

# Biologics

- A biologic is a product that is produced from living organisms or contain components of living organisms.
- Biologic drugs include a wide variety of products derived from human, animal, or microorganisms by using biotechnology.
- Types of biologic drugs include immunostimulants, vaccines, blood, blood components, cells, allergens, genes, tissues, and recombinant proteins.

# IMMUNOSTIMULANTS

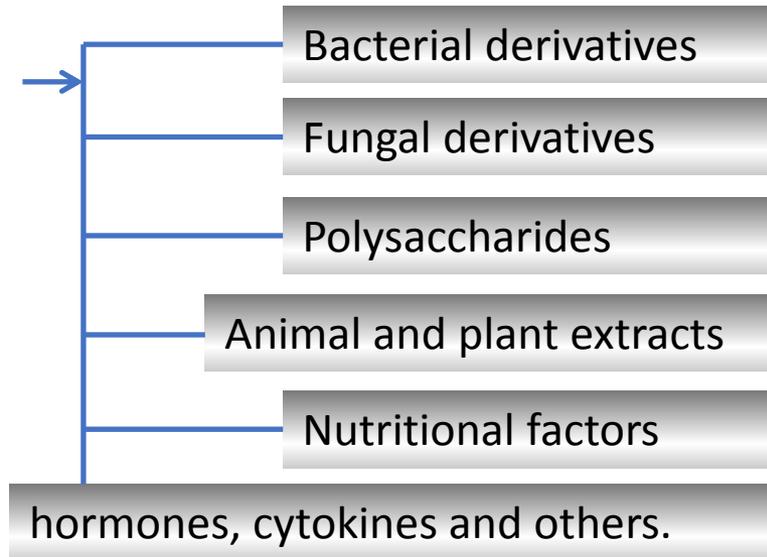
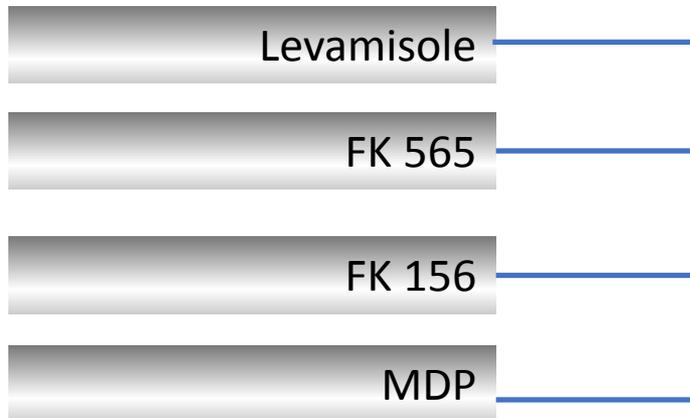
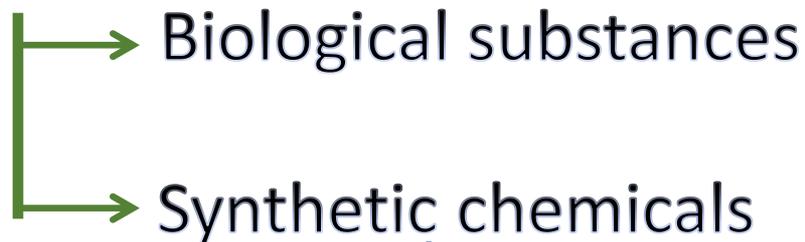
- **Immunostimulants** may be defined as an agent, which stimulate the **non-specific immune mechanism** on their **own** or **specific immune mechanism** when coupled with an **antigen**.
- In general, immunostimulants comprise a group of **biological** and **synthetic compounds** that enhance the non-specific defense mechanisms in animals.
- These Immunostimulants mainly facilitate the function of **phagocytic cells** and increase their antimicrobial activities.

# Characteristics of an ideal Immunostimulants

- ✓ It should be non-toxic, even at a high dose rate.
- ✓ It should be non-carcinogenic.
- ✓ It should stimulate a wide range of non-specific immune responses against bacteria, fungi, virus, protozoa and helminthes.
- ✓ It should be capable of amplifying primary and secondary immune responses to infectious agents.
- ✓ Breakdown products of compound should be biodegradable in the environment.
- ✓ It should be having defined chemical composition or biological activity.
- ✓ It should be inexpensive and either tasteless or palatable.

# Immunostimulants used in aquaculture

## Classification



# Bacterial products

- Freunds Complete Adjuvant (FCA):
  - Preparation of the cell walls of Mycobacteria and related bacteria such as *Nocardia* and *Corynebacteria* suspended in mineral oil.
  - Oil- hold the immunostimulatory substances within oil globules after injection, releasing them slowly into the tissues.
  - Are potent immunostimulants- increase the phagocytic and antibacterial activity.

# Lipopolysaccharide (LPS)

- The outer cell envelope of gram negative bacteria- anchored to the underlying peptidoglycan.
- Molecular structure elicits diverse non-specific actions in the immune system.
- Animals detect the presence of LPS, activates-both macrophages and lymphocytes.
- At a low dose, LPS enhances the disease resistance and act as a prophylactic agent.

# Fungal derivatives (Glucans)

- $\beta$  glucans are cell wall components of mushroom and yeasts.
- The most promising as an immunostimulant.
- Mainly activate the macrophages.
- Stimulation of immune response depends on size of the particle.

# Polysaccharides

- **Chitin**

- Polymer of glucosamine (poly  $\beta$ -(1-4)N-acetyl-glucosamine)
- Chitin is the principal component of crustacean and insect exoskeleton and cell wall of certain fungi.
- Induce the increased macrophage activities
- Increase bactericidal activity

- **Chitosan**

- de-N-acetylated chitin
- Potential killing activity

# Oligosaccharides

- The beneficial oligosaccharides are broadly classified as prebiotics.
- Prebiotics are nondigestible food ingredients that beneficially affect the host. They selectively stimulate the growth and/or activity of bacteria in the colon.
- Among the most common prebiotics, mannan oligosaccharide (MOS) has been recently receiving heightening application in aquaculture.

# Animal and plant extracts

- Ete and Hde:
- Ete - extract from marine tunicate, *Ectavinacida turbinata*
- Hde - glycoprotein fraction of water extract from Abalone, *Haliotis discus hannai*.
- enhances the killing of tumor cells
- enhances phagocytosis
- enhanced NK cell activity.



- EF 203:
- EF 203 is a fermented product of chicken eggs.
- Oral administration stimulates the phagocytic and leucocytes.
- Increases protection to many bacterial infections.

# Plant originated immunostimulants

1. ***Ocimum sanctum*** (Tulsi):Leaves extract of *Ocimum sanctum* affected both specific and non-specific immune responses and disease resistance against *Aeromonas hydrophila*.
2. ***Phyllanthus emblica*** (Amla):Amla fruit pulp contains large proportion of vitamin C, which has also been identified as an immunostimulant.
3. ***Azadirachta indica*** (Neem) : a highly esteemed “wonder” tree of India that is widely dispersed throughout the country. Azadirachtin, a triterpenoid derived from *A. indica*, enhanced respiratory burst activities, the leukocyte count and the primary and secondary antibody response.

4. ***Solanum trilobatum*** (Purple Fruited Pea Eggplant): *Solanum trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activity
5. ***Eclipta alba*** (Bhringraj)
6. ***Zingiber officinale*** (Ginger):The use of Ginger @0.5g /110g of feed reduced mortality.
7. ***Echinacea (purple coneflowers)*** and ***Allium sativum (garlic)***: improve the gain in body weight, survival rate and resistance against challenge infection of *Aeromonas hydrophila*
8. ***Camellia sinensis*** (Green tea):The inclusion of green tea in fish diet up to 0.5 g/kg diet enhanced the protein contents in fish body, while the lowest lipid contents were obtained at 0.0–0.5 g GT/kg diet.
9. ***Aloe vera***:*Aloe vera* supplementation (0.5%) per feed can increases the resistance to *Aeromonas hydrophila* and *A. septicemia*.
10. ***Nyctanthes arbortristis*** (Night-flowering Jasmine)



*Ocimum sanctum*



*Phyllanthus emblica*



*Azadirachta indica*



*Eclipta alba*



*Solanum trilobatum*



*Nyctanthes arbortristis*

# Nutritional factors

- **Vitamin C:**
- An anti-oxidant -maintains many metal co factors in reduced state.
- Deficiency increases disease susceptibility in many species of fishes like rainbow trout and Atlantic salmon affecting the immune system.
- High levels of the Vitamin C (dose level of 3000mg/Kg diet) increases the resistance to bacterial pathogens and also have protective immune responses in fishes (dosage more than 1000mg/Kg).

- **Vitamin E (Tocopherol)**
- Naturally occurring anti oxidant
- Enhances both humoral and cellular immune responses
- Increase phagocytic indices and superoxide anion production by leucocytes.
- Enhance antibody production against bacteria like *Yersinia ruckeri*
- Increase serum complement function and the ability of serum to opsonize bacteria.

# Hormones, cytokines and others

- **Hormones:**

- Growth hormone (GH) and Prolactin (PRL)
  - Directly affect immunocompetent cells (macrophages, lymphocytes and NK cells).
  - Mitogenic activity on lymphocytes and activate NK cells.
  - GH receptors are present on the monocytes and lymphocytes.
  - Enhance the production of superoxide anions by leucocytes.
  - MSH and MCH stimulate phagocytosis by the head kidney leucocytes of rainbow trout in vitro.
- Alpha-melanocyte stimulating hormone ( $\alpha$ -**MSH**), melanin-concentrating hormone (**MCH**).

- **Lactoferrin:**
- Is a polypeptide with a molecular weight of about 87kD.
- Possess two iron binding sites per molecule.
- Activate macrophages .
- Oral administration- increase number of granulocytes in blood and elevates secretion of body mucous in Red sea bream.

# Synthetic compounds

- **MDP (Muramyl Di Peptides):**
- Affects T and B lymphocytes directly
- Stimulates macrophages- to secrete ILs, TNF and colony stimulatory factors, which in turn stimulate the formation of monocytes and granulocytes.
- Potentiates the activity of antibiotics against bacterial infections.
- These enhance the disease resistance in fish.

# Levamisole

(2,3,5,6-tetrahydro-6-phenylimidazo thiazole):

- Anthelmintic
- Treatment of nematode infections in humans and animals.
- Enhances the phagocytic activities of neutrophils, increases the number of phagocytes and leucocytes.
- Active in reducing stress and mortality due to certain infections like *Saprolegnia sp.* in carp.
- Effective adjuvant with a vaccines.
- A narrow dose can create a stimulation where as high doses cause immunosuppression.