$_{1r}$90%	60%	90%

Lower and upper pond parameters recommended combined with details on efficiency range

Probiotics applied in aquaculture (after Irianto & Austin, 2002)

Probiotic identity	*Source*	*Used on*	*Method of application*
Gram-positive bacteria			
Bacillus sp. S11	Penaeus monodon	Penaeus monodon	Premix with feed
Bacillus sp.	Commercial product	Penaeids Water	
Bacillus sp.	Water	Added to water	
Lactobacillus lactis AR21	Mass culture	Brachionus plicatilis	Feed additive
Mixed culture, mostly Bacillus spp.	Commercial product	Brachionus plicatilis	Mixed in water
Gram-negative bacteria			
Vibrio alginolyticus	Beach sand	Penaeids, salmomids	Feed, bath for 10 min
Yeast			
Saccharomyces cerevisiae, S. exiguous, Phaffia rhodozoma	Commercial product	Litopenaeus vannamei	Premix with feed
Microalgae			
Tetraselmis suecica	Commercial product	Penaeids, Salmo salar	Feed

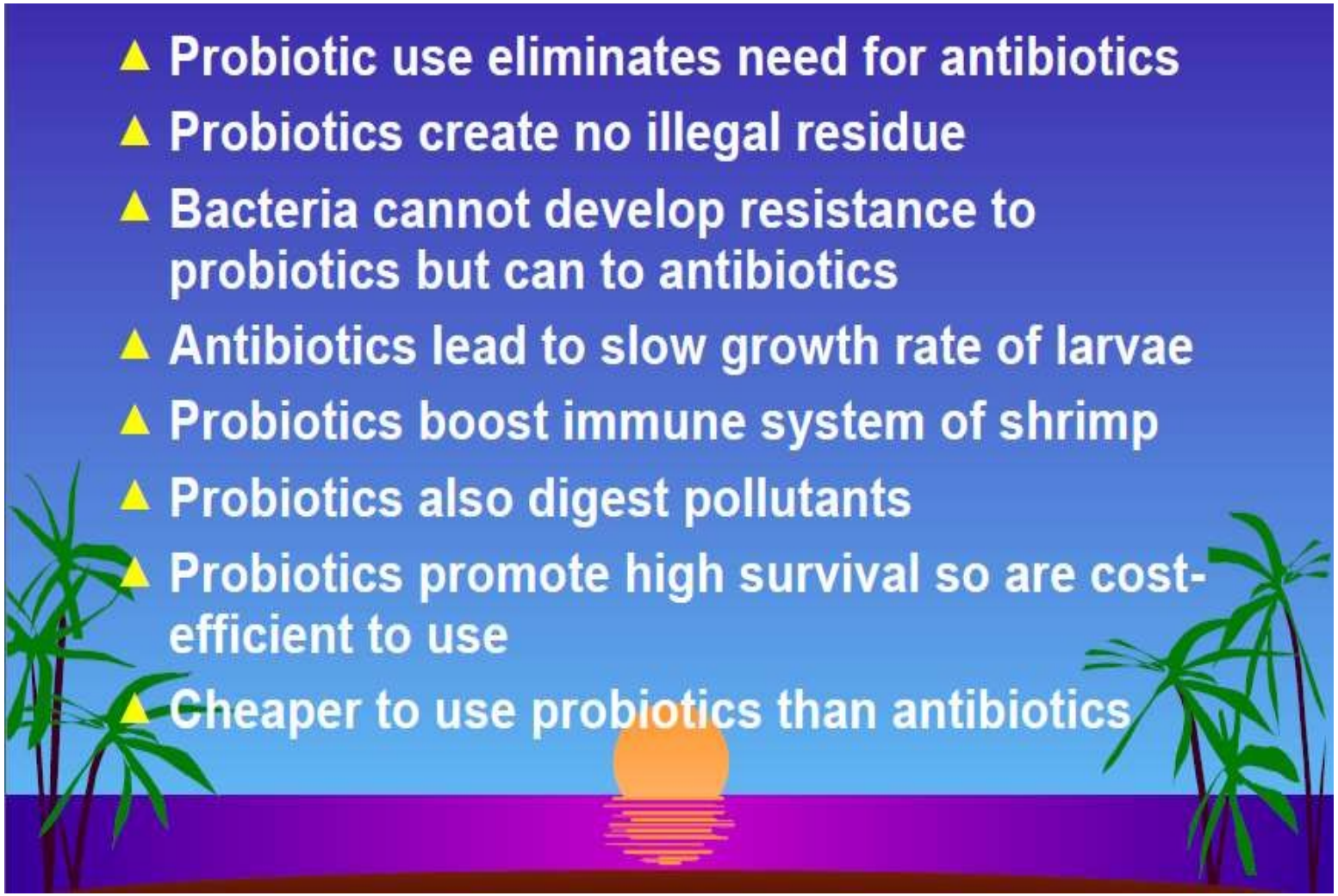
Advantages

- **Produce lactic acid**- lowers the pH of intestines and inhibiting bacterial villains such as *Clostridium, Salmonella, Shigella*, *E. coli,* etc.
- Lead to improved appetite and/or growth performance.
- Decreases the production of a variety of toxic or carcinogenic metabolites.
- Aid absorption of minerals, especially calcium, due to increased intestinal acidity.
- Production of β- D- galactosidase enzymes that break down lactose**.**
- Produce a wide range of antimicrobial substances -acidophilin and bacteriocin etc. help to control pathogenic bacteria .
- Produce vitamins (especially Vitamin B and vitamin K)
- Act as barriers to prevent harmful bacteria from colonizing the intestines

Disadvantages

- There is still no consensus on the most effective dose of a probiotic.
- Probiotic products manufactured by established pharmaceutical companies typically have the stated concentration and bacterial or fungal strain listed on the label.

Probiotics benefit vs Antibiotics



Benefits of pond prebiotics


Thank
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