Subject: Nutrition/4th Semester (CC9T)/Ch-6

Paper: Food Microbiology

Topic Name: Assessing the microbiological Quality of food

(**Indicator Organisms:** Definition, its types and application)

Indicator Organism

Defination: **Indicator organisms** are used as a proxy to monitor conditions in a particular environment, ecosystem, area, habitat, or consumer product. Certain bacteria, fungi and helminth eggs are being used for various purposes. Indicator organisms are microorganisms whose presence in water indicates probable presence of pathogens (disease-causing organisms). Ideally, such microorganisms are nonpathogenic, occur consistently in pathogen-contaminated water, do not multiply in waters, are reliably detectable even at low concentrations, and are present in greater numbers than and have similar survival times to pathogens.

Types

1. Indicator bacteria

Certain bacteria can be used as indicator organisms in particular situations. The presence of bacteria commonly found in human feces, termed **coliform bacteria (e.g. E. coli**), in surface water is a common indicator of faecal contamination. For this reason, sanitation programs often test water for the presence of these organisms to ensure that drinking water systems are not contaminated with feces. This testing can be done using several methods which generally involve taking samples of water, or passing large amounts of water through a filter to sample bacteria, then testing to see if bacteria from that water grow on selective media such as **MacConkey agar**. Alternatively, the sample can be tested to see if it utilizes various nutrients in ways characteristic of coliform bacteria.

Coliform bacteria selected as indicators of faecal contamination must not persist in the environment for long periods of time following efflux from the intestine, and their presence must be closely correlated with contamination by other faecal organisms. Indicator organisms need not be pathogenic

Non-coliform bacteria, such as **Streptococcus bovis and certain clostridia** may also be used as an index of faecal contamination.

2. Indicator fungi

Penicillium species, Aspergillus niger and Candida albicans are used in the pharmaceutical industry for microbial limit testing, bioburden assessment, method validation, antimicrobial challenge tests, and quality control testing. When used in this capacity, **Penicillium and A. niger are compendial mold indicator organisms.**

Molds such as Trichoderma, Exophiala, Stachybotrys, Aspergillus fumigatus, Aspergillus versicolor, Phialophora, Fusarium, Ulocladium and certain yeasts are used as indicators of indoor air quality.

3. Indicator helminth eggs

Helminth eggs (or ova) are a good indicator organism to assess the safety of sanitation and wastewater reuse systems for resource recovery because they are the most environmentally resistant pathogens of all pathogens (viruses, bacteria, protozoa and helminths) and can in extreme cases survive for several years in soil.

Characteristics of an ideal indicator

An Indicator should:

1. Be present whenever the pathogens are present
2. Be present only when the presence of pathogens is an imminent danger (i.e., they must not proliferate to any greater extent in the aqueous environment)
3. Occur in much greater numbers than the pathogens;
4. Be more resistant to disinfectants and to the aqueous environment than the pathogens
5. Grow readily on simple media
6. Yield characteristic and simple reactions enabling as far as possible an unambiguous identification of the group
7. Be randomly distributed in the sample to be examined, or it should be possible to obtain a uniform distribution by simple homogenization procedures; and
8. Grow widely independent of other organisms present, when inculcated in artificial media (i.e., indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria).

Importances or Applications of Indicator Organism

1. One of the most frequent applications of indicators is in public health warning systems. Warning systems include measurement of indicators to assess whether there is a likelihood that pathogenic microorganisms are present at unacceptable risk levels. Warning systems may be related to ingestion of treated drinking water, recreational water contact, or shellfish consumption.

2. Risk levels are codified through enforceable standards, which may be based on a single sample maximum level, an average or median concentration for a specified period of time, or a maximum frequency of samples over a threshold. When a standard is exceeded, actions are taken to reduce exposure, such as increased treatment levels for drinking water, shellfish bed closures, or warnings to avoid recreational water contact.

3. Drinking water warning systems typically focus on treatment adequacy and integrity of the distribution system, rather than on source water quality.

4. There is zero tolerance for fecal coliforms or Escherichia coli in treated drinking water, the presence of which is considered compelling evidence of unacceptable health risk requiring immediate action. However, background levels of microorganisms from natural sources have to be accounted for in monitoring ambient water systems.

5. Sampling frequency is higher and is typically linked to the size of the population served. For example, water supplies serving 50,000 people typically test 2 samples a day, whereas water supplies serving as many as 2.5 million people typically test 420 samples a month, or about 14 samples per day. In contrast, weekly to monthly sampling is typical for ambient recreational waters.

6. Drinking water systems make greater use of rapid real-time physical and chemical surrogates than recreational water systems, such as turbidity and chlorine residual and maintenance of a positive distribution system pressure. This is because they focus on treatment effectiveness as barriers against contamination rather than on natural variability in input sources.

7. Assessment and Coastal Health (BEACH) Act of 2000 was to bring consistency to beach assessments; however, differences between the states continue based on the various approaches for setting standards and their use in closing impaired beaches. Indicator Organisms help in the sectors.

Some Key Indicator Organisms

Coliforms: Gram-negative, non spore-forming, oxidase-negative, rod-shaped facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and gas within 24– 48h at 36±2°C. Not specific indicators of faecal pollution.

Thermotolerant coliforms: Coliforms that produce acid and gas from lactose at 44.5± 0.2°C within 24±2h, also known as faecal coliforms due to their role as faecal indicators.

Escherichia coli (E. coli): Thermophilic coliforms that produce indole from tryptophan, but also defined now as coliforms able to produce β-glucuronidase (although taxonomically up to 10% of environmental E. coli may not). Most appropriate group of coliforms to indicate faecal pollution from warm-blooded animals.

Faecal streptococci (FS): Gram-positive, catalase-negative cocci from selective media (e.g. azide dextrose broth or m Enterococcus agar) that grow on bile aesculin agar and at 45°C, belonging to the genera Enterococcus and Streptococcus possessing the Lancefield group D antigen.

Enterococci: All faecal streptococci that grow at pH 9.6, 10° and 45°C and in 6.5% NaCl. Nearly all are members of the genus Enterococcus, and also fulfil the following criteria: resistance to 60°C for 30 min and ability to reduce 0.1% methylene blue. The enterococci are a subset of faecal streptococci that grow under the conditions outlined above.

Sulphite-reducing clostridia (SRC): Gram-positive, spore-forming, non-motile, strictly anaerobic rods that reduce sulphite to H2S.

Clostridium perfringens: As for SRC, but also ferment lactose, sucrose and inositol with the production of gas, produce a stormy clot fermentation with milk, reduce nitrate, hydrolyse gelatin and produce lecithinase and acid phosphatase.

Bifidobacteria: Obligately anaerobic, non-acid-fast, non-spore-forming, non-motile, Gram-positive bacilli which are highly pleomorphic and may exhibit branching bulbs (bifids), clubs, coccoid, coryneform, Y and V forms. They are all catalase-negative and ferment lactose (except the three insect species; B. asteroides, B. indicum and B. coryneforme) and one of the most numerous groups of bacteria in the faeces of warmblooded animals.

Bacteriophages (phages): These are bacterial viruses and are ubiquitous in the environment. For water quality testing and to model human enteric viruses, most interest in somatic coliphages, male-specific RNA coliphages (F-RNA coliphages) and phages infecting Bacteroides fragilis.

Coliphages: Somatic coliphages attack E. coli strains via the cell wall and include spherical phages of the family Microviridae and various tailed phages in 3 families. The F-RNA coliphages attack E. coli strains via the sex pili (F factor) and are single-stranded RNA non-tailed phages.

Bacteroides fragilis bacteriophages: These infect one of the most abundant bacteria in the gut, belong to the family Siphoviridae with flexible tail (dsDNA, long non-contractile tails, capsids up to 60 nm). Phages to the host strain, B. fragilis HSP40 are considered to be human-specific, but phages to B. fragilis RYC2056 are more numerous and not human-specific.