

## Chapter 25

### Microbiological aspects of fish and fishery products

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The planet earth harbors a total population of 7,530,687,482 (as on 7<sup>th</sup> September 2017 at 1345hrs) in which Asia and Africa share 4,486,207,286 and 1,252,251,830, respectively. In majority of the countries in Asia and Africa surveillance programs for detection and source tracking of human health hazard bacteria in foods is scant and in some cases do not exist. Hence, the task is much more complicated especially catering to needs of food, nutritional security and microbial safety of foods of more than 5,738,459,116 or 76.2 % of the total population of the world. The continued occurrence of foodborne illness is not evidence of the failure of our food safety system. In fact, many of our prevention and control efforts have been and continue to be highly effective. In advanced countries like US where food supply is one of the safest in the world, however, significant food borne illness continues to occur. Despite great strides in the area of microbiological food safety, much remains to be done.

Food-borne disease outbreaks are defined as the occurrence of 2 or more cases of a similar illness resulting from ingestion of a common food or observed number of cases of a particular disease exceeds the expected number. These can be confirmed (when at least one causal agent is identified) or suspected (based on clinical and epidemiological information). Although most cases are sporadic, these diseases draw attention to themselves due to outbreaks, thorough investigation of which can help in identifying control measures. Annual burden of foodborne diseases in the WHO South- East Asia Region includes more than: • 150 million illness • 175 000 deaths • 12 million DALYs Source: FERG Report 2010

The disability-adjusted life year (DALY) is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. It was developed in the 1990s as a way of comparing the overall health and life expectancy of different countries. The DALY is becoming increasingly common in the field of public health and health impact assessment (HIA). It "extends the concept of potential years of life lost due to premature death...to include equivalent years of

'healthy' life lost by virtue of being in states of poor health or disability." In so doing, mortality and morbidity are combined into a single, common metric.

## DALY

Disability Adjusted Life Year is a measure of overall disease burden, expressed as the cumulative number of years lost due to ill-health, disability or early death

$$= \text{YLD} + \text{YLL}$$

Years Lived with Disability      +      Years of Life Lost



*“Despite significant success at improving the safety of the food supply, current science on which safety is based does not sufficiently protect consumers from emerging issues inherent to a complex food supply. The evolving characteristics of food, technology, pathogens and consumers make it unlikely the marketplace will be entirely free of dangerous organisms at all times for all consumers”.* This is the conclusion drawn in the report, *Emerging Microbiological Food Safety Issues: Implications for Control in the 21<sup>st</sup> Century* released at IFT’s International Food Safety and Quality Conference and Expo in Atlanta one and half decades back. This holds good for fish and fishery products too.

The report, drew upon experts specializing in food borne pathogens and microbial evolution, food borne illness, food production and processing, testing methods and regulatory measures, reveals that diligent adherence to current methods that create and monitor the food supply cannot eliminate the risk of food borne illness. The report also offered the recommendations for providing the greatest possible reduction in food safety risks.

Among its seven important issues addressed were:

- Procedures from farm to table to significantly reduce illness due to mishandling,
- Processes to recognize and respond to outbreaks and to reduce their scope.

- Poor habits that make consumers more susceptible to foodborne illness,
- Education and training recommendations necessary for reducing pathogenic influence at every step
- From production to consumption (pond to plate/farm to fork)
- Recommendations to enhance monitoring, data generation, and risk assessment. And
- The current state and future potential of rapidly evolving illness-causing pathogens and other key issues.

In this premise let us examine the fish and fishery products scenario in four parts that include Human health hazard pathogens in fish and fishery products (Part I); Emerging Pathogens in Fish and Fishery Products: Public Safety Issues and Control Measures (Part II), Beneficial Microbes Associated with Aquatic Environment (Part III) and Fish and fishery products safety goals (Part IV).

## **Part I: HUMAN HEALTH HAZARD PATHOGENS IN FISH AND FISHERY PRODUCTS**

Fish are of great concern for export earnings because of their higher nutritive value such as high protein content, with little or no carbohydrate and fat value. But their high water activity, neutral pH, and presence of autolytic enzymes makes fish, shellfish and their products highly perishable in nature. Among different types of spoilage, microbial spoilage due to bacteria and their toxins ranks first which results in loss of about one-third of the world's food production. Microbial spoilage is mainly due to the rapid microbial growth of microorganisms naturally present in fish or from contamination, which can occasionally result in either economic or health-related problems. Generally, fish muscle is sterile at the time of slaughtering/catch, but becomes quickly contaminated by surface and intestinal bacteria, and from equipment and humans during handling and processing.

Food borne pathogens are microorganisms which causes infections from mild illness like gastroenteritis to a range of life threatening cases including death, up on food consumption. *Salmonella* spp., *Listeria* spp., *Shigella* spp., *E. coli*, *Staphylococcus aureus*, *vibrio* species and *Clostridium botulinum* are the major food borne pathogens associated with fish and fishery products. Fecal *coliforms* and fecal *streptococci* in fish and fishery products are indicator organisms for fecal contamination, whereas, the

presence of *staphylococcus* and *coliforms* reflects the poor hygienic handling practices. The presence all pathogenic microflora makes fish and its products unsafe for consumption.

### **I.1. *Salmonella***

*Salmonella* is a genus of rod-shaped (bacillus) Gram-negative bacteria of Enterobacteriaceae family which named after the American Bacteriologist D. E. Salmon. They are motile due to peritrichous flagella. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the type species and is further divided into six subspecies that include over 2,500 serotypes. All groups of *Salmonella* other than *Salmonella typhi* and *S. paratyphi* which causes typhoid are called as Non Typhoidal Salmonella (NTS). NTS usually causes food borne diseases like diarrhea, vomiting, fever, indigestion, enteritis etc. They are generally considered as zero tolerant organisms which means their presence should be nil in food. Compared with other Gram-negative rods, *Salmonella* are relatively resistant to various environmental factors. *Salmonella* grow at temperatures between 8°C and 45°C, at water activities above 0.94 and in pH range of 4-8. The bacterium is heat sensitive and will not survive at high temperature. But they have relatively high resistance towards drying, salting, smoking and even to freezing. *Salmonella* contamination to food is mainly through polluted waters, pets and through feeds used in aquaculture systems etc.

### **I.2. *Vibrio species***

In contrast to most other foodborne pathogens, *Vibrio spp.* have the aquatic habitat as their natural niche. As a result, *vibrios* are most commonly associated with fish and fishery products, as natural contaminants. *Vibrios* are associated with live fish and fishery products as they form part of the indigenous microflora of the environment at the time of fish and fishery products capture or harvest. *Vibrio spp.* are Gram-negative, facultative anaerobic motile curved rods with a single polar flagellum. Among the members of the genus, 12 species have so far been reported to be pathogenic to humans, where eight of these may be associated with foodborne infections of the gastrointestinal tract. Most of these foodborne infections are caused by *V. parahaemolyticus* and *V. cholerae*, and to a lesser extent by *V. vulnificus* and by *V. mimicus*.

### **I.3. *Vibrio cholera***

Among the *Vibrios*, *V. cholerae* is of most concern because of its ability to cause cholera. Cholera is an acute intestinal infection. Its incubation period ranges from a few hours to five days, usually two to

three days. Cholera is transmitted through ingestion of food or water contaminated with the bacterium, especially via faeces or vomitus of infected persons, directly or indirectly. *V. cholerae* is a mesophilic organism that grows in the temperature range of 10 to 43°C, with an optimum growth at 37°C. The pH optimum for growth is 7.6 although it can grow in the pH range of 5.0 to 9.6. *V. cholerae* can grow in the salt range of 0.1 to 4.0% NaCl, while optimum is 0.5% NaCl. Of the more than 200 *V. cholerae* serogroups that exist, only O1 and O139 are associated with the epidemiological features and clinical syndrome of cholera. However, organisms of *V. cholerae* serogroups other than O1 and O139 (non-O1 non-O139 serogroups) have been associated with sporadic cases of foodborne outbreaks of gastroenteritis, but have not spread in epidemic form. The most important virulence factor associated with *V. cholerae* O1 and O139 serogroups is the cholera toxin. Non-O1 non-O139 serogroups are generally nontoxicogenic.

#### **I.4. *Vibrio parahaemolyticus***

Among the potentially pathogenic *vibrios* occurring naturally on fish and shellfish, *V. parahaemolyticus* is the most widespread. *V. parahaemolyticus* is a slightly halophilic bacterium. The optimum growth NaCl concentrations range from 2 to 4% and poor growth is exhibited in media below 0.5% NaCl. The bacterium is inactivated rapidly in distilled water and growth at levels of 10% NaCl is inhibited. The organism grows at a temperature range between 5 and 43°C, with optimum growth at 37°C. The optimum pH range for growth is 7.8 to 8.6, although it can grow in the pH range of 4.8 to 11. The illness caused by *V. parahaemolyticus* food poisoning is a gastroenteritis characterized by watery diarrhea and abdominal cramps in most cases, with nausea, vomiting, fever and headache. The incubation period is usually between 12 and 24 hours and the disease usually resolves in three days. The infection is typically acquired through consumption of contaminated fish and fishery products. These could be raw or inadequately cooked, or that have been cross-contaminated by improper handling.

#### **I.5. *Vibrio vulnificus***

*V. vulnificus* is an opportunistic pathogen that can cause wound infections and a rapidly progressing septicemia with few gastrointestinal signs. *V. vulnificus* is very similar to *V. parahaemolyticus* in cultural characteristics and sensitivity to processing procedures. It differs principally in salt requirement and tolerance, growing in media containing between 0.1 and 5% NaCl. Same as *V. parahaemolyticus*, the organism grows optimally at 37°C although it can grow at a temperature range

between 8 and 43°C. The pH range for growth of *V. vulnificus* is 5 to 10, with an optimum at 7.8. The incubation period is from seven hours to several days

### **I.6. *Vibrio mimicus***

*Vibrio mimicus* are facultative anaerobes and possess a single polar flagellum for movements, and are oxidase-positive. *V. mimicus* are found in aquatic ecosystem, including seawater, freshwater, and brackish water, where it has been found both as a free-living bacterium and in association with zooplankton, crustaceans, filter-feeding mollusks, turtle eggs. *Vibrio mimicus* are responsible for gastroenteritis and are closely related phylogenetically to *Vibrio cholerae*. The ingestion of raw or uncooked fish and fishery products containing *Vibrio mimicus* can cause gastroenteritis and diarrhea.

### **I.7. *Shigella species***

*Shigella* is a genus of Gram-negative, facultative anaerobic, non-sporeforming, non-motile, rod-shaped bacteria genetically closely related to *E. coli*. The genus is named after Kiyoshi Shiga, who first discovered it in 1897. There are four different species of *Shigella* like *Shigella sonnei* (the most common species in the United States), *Shigella flexneri*, *Shigella boydii*, *Shigella dysenteriae*. Most who are infected with *Shigella* develop diarrhea, fever, and stomach cramps starting a day or two after they are exposed to the bacteria. Shigellosis usually resolves in 5 to 7 days. Some people who are infected may have no symptoms at all, but may still pass the *Shigella* bacteria to others. *Shigella* is implicated as one of the pathogenic causes of reactive arthritis worldwide.

### **I.8. *Listeria monocytogenes***

*Listeria monocytogenes* is the species of pathogenic bacteria that causes the infection listeriosis. It is a Gram-positive, facultative anaerobic rod shaped bacterium, capable of surviving in the presence or absence of oxygen which named after Joseph Lister. It is catalase-positive and oxidase-negative, and expresses a beta hemolysin, which causes destruction of red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20–25 °C), the organism does not synthesize flagella at body temperatures (37 °C). *Listeria monocytogenes* has been associated with foods such as raw milk and milk products, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (of all types), and raw and smoked fish. Most bacteria can survive near freezing temperatures,

but cannot absorb nutrients, grow or replicate. *L. monocytogenes* ability to grow at temperatures as low as 0 °C permits exponential multiplication in refrigerated foods. At refrigeration temperature, such as 4 °C, the amount of ferric iron can affect the growth of *L. monocytogenes*.

### **I.9. *Escherichia coli***

*E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. It has been generally recognized as a sanitary indicator organism for faecal contamination of water and fish and fishery products in tropics. *E. coli* is often nonpathogenic, although different strains may cause diseases in gastrointestinal, urinary, or central nervous systems. At least five biotypes are currently known to induce intestinal infection: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), enteropathogenic *E. Coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC).

### **I.10. *Staphylococcus aureus***

*Staphylococcus aureus* is a mesophilic, Gram-positive bacterium associated with warm-blooded animals. They are non-moving small round shaped or non-motile cocci that occur in microscopic clusters resembling grapes. It is a common member of the skin and nasal microflora of humans. Many strains of *S. aureus* may produce enterotoxins which upon ingestion causes a sudden reaction in terms of cramps, abdominal pain and vomiting. Several different enterotoxins may be produced and they have, based on antigenic properties been divided into sero-types A to J. Enterotoxin A is assumed to be the most commonly involved in food-poisoning outbreaks, however, recently type C has become prevalent. *S. aureus* can be detected sporadically on raw fish but is clearly more typical of fish and fishery products that have been heat treated and manually handled, such as crustacean products. Emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine

### **I.11. *Clostridium species***

*Clostridium* is a genus of Gram-positive bacteria, which includes several significant human pathogens, including the causative agent of botulism and an important cause of diarrhea in man. Botulism results

from the ingestion of food containing botulinum toxin produced during the growth of this organism which can even cause the death of organism at microgram level. They are obligate anaerobes capable of producing endospores. *Clostridium botulinum* and *C. perfringens* are the two common food borne pathogens of this group and often associated with the consumption of uncooked, undercooked, smoked, fermented fishes and even with the vacuum packed fish and fishery products.

### I.12. Yeast and Mold (Fungi)

Fungus consists of mainly two types like yeasts (cellular fungi) and molds (filamentous fungi). Several foodborne molds and possibly yeast may be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most of the mycotoxins are heat stable compounds which even cannot destroyed by cooking. Their growth temperature range is very broad (10°C to 35°C), so also their moisture and pH requirements. This usually enables them to invade and virtually grow in or on many food items. Usually they will not see in fresh raw fish since the moisture content is too high. But fishery products like dry fishes, pickles and even fermented fish can be contaminated with fungal spores.

Principal symptoms of bacteria & potential food contamination are shown below.

<b>PRINCIPAL SYMPTOMS OF BACTERIA &amp; POTENTIAL FOOD CONTAMINATION</b>					
<b>(h: hours, d; days, wks: weeks)</b>					
Organi sms respon sible for the food borne illness	Common Name of Illness	<b>Onset Time After Ingesti ng</b>	<b>Signs &amp; Symptoms</b>	<b>Durati on</b>	<b>Food Sources</b>
<i>Bacillus cereus</i>	<i>B. cereus</i> food poisoning	10-16 h	Abdominal cramps, watery diarrhea, nausea	24-48 h	Meats, stews, gravies, vanilla sauce
<i>Campylobacter jejuni</i>	Campylobacteriosis	2-5 d	Diarrhea, cramps, fever, and vomiting; diarrhea may be	2-10 d	Raw and undercooked poultry, unpasteurized



			bloody		milk, contaminated water
<i>Clostridium botulinum</i>	Botulism	12-72 h	Vomiting, diarrhea, blurred vision, double vision, difficulty in swallowing, muscle weakness. Can result in respiratory failure and death	Variabe	Improperly canned foods, especially home-canned vegetables, fermented fish, baked potatoes in aluminum foil
<i>Clostridium perfringens</i>	Perfringens food poisoning	8-16 h	Intense abdominal cramps, watery diarrhea	24h	Meats, poultry, gravy, dried or precooked foods, time and/or temperature-abused foods
<i>Cryptosporidium</i>	Intestinal cryptosporidiosis	2-10 d	Diarrhea (usually watery), stomach cramps, upset stomach, slight fever	May be remitting and relapsing over weeks to months	Uncooked food or food contaminated by an ill food handler after cooking, contaminated drinking water
<i>Cyclospora cayentensis</i>	Cyclosporiasis	1-14 d, usually at least 1 wk	Diarrhea (usually watery), loss of appetite, substantial loss of weight, stomach cramps, nausea, vomiting, fatigue	May be remitting and relapsing over weeks to months	Various types of fresh produce

<i>E. coli</i> ( <i>Escherichia coli</i> ) producing toxin	<i>E. coli</i> infection (common cause of “travelers’ diarrhea”)	1-3 d	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more d	Water or food contaminated with human feces
<i>E. coli</i> O157:H7	Hemorrhagic colitis or <i>E. coli</i> O157:H7 infection	1-8 d	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger. Can lead to kidney failure.	5-10 d	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and vegetables (e.g. sprouts), and contaminated water
Hepatitis A	Hepatitis	28 d average (15-50 d)	Diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain	Variable, 2 wks-3 months	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
<i>Listeria monocytogenes</i>	Listeriosis	9-48 h for gastrointestinal symptoms, 2-6 weeks for	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats

		invasive disease	premature delivery or stillbirth. The elderly or immunocompromised patients may develop bacteremia or meningitis.		
Noroviruses	Variously called viral gastroenteritis, winter diarrhea, acute non-bacterial gastroenteritis, food poisoning, and food infection	12-48 h	Nausea, vomiting, abdominal cramping, diarrhea, fever, headache. Diarrhea is more prevalent in adults, vomiting more common in children.	12-60 h	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters
<i>Salmonella</i>	Salmonellosis	6-48 h	Diarrhea, fever, abdominal cramps, vomiting	4-7 d	Eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables
<i>Shigella</i>	Shigellosis or Bacillary dysentery	4-7 d	Abdominal cramps, fever, and diarrhea. Stools may contain blood and mucus.	24-48 h	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler

<i>Staphylococcus aureus</i>	Staphylococcal food poisoning	1-6 h	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present.	24-48 h	Unrefrigerated or improperly refrigerated meats, potato and egg salads, cream pastries
<i>Vibrio parahaemolyticus</i>	<i>V.parahaemolyticus</i> infection	4-96 h	Watery (occasionally bloody) diarrhea, abdominal cramps, nausea, vomiting, fever	2-5 d	Undercooked or raw seafood, such as shellfish
<i>Vibrio vulnificus</i>	<i>V.vulnificus</i> infection	1-7 d	Vomiting, diarrhea, abdominal pain, blood borne infection. Fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to persons with liver disease or weakened immune systems.	2-8 days	Undercooked or raw seafood, such as shellfish (especially oysters)
Adopted from USFDA (2011)					

## **Part II; EMERGING PATHOGENS IN FISH AND FISHERY PRODUCTS: PUBLIC SAFETY ISSUES AND CONTROL MEASURES**

First we will define the word ‘emerging’ clearly followed by a perusal into major challenges faced in keeping quality of fish and fishery products in relation to pathogenic bacteria. They included Relationship between human health and oceans; Emerging infections of the gastrointestinal tract; Fish and fishery products safety issues of small and medium entrepreneurs; Refrigerated Pasteurized Foods (RPF) and public health implications; identification, assessment and management of food related microbiological hazards: historical, fundamental and psycho-social essentials; information systems in food safety management; Aquaculture practices and potential human health risks: current knowledge and future

priorities; bioterrorism; climate and food safety; challenges to global surveillance of disease pattern; Changes in preference patterns of fish and fishery products consumer and associated changes in risk exposure; technological innovations in food processing; control measures: An example and predictive microbiology

### **II.1. Definition**

The definition the word 'emerging' means, 'to rise out of a state of depression or obscurity; to come to notice; to reappear after being eclipsed'. Thus is the state of infectious diseases throughout the world in the twenty-first century. Not only are new microorganisms being identified every year but some of the old established diseases are again gaining attention. More specifically, emerging diseases have been defined as those 'whose incidence in humans has increased in the past two decades or threatens to increase in the near future'.

### **II.2. Human health**

There has been an increasing recognition of the inter-relationship between human health and the oceans. Traditionally, the focus of research and concern has been on the impact of human activities on the oceans, particularly through anthropogenic pollution and the exploitation of marine resources. More recently, there has been recognition of the potential direct impact of the oceans on human health, both detrimental and beneficial. Areas identified include: global change, harmful algal blooms (HABs), microbial and chemical contamination of marine waters and fish and fishery products, and marine models and natural products from the seas. It is hoped that through the recognition of the inter-dependence of the health of both humans and the oceans, efforts will be made to restore and preserve the oceans.

### **II.3. Emerging infections of the gastrointestinal tract**

Infections account for significant GI morbidity and mortality worldwide. New organisms are being identified, associated with diarrheal illness and some with other gastrointestinal illness as well. Among GI viruses, Sapovirus is now recognized to cause diarrhoea, especially in children. A hyper virulent strain of *Clostridium difficile* has caused epidemics in many countries. Newly identified bacterial species that may cause diarrhoea included *Campylobacter concisus*, *Arcobacteria*, *Edwardsiella tarda*, *Aeromonas*, *Plesiomonas*, *Laribacte* and *Helicobacteria*.

#### **II.4. Aquaculture practices and potential human health risks: current knowledge and future priorities.**

Annual global aquaculture production has more than tripled within the past 15 years, and by 2015, aquaculture is predicted to account for 39% of total global fish and fishery products production by weight. Given that lack of adequate nutrition is a leading contributor to the global burden of disease, increased food production through aquaculture is a seemingly welcome sign. However, as production surges, aquaculture facilities increasingly rely on the heavy input of formulated feeds, antibiotics, antifungals, and agrochemicals. In the context of this current knowledge concerning major chemical, biological and emerging agents that are employed in modern aquaculture facilities and their potential impacts on public health were reviewed.

Studies indicate that current aquaculture practices can lead to elevated levels of antibiotic residues, antibiotic-resistant bacteria, persistent organic pollutants, metals, parasites, and viruses in aquacultured finfish and shellfish. Specific populations at risk of exposure to these contaminants include individuals working in aquaculture facilities, populations living around these facilities, and consumers of aquacultured food products.

Additional research is necessary not only to fully understand the human health risks associated with aquacultured fish versus wild-caught fish but also to develop appropriate interventions that could reduce or prevent these risks. In order to adequately understand, address and prevent these impacts at local, national and global scales, researchers, policy makers, governments, and aquaculture industries must collaborate and cooperate in exchanging critical information and developing targeted policies that are practical, effective and enforceable.

#### **II.5. Bioterrorism**

The bioterrorism potential of *Brucella*, *Burkholderia mallei* & *pseudomallei*, *Chlamydia psittaci*, *Coxiella burnetii*, *Rickettsia prowazekii*, Epsilon toxin of *Clostridium perfringens*, ricin toxin, Staphylococcal enterotoxin B, Alpha viruses and Bunya viruses, Flavi viruses needs special attention. Foodborne and waterborne pathogens that included bacteria, viruses and protozoans and the bioterrorism potential is equally important.

## **II.6 Climate and food safety**

According to general consensus, the global climate is changing, which may also affect agricultural and livestock production. The potential impact of climate change on food security is a widely debated and investigated issue. Nonetheless, the specific impact on safety of food and feed for consumers has remained a less studied topic. Therefore, various food safety issues that are likely to be affected by changes in climate, particularly in Europe were assessed.

Amongst the issues identified are mycotoxins formed on plant products in the field or during storage; residues of pesticides in plant products affected by changes in pest pressure; trace elements and/or heavy metals in plant products depending on changes in their abundance and availability in soils; polycyclic aromatic hydrocarbons in foods following changes in long-range atmospheric transport and deposition into the environment; marine biotoxins in fish and fishery products following production of phycotoxins by harmful algal blooms; and the presence of pathogenic bacteria in foods following more frequent extreme weather conditions, such as flooding and heat waves. Research topics that are amenable to further research are highlighted.

## **II.7 Challenges to global surveillance of disease pattern**

Surveillance systems for foodborne disease vary in capacity by country, especially for marine-related illnesses. Generally, the more developed the country is, the more funding that is put into its surveillance programs, but no country has an outstanding system that could serve as a model for all others. An additional problem is lack of consistency. Approaches to surveillance and available resources change over time, so that apparent trends may reflect more of an administrative function. Most countries have some passive system that allows data on foodborne illnesses to be sent to centralized authorities where summaries are generated. However, these depend on the uneven quality of the source data that vary according to the resources allocated at the local level. Active surveillance systems collect data targeted to answer specific epidemiological questions more efficiently, but at such a high cost that most countries do not have the resources, except on occasional basis. There is also the issue of what to do with the collected data. There has to be a conscious effort to translate the problems identified from the surveillance programs to consider strategies for prevention and control of foodborne disease. Otherwise, there is little value in having these kinds of monitoring programs. Another problem is lack of coordination in surveillance systems between most countries, so that information can be

rapidly and efficiently shared. That being said, surveillance over the years had generated much interesting information on how disease agents are transmitted through the food supply, and where contamination and growth by pathogens in the food production and preparation chain typically occur. In addition, attempts are being made to create regional networks in different parts of the world usually initiated by organizations like WHO and PAHO (Pan American Health Organization). The kinds of information collected and programs being introduced are discussed in examples taken from both the developed and less developed world, followed by a series of recommendations for improving surveillance on a global basis. A recent burden in the surveillance system is the potential for a deliberate attack on the food supply with agents not usually involved with foodborne illness. At least in the US, a major concern is for the rapid detection and containment of a massive contamination of the food supply

## **II.8 Fish and fishery products safety issues small and medium entrepreneurs**

Providing small food and catering operators, constituting a segment of the small and medium size enterprises (SME), with adequate guidance to ensure microbiologically safe fish and fishery products at the moment of ingestion constitutes a difficult endeavor. It culminates in street vending of foods, particularly in areas with poor sanitary environmental conditions and high ambient temperatures. The natural occurrence of pathogens on raw fish and fishery products is often compounded by an unreliable water supply, poor temperature control and lack of even a rudimentary knowledge of applied food microbiology. The mission statement of the **Codex Alimentarius Commission** nonetheless includes providing the entire sector of food and catering enterprises world-wide with Codes to enable the plentiful supply of unconditionally safe food.

A construct has been devised to gradually but substantially enhance the microbiological safety of products offered by SMEs- in essence complying with the HACCP maxim. Lord Plumb's extension of Bauman's HACCP model to longitudinally integrated safety assurance (LISA) has been chosen as the lead policy. Wilson's Triad, ensuring control of contamination and proliferation throughout the entire food chain provides the essential guidelines. An innovation consists of introducing the concept of attention points, where critical practices or sites cannot yet be brought under control. In this way the mental preparedness to pursue further improvements as required is perennially stimulated.



## **II.9 Public safety issues:**

### **Public health implication of refrigerated pasteurized ('sous-vide') foods.**

Food that upon pasteurization is stored in hermetically sealed containers at food temperatures not exceeding 3°C could be designated by the generic term Refrigerated Pasteurized Foods of Extended Durability, REPFEDs. If not properly processed or protected against recontamination, or if temperature-abused, REPFEDs may present serious health risks. However, control is possible through sound microbial ecology, supported by expert risk assessment, allows the design and introduction of longitudinally integrated manufacture, distribution, handling by outlets and consumers and culinary preparation, which result in the assurance of the wholesomeness of the commodity as eaten. The progress, including intrinsic preservation by the incorporation of starter cultures, bacteriocins or particular enzymes, opened new vistas for attractive developments. Once microbiological safety has been built into the REPFED-line, monitoring can be limited to,

- (i) Real-time tests particularly applied to the factory environment; and
- (ii) Rapid, simple examination for marker organisms of freshly manufactured products versus those approaching expiration dates.

This kind of audits will allow rapid retrieval of incidental process failure and its rectification. It also serves to substantiate measurements of food temperature and spot checks on intrinsic inhibitory attributes. The application of scientific knowledge and technological expertise should primarily be entrusted to the industry itself, heeding Lord Plumb's strategy of "partnership along the food production chain from farm to fork". It should be supported and validated by Public Health Authorities. At all stages safety communication with the public should be ensured.

### **Identification, assessment and management of food related microbiological hazards: historical, fundamental and psycho-social essentials.**

Microbiological risk assessment aimed at devising measures of hazard management, should take into account all perceived hazards, including those not empirically identified. It should also recognize that safety cannot be "inspected into" a food. Rather hazard management should be the product of intervention strategies in accordance with the approach made mandatory in the EU Directive 93/43 and the USDA FSIS Pathogen Reduction HACCP system; Final Rule. It is essential too that the inherent

variability of the biological attributes affecting food safety is recognized in any risk assessment. The above strategic principles may be conceptualized as a four-step sequence, involving

- I. Identification and quantification of hazards;
- II. Design and codification of longitudinally integrated (“holistic”) technological processes and procedures to eliminate, or control growth and metabolism of, pathogenic and toxinogenic organisms;
- III. Elaboration of microbiological analytical standard operating procedures, permitting validation of “due diligence” or responsible care, i.e. adherence to adopted intervention strategies. This should be supported by empirically assessed reference ranges, particularly for marker organisms, while the term “zero tolerance” is refined throughout to tolerable safety limit;
- IV. When called for, the need to address concerns arising from lay perceptions of risk which may lack scientific foundation.

**In relation to infectious and toxic hazards in the practical context the following general models for quantitative holistic risk assessment are presented:**

- a. The first order, basic lethality model;
- b. Second approximation taking into account the amount of food ingested in a given period of time;
- c. Further adjustment accounting for changes in colonization levels during storage and distribution of food commodities and the effects of these on proliferation of pathogens and toxin production by bacteria and moulds.

#### **II.9.1 Guidelines are provided to address:**

- I. Unsubstantiated consumer concern over the wholesomeness of foods processed by an innovative procedure; and
- II. Reluctance of small food businesses to adopt novel strategies in food safety. Progress here calls for close cooperation with behavioural scientists to ensure that investment in developing measures to contain risk deliver real benefit.

## II.10 Information systems in food safety management

Information systems are concerned with data capture, storage, analysis and retrieval. In the context of food safety management, they are vital to assist decision making in a short time frame, potentially allowing decisions to be made and practices to be actioned in real time.

Databases with information on microorganisms pertinent to the identification of foodborne pathogens, response of microbial populations to the environment and characteristics of foods and processing conditions are the cornerstone of food safety management systems. Such databases find application in:

- I. Identifying pathogens in food at the genus or species level using applied systematics in automated ways.
- II. Identifying pathogens below the species level by molecular subtyping, an approach successfully applied in epidemiological investigations of foodborne disease and the basis for national surveillance programs.
- III. Predictive modelling software, such as the Pathogen Modeling Program and Growth Predictor (that took over the main functions of Food Micromodel) the raw data of which were combined as the genesis of an international web based searchable database (ComBase).
- IV. Expert systems combining databases on microbial characteristics, food composition and processing information with the resulting “pattern match” indicating problems that may arise from changes in product formulation or processing conditions.
- V. Computer software packages to aid the practical application of HACCP and risk assessment and decision trees to bring logical sequences to establishing and modifying food safety management practices.

In addition, there are many other uses of information systems that benefit food safety more globally, including:

- I. Rapid dissemination of information on foodborne disease outbreaks via websites or list servers carrying commentary from many sources, including the press and interest groups, on the reasons for and consequences of foodborne disease incidents.

- II. Active surveillance networks allowing rapid dissemination of molecular subtyping information between public health agencies to detect foodborne outbreaks and limit the spread of human disease.
- III. Traceability of individual animals or crops from (or before) conception or germination to the consumer as an integral part of food supply chain management.
- IV. Provision of high quality, online educational packages to food industry personnel otherwise precluded from access to such courses.

## **II.12 Control Measures**

### **Predictive food microbiology**

Predictive food microbiology (PFM) is an emerging multidisciplinary area of food microbiology. It encompasses such disciplines as mathematics, microbiology, engineering and chemistry to develop and apply mathematical models to predict the responses of microorganisms to specified environmental variables. A critical review on the development of mathematical modelling with emphasis on modelling techniques, descriptions, classifications and their recent advances was given. The accuracy of predictive food microbiology will increase as understanding of the complex interactions between microorganisms and food becomes clearer. However, the reliance of food microbiology on laboratory techniques and skilled personnel to determine process and food safety is equally important.

The predictive microbiology was taken into consideration in the context of the Food Micro 2002 theme, “Microbial adaptation to changing environments”. To provide a reference point, the state of food microbiology knowledge in the mid-1970s is selected and from that time, the impact of social and demographic changes on microbial food safety is traced. A short chronology of the history of predictive microbiology provided context to discuss its relation to and interactions with hazard analysis critical control point (HACCP) and risk assessment. The need to take account of the implications of microbial adaptability and variable population responses is couched in terms of the dichotomy between classical versus quantal microbiology. The role of population response patterns and models as guides to underlying physiological processes draws attention to the value of predictive models in development of novel methods of food preservation. It also draws attention to the inconsistencies in food industry that is required to balance the “clean, green” aspirations of consumers with the risk, to safety or shelf life, of removing traditional

barriers to microbial development. Further the discussion is dominated by consideration of models and responses that lead to stasis and inactivation of microbial populations. This highlights the consequence of change on predictive modelling where the need is to develop interface and non-thermal death models to deal with pathogens that have low infective doses for general and/or susceptible populations in the context of minimal preservation treatments. The challenge is to demonstrate the validity of such models and to develop applications of benefit to the food industry and consumers as was achieved with growth models to predict shelf life and the hygienic equivalence of food processing operations.

The future of predictive microbiology depends on exploring the balance that exists between science, applications and expectations. Attention is drawn to the development of predictive microbiology as a sub-discipline of food microbiology and of technologies that are required for its applications, including a recently developed biological indicator. As we move into the era of systems biology, in which physiological and molecular information will be increasingly available for incorporation into models, predictive microbiologists will be faced with new experimental and data handling challenges. Overcoming these hurdles may be assisted by interacting with microbiologists and mathematicians developing models to describe the microbial role in ecosystems other than food. Coupled with a commitment to maintain strategic research, as well as to develop innovative technologies, the future of predictive microbiology looks set to fulfill “great expectations.

### **II.13 Technological innovations in food processing**

During the last 25 years, consumer demands for more convenient and varied food products have grown exponentially, together with the need for faster production rates, improved quality and extension in shelf life. These requests together with the severity of the traditional food processing technologies were driving forces for improvements in existing technologies and for the development of new food preservation technologies. Therefore, many technological developments have been directed towards unit operations such as pasteurization, sterilization, cooking and drying, and currently the new technological approaches for food preservation are serious candidates to replace the traditional well-established preservation processes.

The environmental impact that some of the most promising novel food preservation technologies may represent in terms of energy efficiency, water savings and reduced emissions. The emergence of novel thermal and non-thermal technologies allows producing high quality products with

improvements in terms of heating efficiency and, consequently, in energy savings. Most of these technologies are locally clean processes and therefore appear to be more environment-friendly, having less environmental impact than the traditional ones. Novel processing technologies are increasingly attracting the attention of food processors once they can provide food products with improved quality and a reduced environmental footprint, while reducing processing costs and improving the added-value of the products.

### **Part III: BENEFICIAL MICROBES ASSOCIATED WITH AQUATIC ENVIRONMENT**

Microorganisms have major roles in aquaculture, particularly with respect to productivity, nutrient cycling, the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent. Management of the activities of microorganisms in food webs and nutrient cycling in ponds is necessary for optimizing production. Bacteria, micro algae and protozoans all have major roles in aquatic ecosystems. Of these microorganisms, microalgae and the blue-green algae, or the cyanobacteria as they are now called, are the most easily seen and the best known. Until the last decade the heterotrophic bacteria were often overlooked, possibly because the older methods used to study them are not adequate for natural environments. The classical methods of clinical microbiology, especially counting colonies of bacteria on nutrient-rich agar, were designed to isolate or count particular pathogenic bacteria. Some advances were made with these techniques, but the extent of the role of bacteria and protozoans in the decomposition of organic matter and the cycling of important elements has been fully recognized only recently.

In the last 15 years, major advances have been made in microbial ecology with new methods that have been developed for studying aquatic microorganisms and their activities. Epifluorescence microscopy has become a powerful tool for counting the total numbers of bacteria, estimating their biomass and determining their distribution in water, in sediments and on surfaces of submerged plants and man-made structures. This technique has shown that bacteria are anywhere from 10 to 100000 times more numerous than the classical microbiological techniques showed.

Biochemical techniques involving small compounds labelled with radioactive atoms (especially tritium and  $^{14}\text{C}$ ) have been used to study the activities, growth rates and productivity of microorganisms in aquatic ecosystems. These methods enable microbial ecologists to study the

functions of ecological groups of bacteria in their natural environment. The new tools of the microbial ecologist have an important application to aquaculture. By quantifying the productivity of bacteria, we can make scientifically-based judgments about the functional roles of bacteria, and thus improve pond management to optimize productivity.

Several factors influence the accuracy of bacterial productivity determinations, and these should be assessed before applying values in a model. These include the type of method used; the species composition i.e. variations in biochemistry; the physiological state of the bacteria, e.g. whether they are starved, or growing slowly or rapidly. Factors for converting growth rate to production or carbon flux depend on bacterial size and growth efficiency and these vary with species composition of the active populations (many bacteria in natural water bodies are in a non-growing state).

**Figure: Microbial processes happening in aquatic environment**

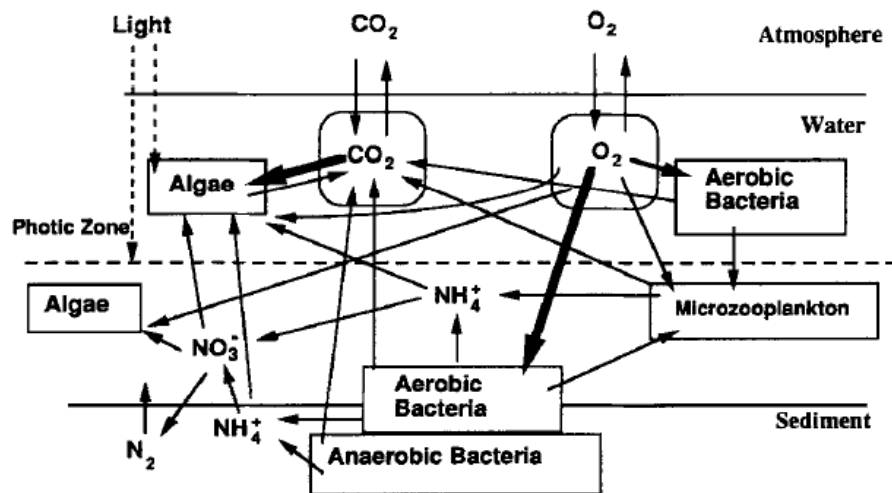


Fig. 1. Flows of oxygen, carbon dioxide and some nutrients between the principal microorganisms in a pond. In the photic zone, which is generally where the light intensity is more than 1% of the surface intensity, oxygen is produced by phytoplankton at a greater rate than it is consumed by respiration. Net mineralisation of nitrogen as ammonium by bacteria is only greater than uptake if the C:N ratio of detritus is less than 10:1. Where the C:N ratio is greater, bacteria will be net immobilisers of nitrogen and compete with the algae for it; microzooplankton mineralise the nitrogen when they feed on bacteria.

### III.1 Bacterial disease and probiotics

Disease control needs a new approach, which is both cost-effective and environmentally safe. The use of beneficial bacteria (probiotics) to displace pathogens (by competitive processes or by release of growth inhibitors) is now gaining acceptance in the animal industry as a better, cheaper and more effective remedy than administering antibiotics to promote health of animals. The probiotic control of pathogens in fish

culture has considerable. It is likely that antibiotic usage in hatcheries and ponds can be replaced by bacteria that inhibit pathogens. Antibiotics are often ineffective either because resistant bacteria develop or the pathogens are in a non-growing phase when they are insensitive to antibiotics.

Knowledge of the precise relationships between water column bacteria and those growing in or on the animals is essential for making informed decisions on management and operational procedures. Further research is needed on the nature of the bacterial populations associated with aquatic animals, both in the wild and in culture, in order to provide a basis for understanding the impact of control measures in hatcheries and ponds and on water released to the natural environment. If we can manipulate the bacterial species composition and in particular control pathogen numbers, larval survival and fitness will be enhanced in hatcheries and grow-out productivity will be increased, and the risk of contaminating natural populations with antibiotic-resistant bacteria will be lowered. Many commercial products of viable bacteria are being used to control water quality in ponds; (one term for this is bio-augmentation). The ecological processes that govern the presumed efficacy of many of these preparations are not well defined, although it is presumed that the added bacteria produce greater quantities and a greater range of exoenzymes for breaking down organic compounds. Many users report that the products are effective, so a study of the ecology of the bacteria involved in those processes will lead to better control of water quality in ponds and the development of better products. This would have a flow-on to improvements to the microbial amelioration of pollution, not only in aquaculture, but in other industries that have an impact on coastal environments.

### **III.3 Probiotics are classified into following two groups**

**III.3.1 Feed Probiotics:** Some bacterial, fungal strains can be blended with feeding pellets or by encapsulating into live feed stock or administered orally to feed rearing animals to prevent disease and enhance essential microbial flora of the gut. Viability of strains should be tested before feeding animals. Probiotics like lactic acid bacteria applied in the feed of fry of Atlantic cod, showed adequate growth, survival and immune response.

**III.3.2 Water probiotics:** water probiotics are applied to reduce organic pollutants and various contaminants in water by directly applying to rearing medium. These improve water quality by converting organic matter to smaller units.



Breakdown of organic matters evolve simpler substances like glucose and amino acids that are used as food for beneficial bacteria which reduce the accumulation of organic pressure and provide congenial environment to farmed stock. Probiotic bacteria such as *Bacillus* sp. can convert organic matter to CO<sub>2</sub> so that organic effluent can be minimized in aquatic system. By using nitrifying bacteria, the quantity of nitrate, nitrite, ammonia is reduced to a large extent. These lead to purify the water in the hatchery enhancing larval survival and growth.

### **III.4 Significance of Probiotics in Aquaculture**

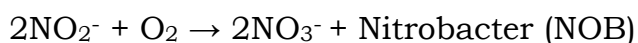
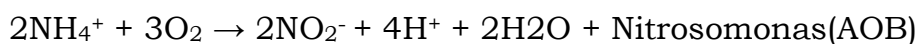
Probiotics use in aquaculture show great impact on aquatic organisms. Probiotics decrease accumulation of organic load and maintain water quality in an efficient way. A modern probiotic organism can easily fulfill the desires of sustainable aquaculture development because it can heighten two major key factors of growth performance and disease resistance. Lactic Acid Bacteria, a popular probiotic strain, can be applied to control bacterial pathogen. In addition, another well-known probiotic organism, *Bacillus* sp. is used to diminish metabolic waste in aquatic system. Many strains of *Aeromonas* sp., *Pseudomonas* sp., *Vibrio* sp. act against infectious hematopoietic necrosis virus to show antiviral activity. These probiotic organisms may be used singly or in combination such as incorporation of individual or combined supplementation of *Lactobacillus rhamnosus* and *Lactobacillus sporogenes* enhance health and disease resistance. Probiotics do not cause water pollution because of their eco-friendly nature, thus more and more suitable for aquaculture system. They not only promote animal health but also maintain consumer health safety. Uses of probiotics and their target aquatic organisms are briefly dealt in Table 1.

**Table 1:** Uses of Probiotic in aquaculture system (Cruz *et al.* [49])

Uses of Probiotic	Probiotic Species	Gram Positive/negative Bacteria	Target aquatic species
Water quality	<i>Bacillus</i> sp.	+ve	<i>Penaeus monodon</i>
	<i>Vibrio</i> sp. NE 17	-ve	<i>Macrobrachium rosenbergii</i>
	<i>Lactobacillus acidophilus</i>	+ve	<i>Clarias gariepinus</i>
Control of diseases	<i>Enterococcus faecium</i> SF 68	+ve	<i>Anguilla Anguilla</i>
	<i>Pseudomonas fluorescens</i>	-ve	<i>Oncorhynchus mykiss</i>
	<i>Lactococcus lactis</i>	+ve	<i>Epinephelus coioides</i>
	<i>Pseudomonas</i> sp.	-ve	<i>Oncorhynchus mykiss</i>
	<i>Bacillus</i> sp.	+ve	Penacids
	<i>Vibrio alginolyticus</i>	-ve	Salmonids
Growth promoter	<i>Lactobacillus lactis</i> AR21	+ve	<i>Brachionus plicatilis</i>
	<i>Bacillus</i> sp.	+ve	Catfish
	<i>Streptococcus thermophiles</i>	+ve	<i>Scophthalmus maximus</i>
	<i>Bacillus coagulans</i>	+ve	<i>Cyprinus carpio koi</i>
	<i>Bacillus</i> NL 110	+ve	<i>M. rosenbergii</i>
Digestion	<i>Lactobacillus acidophilus</i>	+ve	<i>Clarias gariepinus</i>
	<i>Vibrio</i> NE 17	+ve	<i>M. rosenbergii</i>
	<i>Lactobacillus helveticus</i>	+ve	<i>Scophthalmus maximus</i>
Improvement of immune response	<i>Clostridium butyricum</i>	+ve	Rainbow trout
	<i>L. casei</i>	+ve	<i>Poecilopsis gracilis</i>
	<i>L. acidophilus</i>	+ve	<i>Paralichthys olivaceus</i>

### III.4 Ammonia oxidizing bacteria:

Nitrifying bacteria were involved nitrification process to convert ammonia tonitrate via nitrite, and then they are applied in ammonia pollutedwater treatment technology especially in aquaculture. Nitrification is widely used to remove ammonia fromwastewater by biological oxidation. Wastewaters containinghigh concentrations of ammonia create environmentalproblems because ammonia may be toxic to aquatic organismsand can cause fertilization of lakes and reservoirswhich leads to algal growth and eutrophication. The overall biochemical process of oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ , then finally to  $\text{NO}_3^-$  is knownas nitrification. Nitrification is performed by the group ofbacteria known as nitrifiers. The nitrifying process takesplace in two steps and each step is carried out by a specificgroup of nitrifying organisms. The two microbes involvedhave been identified in many studies and are the aerobic autotrophic main genera *Nitrosomonas* and *Nitrobacter*. The reactions are as follows:



AOB performs the first step by oxidizing ammonium to nitrite. NOB completes the oxidation by converting the nitrite to nitrate.

Nitrification is a two-step process where ammonia is first oxidized to nitrite by ammonia-oxidizing bacteria and/or archaea, and subsequently to nitrate by nitrite-oxidizing bacteria. Already described by Winogradsky in 1890, this division of labour between the two functional groups is a generally accepted characteristic of the biogeochemical nitrogen cycle. Complete oxidation of ammonia to nitrate in one organism (complete ammonia oxidation) is energetically feasible, and it was postulated that this process could occur under conditions selecting for species with lower growth rates but higher growth yields than canonical ammonia-oxidizing microorganisms. Still, organisms catalyzing this process have not yet been discovered. *Nitrospira* species encodes the enzymes necessary for ammonia oxidation via nitrite to nitrate in their genomes, and indeed completely oxidize ammonium to nitrate to conserve energy. Their ammonia monooxygenase (AMO) enzymes are phylogenetically distinct from currently identified AMOs, rendering recent acquisition by horizontal gene transfer from known ammonia-oxidizing microorganisms. The recognition of a novel amoA sequence group will lead to an improved understanding of the environmental abundance and distribution of ammonia-oxidizing microorganisms.

### III.5 Sulphur oxidizing bacteria

Sulphur-oxidizing bacteria play an important role in the detoxification of sulphide in water and sediments. Sulphur reducing bacteria are anaerobic micro-organisms that are wide spread in anoxic habitats, where they use sulphate as a terminal electron acceptor for the degradation of organic compounds, resulting in the production of sulphide. Subsequently, the sulphide can be oxidized under anoxic conditions by chemolithotrophic sulphur bacteria or under anoxic conditions by phototrophic sulphur bacteria. Sulphur oxidizing chemolithotrophs growth is primarily aerobic, that is, using molecular oxygen as terminal electron acceptor. However, some species (*Beggiatoa* sp., *Thioploca* sp., *Thiobacillus denitrificans*, *Thiomicrospira denitrificans*) oxidize H<sub>2</sub>S and aerobically coupling it to nitrate reduction. The large inputs of organic matter support high rates of heterotrophic metabolism. Since oxygen is usually depleted below a few millimeter depths, even where the sediment surface is exposed to air, anaerobic metabolism

predominates with decomposition mediated primarily by fermentative and sulphate reducing bacteria. Sulphide formed as the product of bacterial sulphate reduction usually undergoes rapid diagenetic transformations in coastal sediments. Hence, microbial sulphur transformation is a key process for the biogeochemical sulphur cycle in marine sediments and closely linked to the cycling of other elements like oxygen, nitrogen, and carbon.

The major processes of transformation involved in the cycling of sulphur in the environment are:

1. Mineralization of organic sulphur to the inorganic form, hydrogen sulphide, H<sub>2</sub>S.
2. Immobilization
3. Oxidation and
4. Reduction

### **III.5.1 Mineralization**

The breakdown/decomposition of large organic sulphur compounds to smaller units and their conversion into inorganic compounds (sulphates) by the microorganisms.

### **III.5.2 Immobilization**

Immobilization involves microbial conversion of inorganic sulphur compounds to organic sulphur compounds. In the process of immobilization, microorganisms absorb inorganic sulphate and convert it into organic form for the synthesis of microbial tissue. If an abundant supply of carbon is available for energy then the entire inorganic sulphate in soil will be converted to organic form, but if little carbon is available then inorganic sulphate will be released from the organic matter.

### **III.5.3 Oxidation**

Sulphate on the reductive side functions as an electron acceptor in metabolic pathways is used by a wide range of microorganisms and is converted to sulphide. Reduced sulphur compounds such as sulphide serve as electron donors for phototrophic or chemolithotrophic bacteria which convert these compounds to elemental sulphur or sulphate. When plant and animal proteins are degraded, sulphur is released and accumulates in the soil which is then oxidized to sulphates in the presence of oxygen and under anaerobic condition organic sulphur is decomposed to produce hydrogen sulphide (H<sub>2</sub>S). H<sub>2</sub>S can also

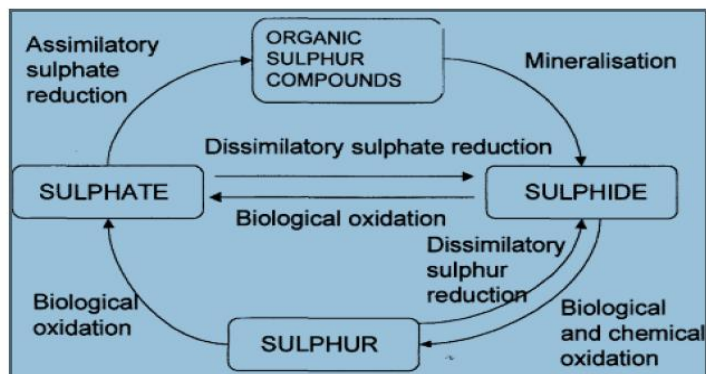
accumulate during the reduction of sulphates under anaerobic conditions which can be further oxidized to sulphates under aerobic conditions.

The biological oxidation of elemental sulphur and inorganic sulphur compounds (such as H<sub>2</sub>S, sulphite and thiosulphate) to sulphate (SO<sub>4</sub>) is brought about by direct and indirect methods. In the direct approach photoautotrophic or chemolithotrophic sulphide oxidizing bacteria use sulphide as an electron donor and convert it to sulphur or sulphate. Photoautotrophs use CO<sub>2</sub> as the terminal electron acceptor, while with chemolithotrophs, oxygen (aerobic species) or nitrate and nitrite (anaerobic species) serve as terminal electron acceptors. In the indirect method oxidation of reduced sulphur compound is carried out chemically by ferric iron as the oxidizing agent, and iron oxidizing bacteria are used to regenerate the ferric iron for further use.

The purple sulphur bacteria encompass many genera such as *Chromatium*, *Thioalkalicoccus*, *Thiorhodococcus*, *Thiocapsa*, *Thiocystis*, *Thiococcus*, *Thiospirillum*, *Thiodictyon*, and *Thiopedia*. The colourless sulphur bacteria encompass many genera such as *Thiobacillus*, *Acidithiobacillus*, *Achromatium*, *Beggiatoa*, *Thiothrix*, *Thioplaca*, *Thiomicrospira*, *Thiosphaera*, and *Thermothrix* etc. *Achromatium*, a spherical sulphur oxidizer, is common in freshwater sediments containing sulphide.

### III.5.4 Reduction

Two physiological types of sulphate reduction are recognized. The first is assimilatory or biosynthetic sulphate reduction in which organisms reduce only enough sulphates to meet their nutritional requirements for sulphur. The assimilatory pathway generates reduced sulphur compounds for biosynthesis of amino acids and proteins. This pathway is considered to be in the pathway for the biosynthesis of cysteine and is usually under both coarse and fine metabolic regulation. The second sequence involved in the reduction of sulphate is the dissimilatory or respiratory pathway of sulphate reduction in which sulphate in the absence of oxygen serves as a terminal electron acceptor for anaerobic respiration. Sulphate can be reduced to hydrogen sulphide (H<sub>2</sub>S) by sulphate reducing bacteria (*Desulfovibrio* and *Desulfatovaculum*) and may diminish the availability of sulphur. This is “dissimilatory sulphate reduction” which is not at all desirable from soil fertility. Dissimilatory sulphate-reduction is favoured by the alkaline and anaerobic conditions of soil and sulphates are reduced to hydrogen sulphide.



**Figure: Sulphur cycle in aquaculture pond**

Knowledge of the precise relationships between water column bacteria and those growing in or on the animals is essential for making informed decisions on management and operational procedures. Further research is needed on the nature of the bacterial populations associated with aquatic animals, both in the wild and in culture, in order to provide a basis for understanding the impact of control measures in hatcheries and ponds and on water released to the natural environment. If we can manipulate the bacterial species composition and in particular control pathogen numbers, larval survival and fitness will be enhanced in hatcheries and grow-out productivity will be increased, and the risk of contaminating natural populations with antibiotic-resistant bacteria will be lowered.

#### **Part IV: FISH AND FISHERY PRODUCTS SAFETY GOALS**

Fish and fishery products safety goals must achieve more than end-product probes. The absence of pathogens in final-product testing does not ensure food free of virulent microorganisms, according to a new expert report on food safety issues, and as pathogen contamination decreases this form of testing becomes more deficient. So as today's food safety continues to improve, more emphasis should be placed on monitoring processing capabilities and conditions through the application of science-based food systems.

The microbiological testing of finished fish and fishery products and can be misleading for the following reasons 1. Due to statistical limitations based on the amount of product sampled,

2. The percentage of product contaminated, and
3. The uniformity of the contamination distributed throughout the food.

The above mentioned negative results imply an absence of pathogens in foods, the report states, and can cause consumers to assume proper food selection and handling practices are unnecessary. Instead, the report urges everyone along the farm-to-fork fish and fishery products chain to be responsible for an important role in food safety management.

According to Douglas L. Archer of the University of Florida who contributed to IFT report “Current safety evaluations focus on microbes that may or may not be harmful to humans,” he added, “For example, some subtypes of *Listeria monocytogenes* found in or on food may not be associated with food borne illness. Yet their mere detection can be grounds for legal action against the manufacturer and force recalls of food that is unlikely to cause illness in the general population.”

The need science-based approach called Food Safety Objectives that would place specific values on public health goals, with reassurances those values are reached at key points along the pond to plate process. Those values would be flexible as hazards and public health goals change, science progresses, and unfettered data sharing improves, allowing for the quickest implementation of new safety improvements as they evolve, and a safer food supply.

The report urges intentional interaction of public health, regulatory, industrial and consumer agencies, calling the implementation of a flexible, science-based approach involving all these parties “as the best weapon against emerging microbiological food safety issues.”

#### **IV.1 Steps in fish and fishery products Safety Management**

Foodborne illness in India is a major and complex problem that is likely to become a greater problem as we become a more global society where every 5<sup>th</sup> person walking on this planet is going to be Indian. Nearly 10 million foodborne illnesses occur per year in India. To adequately address this complex problem, the need is to develop and implement a well-conceived strategic approach that quickly and accurately identifies hazards, ranks the hazards by level of importance, and identifies approaches for microbial control that have the greatest impact on reducing hazards, including strategies to address emerging hazards that were previously unrecognized.

#### **IV.2 Policy Development**

Scientific research has resulted in significant success in improving fish and fishery products safety, but the current science supporting the

safety of our fish and fishery products supply is not sufficient to protect us from all the emerging issues associated with the complexity of the food supply. As new issues emerge, some will be best addressed through the application of control technologies during fish and fishery products production and processing, but others may be best addressed at the consumer level through modification of exposure or susceptibility.

Food safety policies should be developed as part of national initiatives, with input from all stakeholders. In addition, international coordination of food safety efforts should be encouraged. Globalization of the food supply has contributed to changing patterns of food consumption and foodborne illness, and global food trade has the potential to introduce pathogens to new geographic areas.

To achieve the maximum benefits, our food safety efforts and policies must be carefully prioritized, both in terms of research and in application of controls. As scientific advances provide a better picture of pathogenicity, the need of the hour is whether to focus the efforts on those pathogens that cause many cases of minor illness or instead focus on those pathogens with the greatest severity, despite the relatively low number of cases. In the move toward making decisions based on risk, the food safety policies need to weigh these issues, and communicate information about risk to all stakeholders, especially the public.

The body of scientific knowledge must be further developed, with the research efforts carefully prioritized to yield the greatest benefit. Food safety and regulatory policies must be based on science and must be applied in a flexible manner to incorporate new information as it becomes available and to implement new technologies quickly. The fish and fishery products industry, regulatory agencies and allied professionals should develop partnerships to improve food safety management.

## **To conclude**

**IV. 3 Fish and fishery products Supply and exports:** The amount of exported fish and fishery products has increased significantly, and this trend is likely to continue. Consistent, widespread application of food safety systems, including Hazard Analysis and Critical Control Points systems and good manufacturing (GMP), must be encouraged for international trade.

**IV. 4 New Fish and fishery products Processing Technologies and Novel sea foods** Scientists continue to be challenged to adequately address all the parameters associated with the introduction of a novel fish and fishery products or alternative processing technology. Once



developed, new technologies must be appropriately used and regulated to ensure their proper application and the product's safety.

**IV.5 Increases in Organic Foods.** The use of manure as a fish pond fertilization is a significant concern. Methods are needed to reduce the presence of pathogens in manure and to effectively eliminate them before they contaminate the aquatic environment and fish.

**IV.6 Changes in Food Consumption.** People's changing dietary patterns affect their risk of foodborne illness. The control and prevention methods will need to be adapted to these changing dynamics. For example, in India the number of high end consumers who prefer ready to eat foods are more than 300 million which is more or less equivalent to Europe

**IV.7 At-Risk populations.** It is likely that the number of persons at higher risk for foodborne disease will continue to increase with time. The population of India is going to be 150 crores. In addition, there are an increasing number of transplant recipients, people undergoing treatment for cancer, people with AIDS, and others with compromised immune system function.

**IV.8 Pathogen Evolution.** Microbial evolution has always happened and will continue to occur. Improved surveillance and new genomic technologies offer the potential to identify new potential foodborne pathogens before they cause significant illness. Another hope for the future is a better understanding of how human actions affect foodborne pathogens.

**IV.9 Consumer Understanding.** Education and risk communication will be necessary to share with consumers our growing knowledge of food safety risks and to encourage behavior modification, where needed.

**Integrated Food Safety System.** A farm to- fork or pond to plate table food safety system must involve many interested parties working together toward a common goal. The challenge is to build a system that applies science in a predictable, consistent, and transparent manner to enable harmonization within and between countries.

### **Further reading**

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