Aquaculture:
Disease Control in Fish Farming based on Probiotics

Mommy: do fish get sick like us? And how do we cure them?

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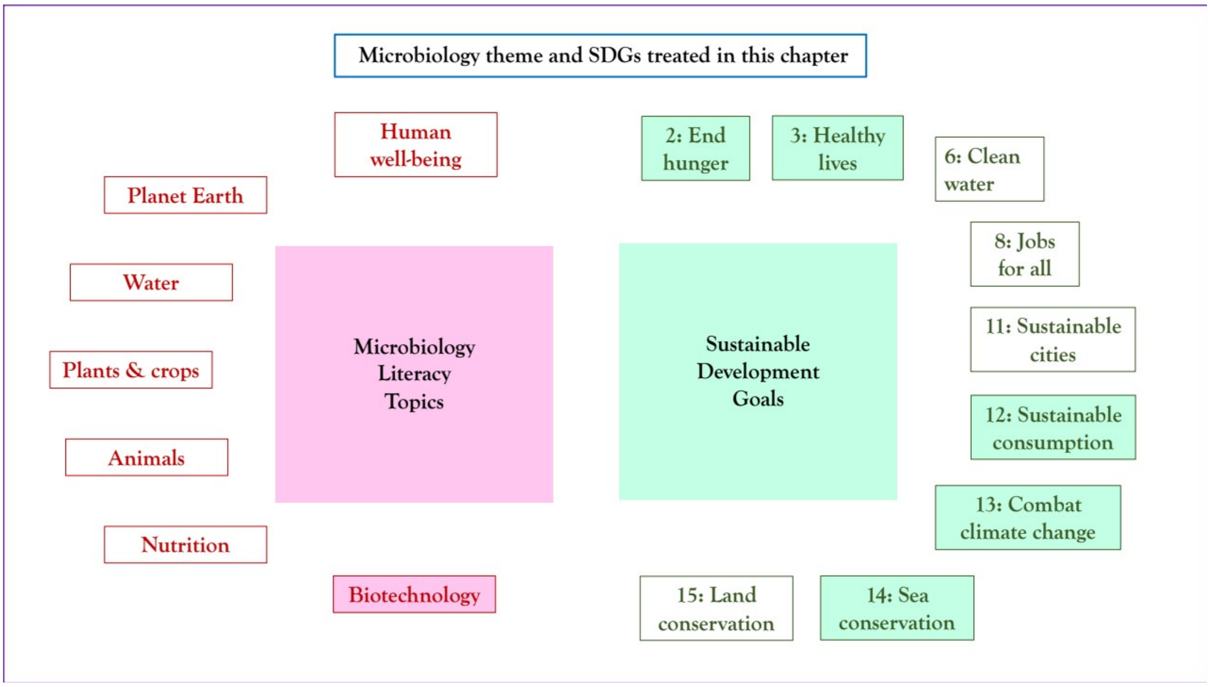
Storyline

Providing sustainably produced, healthy and safe food for the growing world population is a challenge. Fish and shellfish provide high-quality proteins for humans, but catches from wild fish have stagnated since the late 80'ies. Fortunately, fish and shellfish can be brought into culture and "farmed" like other animals or plants. Growing fish (or plants) in water is called aquaculture. Today (2020), half of the fish we consume, has been produced by aquaculture. Compared to the production of other food animals, like cattle, pigs, chicken, etc., aquaculture is associated with relatively low emissions of greenhouse gases. Aquaculture is therefore also an attractive system of animal food production from a climate perspective. However, infectious diseases are a major problem in the farming of fish and shellfish, significantly reducing yields, and can spread rapidly. More than half of the aquaculture-relevant infectious diseases are caused by bacteria and, therefore, treatment with antibiotics has been used as a major disease control strategy. This, unfortunately, results in development and spread of antibiotic resistance in the bacterial population, and this will in turn render the fish diseases untreatable. Importantly, antibiotic resistance in farmed fish-associated bacteria may spread to human pathogens, rendering human diseases untreatable. The World Health Organization (WHO) has highlighted antibiotic resistance as one of the top challenges of humanity. Another strategy to protect fish from infectious diseases is through vaccination. Vaccines have been developed against several bacterial pathogens of fish and have, in some fish species, been hugely successful, almost eliminating the use of antibiotics. However, fish larvae and shellfish (e.g. shrimp, crab, mussels) do not have a developed immune system and vaccines do not therefore work. Alternative, sustainable ways of preventing infections in aquaculture are needed. A probiotic is a living microorganism that, when added to a host, provides a health benefit, such as disease resistance. The probiotic may improve the host health in several ways: it can provide essential nutrients, stimulate the innate immune system, or inhibit or even kill a fish pathogen. The use of probiotic beneficial, non-pathogenic bacteria is a promising strategy for disease control in aquaculture.

The Microbiology and Societal Context

The microbiology: the aquaculture microbiome and its positive and negative influence on fish and shellfish;, bacteria as disease agents in fish/shellfish production; antibiotic resistance; probiotic bacteria in aquaculture; mechanisms underlying the beneficial effects of probiotics; *and, peripherally, for completeness of the story*: food production; climate aspects of aquaculture; policy decisions on health and nutrition; regulatory issues related to food and farming.
Sustainability issues: health; food production; economy and employment; environmental pollution; climate gases and global warming.



1. Microorganisms on and in fish and shellfish. Fish and shellfish (both crustaceans, like shrimps, lobster and crabs, and mollusks, like mussels, oysters, clams) live submerged in water, either freshwater or seawater. As all other living organisms, their outer (skin and gills) and inner (gastrointestinal tract) surfaces are colonized by a complex mixture of microorganisms, their microbiome. Also, their surrounding water contains a lot of microorganisms, for instance seawater contains approx. 1 million bacterial cells per ml. The commensal, or harmless, bacteria living on fish and shellfish can use cell debris, the dead cells, and exudates, the organic secretions, released from the fish surface, as food, and so it is beneficial for bacteria to colonize living animals rather than living as free (planktonic) cells in open waters that are often very low in nutrients, so-called oligotrophic environments. The majority of this microbial community has no effect on the host, but has merely found a niche of nutrients, surface and temperature where they have an advantage. However, a few of the bacteria are (or can become) pathogenic to the host, i.e. cause disease, and others can provide the host with benefits, e.g. disease protection.

2. Roles of microorganisms in aquaculture. Aquaculture is the farming of organisms (animals or plants) in water. This can be fish farming but also growing seaweed or shellfish. Aquaculture has surpassed catches of wild fish as a supplier of food (Figure 1). Aquaculture systems typically have high loads of microorganisms, as these are environments with high levels of nutrients (cell debris and feces from the fish, food left-overs) where heterotrophic bacteria that exploit organic wastes thrive. Such bacteria play several roles in the system. First and foremost, they digest organic matter and may improve water quality, especially by removing nitrogen compounds. A lot of ammonia is generated in intensive fish and shellfish farming and several bacteria can remove ammonia by oxidizing it to nitrite and nitrate. Both ammonia and nitrite can be toxic to fish, so it is important that a full oxidation to nitrate takes place. Commercial products are available containing bacteria from the genera *Nitrosomonas* (oxidize ammonia to nitrite) and *Nitrospora* (oxidize nitrite to nitrate) and these can be added as water treatment to facilitate full oxidation of ammonia.

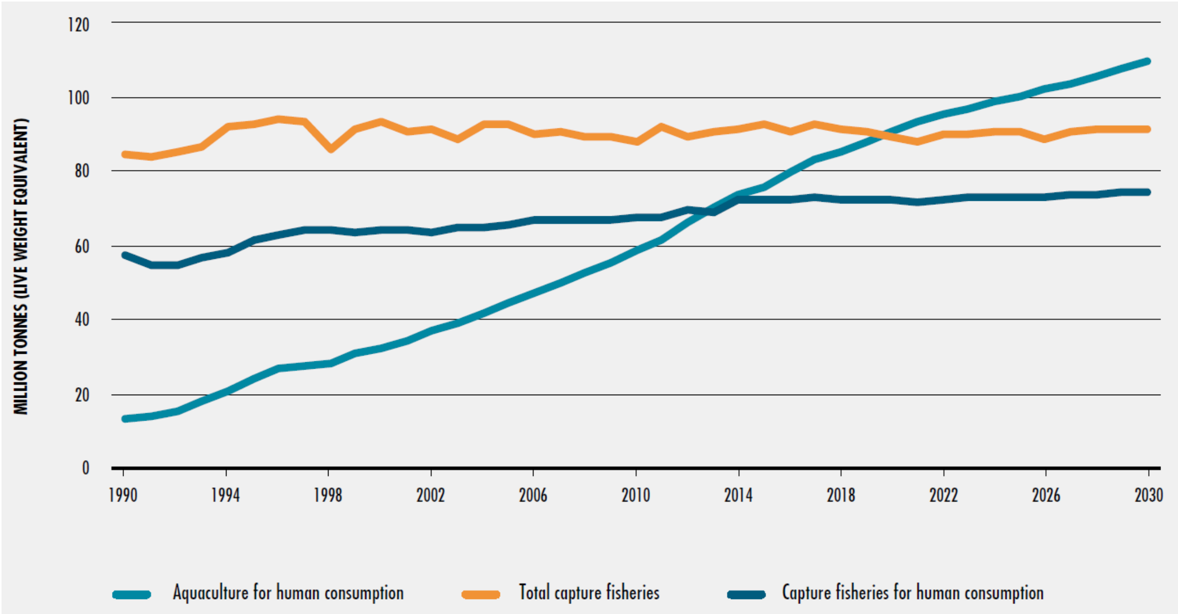


Figure 1. Global aquaculture and capture fisheries production from 1990 with a projection for future production until 2030. Dittmann (2019) modified from FAO (2018)

Some bacteria present in the system are so-called opportunistic pathogens. This means that they do not necessarily live by causing disease, but they may do so if the host immune system is weakened or the environmental conditions facilitate growth of these bacteria to high numbers. Other bacteria are true pathogens, meaning that they can cause disease in even healthy hosts. Some true pathogens can cause disease even when present in low numbers. True pathogens are typically not present at all times, but may be introduced with water or with algae, rotifers or tiny shrimp that are used as live feed for small fish larvae.

And some bacteria (or other microorganisms) may actually benefit the health and growth of the fish and shellfish. They do this by a multitude of different mechanisms, such as stimulating the host immune system or antagonizing infectious agents.

3. Microbial diseases in aquaculture. Because aquaculture involves unnaturally high fish/shellfish densities, and the water environment provides complete connectivity, disease transmission is rapid and an entire production may be lost. For instance, infectious diseases have destroyed shrimp aquaculture operations in several Asian countries. Fish and shellfish infectious diseases can be caused by several different types of pathogens: parasites, fungi, bacteria and virus. Infectious bacteria are responsible for more than 50% of fish and shellfish diseases. Some pathogens are specific to one or two hosts, whereas others may infect a broad range of hosts. Many different bacterial genera and species can cause disease, but the most important belong to the genus *Vibrio*. This genus is perhaps best known because of one of the human pathogenic species, *Vibrio cholerae*, which causes cholera outbreaks when drinking water supplies become contaminated with human waste during natural catastrophes or wars, but several other species are important fish pathogens, including *Vibrio anguillarum* (Figure 2) that can infect more than 50 different fish species. When causing an infection, the pathogen must be able to attach to the host cells, invade the host and then multiply, thereby causing disease. This requires that the bacterium produce many different molecules that facilitate these processes. For instance, surface proteins on the bacterium may enable it to attach to the host (fish cells), and different bacterial enzymes may then allow the bacterium to degrade the host tissue. Collectively, all the molecules and behaviors that are involved in virulence, the ability of the bacterium to infect and cause disease, are called virulence factors.

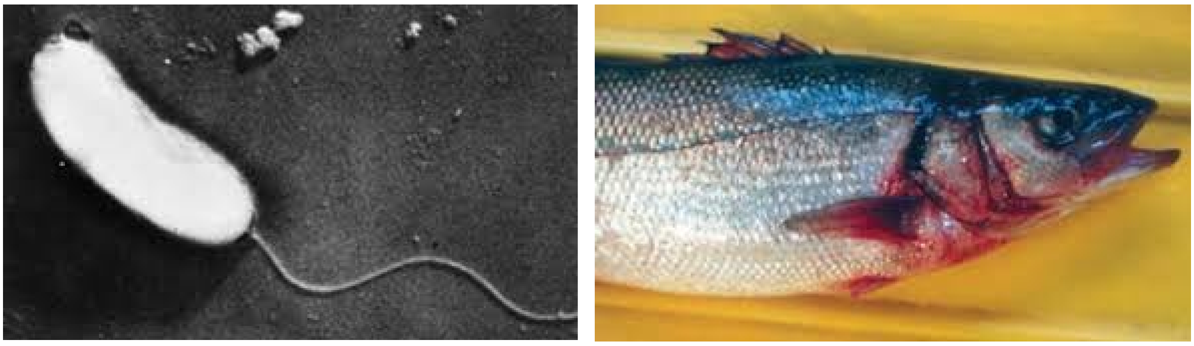


Figure 2. *Vibrio anguillarum* is an important fish pathogen causing wounds and bleeding of the fish. On the left: a scanning electron micrograph of the bacterium (length 3 um), note the flagellum that the bacterium uses for its motility (Actis et al. 1999); on the right, a fish infected with the bacterium (Austin and Austin 2007).

4. Control of microbial diseases in aquaculture. Infectious diseases require that the pathogenic agent reaches the host in sufficient levels to cause disease. Thus, maintaining a high level of hygiene and good water quality is the first level of defense. Also, fish (and other organisms) that are stressed are more susceptible to diseases, and crowding is a major cause of stress. Disease resistance will therefore be highest if fish are not too cramped, water quality is kept high and feeding is optimal: this is good aquaculture management. Next to good hygiene and management, vaccination is a key strategy for disease control. Vaccination involves the introduction into the body of an inactivated pathogen, or a component of a pathogen, which induces an immune response and the production of specific protective antibodies and/or immune cells against the infectious agent. When possible, this can be a very successful strategy. For instance, large amounts of antibiotics were used to treat infectious diseases of salmon until vaccines were developed., which then allowed a massive increase in production of salmon with very low usage of antibiotics (Figure 3).

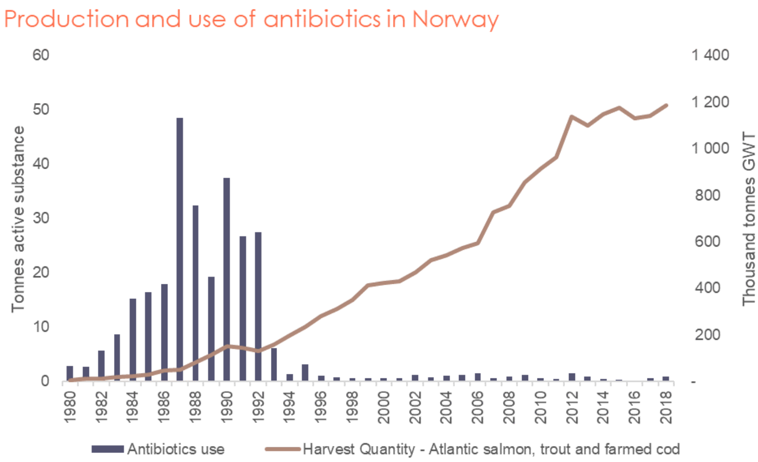


Figure 3: Salmon production and antibiotic usage in Norway before and after introduction of vaccines against the most important salmon pathogenic bacteria. From Salmon Farming Industry Handbook 2019; data from Kontali Analyse, Norsk medisinaldepot, Norwegian Institute of Public Health

However, some aquaculture organisms, such as shellfish (crustaceans and mollusks) do not have a well developed adaptive immune system, i.e. an immune system capable of producing specific protective antibodies and immune cells, and can therefore not be vaccinated. Also, when fish are very young – larvae – their immune system is not yet fully developed and vaccines do not work. Therefore, when shellfish and fish larvae suffer infectious disease episodes, antibiotics are used to treat; they may even be used prophylactively, to prevent disease. However, bacteria rapidly develop or acquire resistance, and are no longer are inhibited or killed by the antibiotic. They may use enzymes to inactivate the antibiotic, or have pumps in their membrane enabling them to expel the antibiotic, or they may modify the cellular target, that is the particular molecule(s) in the cell that the antibiotic attacks, thereby rendering themselves insensitive to the antibiotic. Since the ability to resist antibiotics can spread rapidly between bacteria, and is increasingly being found in bacteria that infect humans, we are facing a great risk of soon being unable to treat even simple bacterial infections. Therefore, other antibacterial strategies must be developed. One such strategy is the use of probiotics.

5. Probiotics as disease control agents in aquaculture. The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Originally, probiotic bacteria were thought of as edible components that would exert their effect in the gastrointestinal tract. However, the concept has now been widened to also include applications outside the GI tract. This is relevant in aquaculture where probiotics may act in the GI tract, but may also be effective on the skin or gills. Many different bacteria have been tested as fish probiotics. This include both organisms used for humans and warm-blooded animals, such as lactic acid bacteria, but also bacteria isolated from the aquaculture environment and tested their probiotic potential. Probiotic bacteria may act in many different ways (Figure 4) and, in many cases, the mechanisms are not known. For instance, some probiotic bacteria may stimulate the immune system of the host, and this subsequently provides the fish with protection against infectious pathogens. But little is known about how the probiotic bacteria stimulate the fish immune system.

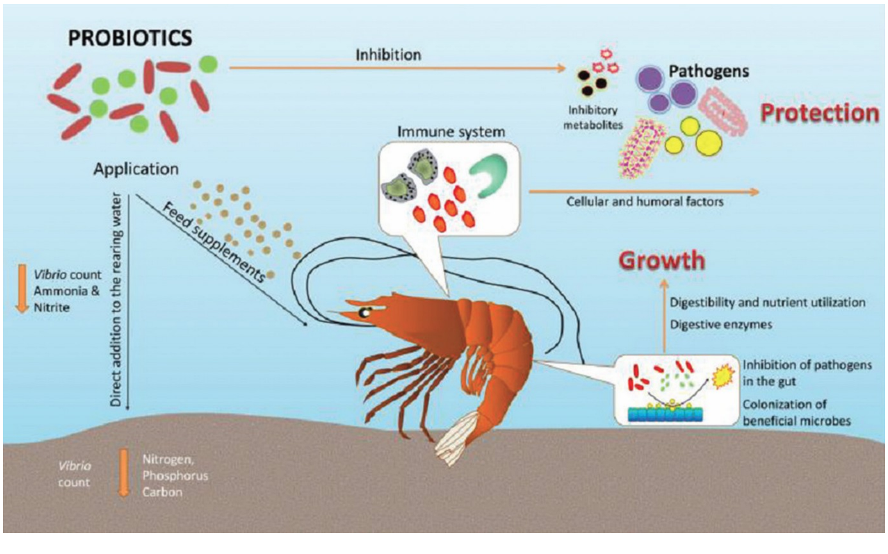


Figure 4. Probiotic bacteria may improve health in many ways: by killing pathogens, by stimulating the immune system, by removing toxic ammonia or by digesting fibre-like compounds providing more food for the fish (Jamal et al. 2019)

Mostly, potential probiotic bacteria are tested if they can antagonize the pathogen, and this is typically done in laboratory setups where the growth of the pathogen with and without the addition of the probiotic bacterium is measured. This can be done in petri-dishes where the pathogen is embedded in a gellified nutrient substrate and grows to form a turbid layer except for those zones where the antagonizing probiotic bacterium has been added (Figure 5, left). This assay is very similar to the setup that allowed Alexander Fleming to discover penicillin: he noticed that staphylococcal bacteria on a nutrient plate were killed when exposed to a filamentous fungus. To know if a bacterium really has a probiotic effect, it must be tested in (or on) real animals and a health benefit measured. Some studies have therefore also tested if a potential probiotic bacterium can reduce (or eliminate) disease in a fish or fish larvae challenged with a pathogen (Figure 5, right).

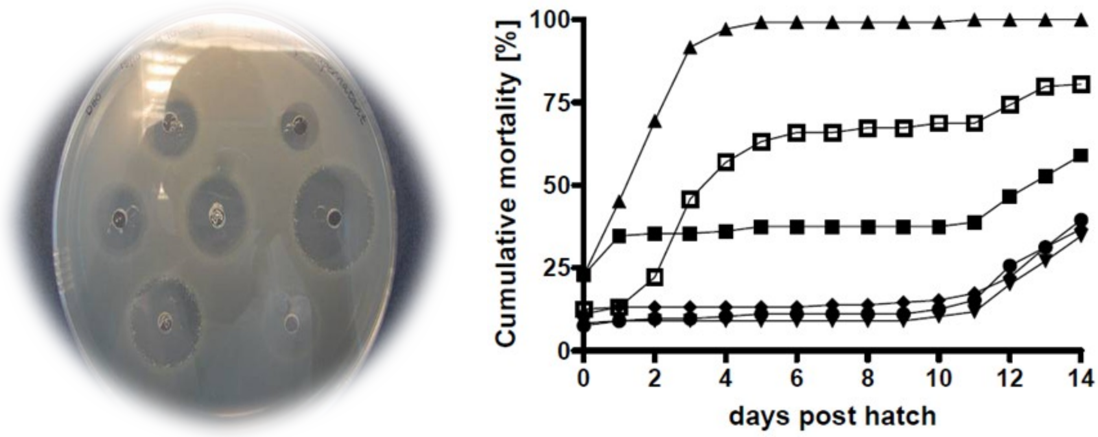


Figure 5. Marine roseobacter bacteria can inhibit the fish pathogen, *Vibrio anguillarum* (left), and can prevent fish larvae from dying of vibriosis (right). Cod larvae were simultaneously inoculated with rosebacter wild type and *V. anguillarum* (●), or with a roseobacter mutant that does not produce antibacterial compound (□). Unexposed larvae and larvae exposed to single bacterial strains acted as controls: Negative Control (■), only *V. anguillarum* (▲), only roseobacter (▼), and only roseobacter mutant (◆) (D'Alvise et al. 2012)

Relevance to Sustainable Development Goals and Grand Challenges

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable food production.** The world population is growing and needs food. Fish and shellfish are one of many sources of high-quality protein and were for centuries harvested from the wild from lakes and sea. However, we have overfished and over-exploited the natural resources and today (and in the future), fish and shellfish protein will increasingly be derived from aquaculture. Thus, this is an essential means of feeding the world population.

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages.** Fish are an essential component of the healthy "Mediterranean diet". The lipid fraction of many fish contains a high proportion of omega-3-fatty acids that reduce risk of heart attack and stroke.

- **Goal 12. Ensure sustainable consumption and production patterns.** Using antibiotics for treatment of diseases in aquaculture is not sustainable as it leads to development and spread of bacterial antibiotic resistance. In the worst-case scenario, this leads to infectious diseases of man, animals and plants being untreatable, and humanity returning to the high frequency of deaths by infectious diseases seen before the discovery of antibiotics. Thus, novel non-antibiotic-based disease treatments are part of developing sustainable production patterns.

- **Goal 13. Take urgent action to combat climate change and its impacts.** Compared to other forms of high-quality protein food production, notably red meat, fish and shellfish production involves lower levels of greenhouse gas emissions. Thus, from a climate perspective, it makes sense to increase fish and shellfish production and reduce other protein food productions. Developing sustainable disease control measures also contributes to reduction of climate effects. Rearing fish obviously is energy requiring (e.g. for food production, water treatment, etc.), and diseases that kill mature fish render the energy investment in their production wasted.

- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development.** Seafood makes an important contribution to a healthy diet, particularly oily fish. However, we have over-fished and over-exploited the wild populations so, for sustainability, we need the environmental benefits and reduction in harvest pressure on oceans and lakes that aquaculture can deliver.

Pupil Participation

1. *Class discussion*

a. Do you live close to a coast-line or lakes?
b. Can you easily find fish and shellfish in your shopping area?
c. Where do the fish and shellfish you eat come from?
d. Give some examples of fish and shellfish, and some fish-based food products
e. Are fish or shellfish, or component of these, used for other purposes than food?
f. Which fish and shellfish have you eaten in the last two days?
g. Could you eat less meat?
h. What is a pescatarian?

2. *Pupil stakeholder awareness*

a. How will antibiotic resistance affect your life?
b. How can we, as society, reduce antibiotic resistance?
c. How could we help fish farmers treat sick fish?

3. *Exercises*

a. What options are there for using less antibiotics in fish farming?
b. Antibiotic resistance is a major risk and challenge – which of our behaviours contribute to development of antibiotic resistance?
c. Beneficial bacteria can be used in aquaculture; can you describe other areas (organisms) where probiotics are used?

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Adaptive immune system: also referred as the acquired immune system, is a subsystem of the immune system that is composed of specialized, systemic cells and processes that eliminates pathogens by preventing their growth. Acquired immunity creates immunological memory after an initial response to a specific pathogen, and leads to an enhanced response to subsequent encounters with that pathogen. This process of acquired immunity is the basis of vaccination.

Antibiotic: is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill (bacteriolytic) or inhibit the growth (bacteriostatic) of bacteria.

Aquaculture is the farming of fish, crustaceans, molluscs, aquatic plants, algae, and other organisms. Aquaculture involves cultivating freshwater and saltwater populations under controlled conditions, and can be contrasted with commercial fishing, which is the harvesting of wild fish.

Cell debris is all the leaked (organic) material that results from a cell that dies and disintegrates

Challenge trial: experiments where organisms (participants) are deliberately exposed to infection, in order to study diseases and test vaccines or treatments.

Commensal: Living in a relationship in which one organism derives food or other benefits from another organism without hurting or helping it. Commensal bacteria are part of the normal microbiota of all living organisms.

Crowding: organisms having to live/stay very close to one another; it gets crowded

Exudate: is a fluid emitted by an organism through pores or a wound, a process known as exuding or exudation.

Greenhouse gas is a gas that absorbs and emits radiant energy within the thermal infrared range. Greenhouse gases cause the greenhouse effect on planets. The primary greenhouse gases in Earth's atmosphere are water vapor (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3).

Heterotrophic bacteria are bacteria that cannot produce their own food, instead taking nutrition from other sources of organic carbon, mainly plant or animal matter. Living organisms that are heterotrophic include all animals and fungi, some bacteria and protists, and many parasitic plants.

Immune cells are the cells of the immune system, including lymphocytes (T-cells, B-cells and NK cells), neutrophils, and monocytes/macrophages. These are all types of white blood cells.

The **Immune system** is a complex network of cells and proteins that defends a body (a host) against infection. The immune system keeps a record of every germ (microbe) it has ever defeated so it can recognise and destroy the microbe quickly if it enters the body again.

The **Innate immune system** is one of the two main immunity strategies found in vertebrates (the other being the adaptive immune system). The innate immune system is an older evolutionary defense strategy, relatively speaking, and is the dominant immune system response found in plants, fungi, insects, and primitive multicellular organisms. It relies on a series of non-specific defenses (enzymes, peptides, oxidative radicals) elicited when a host encounters „foreign“ cells, for instance infecting bacteria

Larvae: is a distinct juvenile form many animals undergo before metamorphosis into adults. Animals with indirect development such as insects, amphibians, fish or cnidarians typically have a larval phase of their life cycle.

Live feed is an important basic diet for newly-hatched fish and shrimp larvae as they still have an incomplete digestive system and are lacking in enzymes. They are still at a very young stage to generate their own required nutrients or convert them from any pre-cursor obtained from a diet. Live feed organisms are algae, rotifers, Artemia and copepods.

Microbiome is the aggregate of all microorganisms that reside on or within a particular niche, for instance on other living organisms

Mollusks (mollusca) are an important phylum of invertebrate animals. Most of them are marine. They have huge numbers in-shore, that is, in shallow water. They are the largest marine phylum, with about 85,000 living species, 23% of all named marine organisms. They also occur in freshwater and on land. Typical examples are mussels and oysters

Opportunistic pathogen. An infectious microorganism that is normally a commensal or does not harm its host but can cause disease when the host's resistance is low.

Pathogen is in the oldest and broadest sense, is anything that can produce disease. A pathogen may also be referred to as an infectious agent.

Probiotics are live microorganisms promoted with claims that they provide health benefits to the host

Prophylactics consists of measures taken for disease prevention, for instance vaccination

Protective antibodies are part of the adaptive immune system. These are proteins produced by B-cells. They specifically recognizes and inactivates an infectious agent

Resistance: bacteria or other infectious agents that have evolved to tolerate antibiotics (or other antimicrobials) and can survive treatments that otherwise would kill or inhibit the agent.

Stress: chemical, physical and biological treatments that are damaging to the bacterium, for instance high salt, low temperature, UV-irradiation

Vaccine is a biological preparation that provides active acquired immunity to a particular infectious disease

Virulence is the ability of a pathogen to invade, infect and cause disease in a host