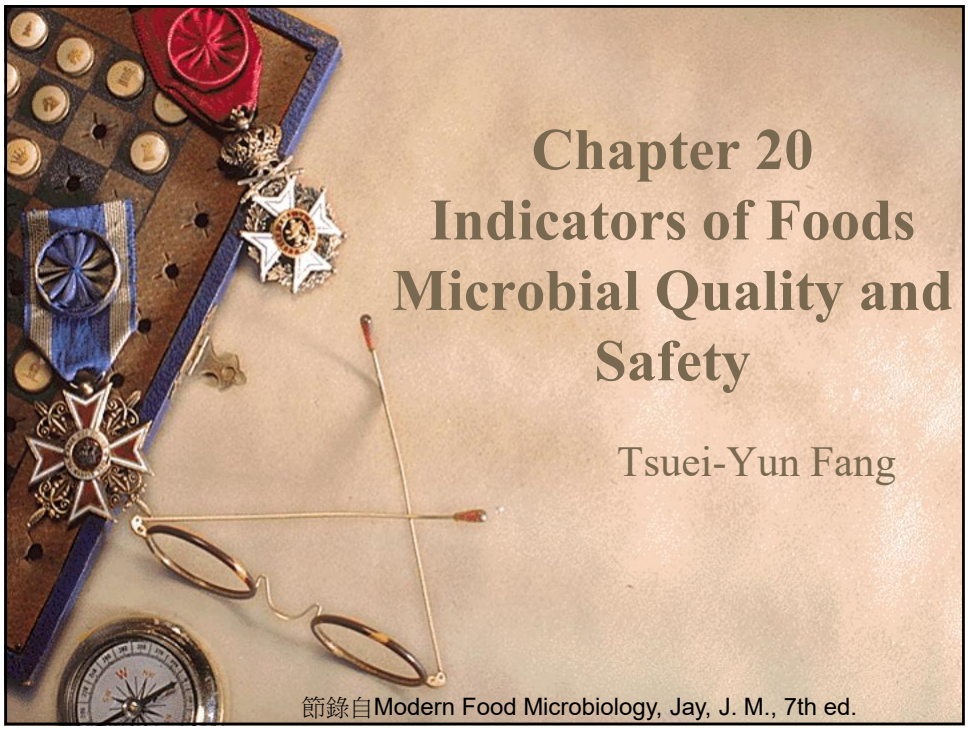


Introduction

- **Indicator organisms:**
  - reflect the **microbiological quality** of foods
    - **shelf life**
    - **safety**
- In general, indicators are most often used to assess **food safety/sanitation**.

INDICATORS OF PRODUCT QUALITY

Organisms and/or their metabolic products

Indicator organisms should meet the following criteria:

1. **present and detectable** in all given foods to be evaluated.
2. their growth and numbers should have a **direct negative correlation** with product quality.
3. should be **easily detected and enumerated** and be **clearly distinguishable** from other organisms.
4. should be **enumerable in a short period of time**, ideally within a working day.
5. Their **growth should not be affected adversely** by other components of the food microbiota

INDICATORS OF PRODUCT QUALITY

- **Metabolic products** may be used to assess and predict **microbial quality** in some products.
  - **negative predictor**
  - **e.g. diacetyl, ethanol, histamine, lactic acid**
- Where product quality is significantly affected by the presence and quantity of certain metabolic products, they may be used as **quality indicators**.

INDICATORS OF FOOD SAFETY

Microbial indicators are employed more often to assess **food safety** and **sanitation** than quality.

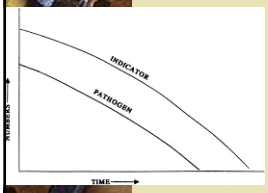
Ideally, a food safety indicator should meet certain important criteria. It should

1. **be easily and rapidly detectable**
2. **be easily distinguishable** from other members of the food biota (生物群)
3. have a history of **constant association with the pathogen** of concern
4. always be **present when the pathogen of concern is present**.

Ideally, a food safety indicator should meet certain important criteria. It should

5. be an organism whose **numbers** ideally should **correlate with those of the pathogen** of concern

6. possess **growth requirements** and a **growth rate** equaling those of the pathogen



7. have a **die-off rate** that at least parallels that of the pathogen and ideally persists slightly longer than the pathogen of concern

8. **be absent from foods that are free of the pathogen** except perhaps at certain minimum numbers

INDICATORS OF FOOD SAFETY

In the historical use of **safety indicators**, however, the pathogens of concern were assumed to be of **intestinal origin**, resulting from either direct or indirect **fecal contamination**.

Sanitary indicators were used to detect **fecal contamination of waters** and the possible **presence of intestinal pathogens**.

INDICATORS OF FOOD SAFETY

The **first fecal indicator** was

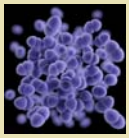
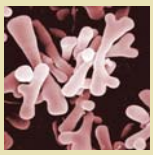
Escherichia coli \ˌesh-ə-ˈrik-ē-ə\\ˈkō-ˌlī\

When the concept of **fecal indicators** was applied to food safety, criteria were

1. Ideally the bacteria selected should demonstrate **specificity ➔ occurring only in intestinal environments**.
2. They should occur in **very high numbers in feces**.
3. They should possess a **high resistance to the extraenteral (腸道外) environment** ➔pollution can be assessed
4. They should permit relatively **easy and fully reliable detection** ➔even in very low numbers.

INDICATORS OF FOOD SAFETY

Following the practice of employing ***E. coli* as an indicator of fecal pollution of waters**, other organisms were also suggested for **food sanitary indicator**

- **1. Coliforms**
- **2. Enterococci**
- **3. Bifidobacteria**
- **4. Coliphages/Enteroviruses**


Coliforms (大腸菌群)

Strains

- coliforms are **gram-negative asporogenous (**不產孢子的**) rods** that ferment lactose within 48 h
- coliforms are represented by **four** genera of the family **Enterobacteriaceae** (腸內細菌科): ***Citrobacter*** (檸檬酸桿菌屬)**,** ***Enterobacter*** (腸桿菌屬)**,** ***Escherichia*** (大腸桿菌屬)**,** and ***Klebsiella*** (克萊桿菌屬).

Coliforms

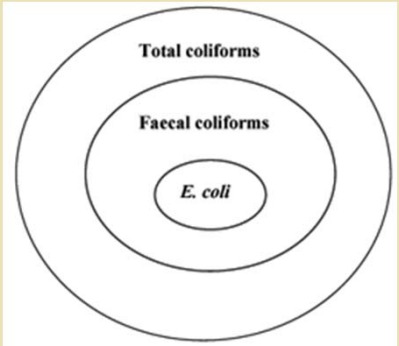
The **IMViC** formula is the classical method used to determine the coliform population

- I = indole production (the presence of tryptophanase)
  orange-yellow ➔ pink
- M = methyl red reaction
  yellow ➔ red (acid production)
- V = Voges-Proskauer reaction
  colorless ➔ pink (production of acetoin, the precursor of 2,3-butanediol)
- C = citrate utilization green ➔ blue

Bacteria	I	M	V	C
E. coli type I	+	+	-	-
E. coli type II	-	+	-	-
E. aerogenes	-	-	+	+
Citrobacter	±	+	-	+
K. pneumoniae	±	- (+)	+	+

Coliforms

- **Fecal coliforms** are defined by the **production of acid and gas in EC broth between 44˚C and 46˚C**, usually 44.5˚C or 45.5˚C (EC broth, for *E. coli* growth).



EC broth
bile salts, 1.5 g/L
casein digest (pancreatic), 20 g/L
dipotassium phosphate, 4 g/L
lactose, 5 g/L
potassium phosphate, 1.5 g/L
sodium chloride, 5 g/L

Coliforms

- ***Growth***
- 1) like most other nonpathogenic G(-) bacteria, coliforms grow well on many media and in many foods.
- 2) grow at **-2˚C ~ 50˚C**. In foods, growth is poor or very slow at 5˚C
- 3) grow over a pH range of **4.4-9.0**.
- 4) grow on **minimal medium** ➔ carbohydrate (e.g. glucose) + inorganic nitrogen (e.g. NH_4SO_4)
- 5) grow well on nutrient agar and produce visible colonies within 12-16 h at 37˚C.

nutrient agar ingredients (g/L)
Peptic digest of animal tissue 5.00
Beef extract 3.00
Agar 15.00

Coliforms

- ***Growth***
- 6) grow in the presence **of bile salts**, which inhibit the growth of G(+) bacteria ➔ selective isolation
- 7) **Ferment lactose** with production of **gas**
- 8) the attractive properties of ***E. coli* as a fecal indicator for water**➔*E. coli* generally dies off about the same time as the more common intestinal bacterial pathogens
- 9) However, various pathogens may persist after *E. coli* is destroyed in treated water and in foods that are **frozen, refrigerated, or irradiation**.

Coliforms

Detection and Enumeration

- Many methods have been developed

Distribution

- 1) *Escherichi coli* ➔ the **intestinal tract** of most warm-blooded animals
- 2) *Enterobacter aerogenes* ➔ **vegetation** and occasionally the intestinal tract.
- 3) Coliforms are also present in dust and air, on hands, and in and on many foods.

Coliforms

Coliform Criteria and Standards

1) It is virtually **impossible to eliminate all coliforms** from fresh and frozen foods.

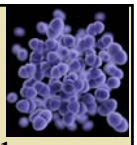
2) Low numbers of coliforms are permitted in some foods ➔ **1 ~100/g or 100/mL.**

Coliforms

Some Limitations for Food Safety Use

- **Coliform tests are not good for**
  - i) **frozen blanched vegetables** ➔ ***Enterobacter*** have common associations with vegetation
  - ii) **poultry products** ➔ **salmonellae** may exist in a flock (毛髮) prior to slaughter
  - iii) **meats** ➔ the widespread occurrence of psychrotrophic enterics and ***Aeromonas* spp.** **(產氣單胞菌)** in meat environments

Enterococci



- Features of the classical enterococci that led to their use as **pollution indicators for water** are the following:
  1. **do not multiply in water**, especially if the organic matter content is low.
  2. generally **less numerous in human feces than *E. coli***, with ratios of fecal coliforms to enterococci of 4.0 or higher. The classical enterococcal tests presumably reflect **more closely the numbers of intestinal panthogens** than fecal coliforms.
  3. The enterococci **die off at a slower rate** than coliforms in waters and thus would normally outlive the pathogens.

Enterococci

Growth Requirements

1) Most of the enterococci **grow at 45˚C** and some, at least ***E. faecalis*** **and** ***E. faecium*****, grow at 50˚C.**

2) Some species grow at a pH of 9.6 and in 40% bile (coliforms pH 4.4-9.0).

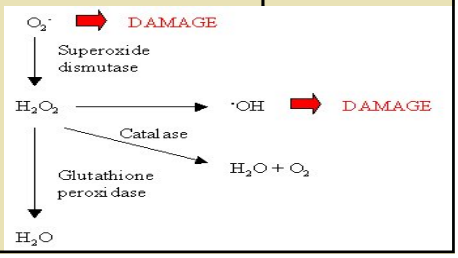
3) enterococci are more fastidious in having nutritional requirements for more growth factors, especially **B vitamins and certain amino acids**.

Enterococci

Growth Requirements

4) They **grow over a much wider range of pH** than all other foodborne bacteria.

5) Although they are **aerobes**, they **do not produce catalase** (except a pseudocatalase by some strains when grown in the presence of O_2), and they are **microaerophiles** that grow well under conditions of low oxidation-reduction potential (Eh).



Enterococci

100% of human and pig feces samples contained enterococci, whereas only 86-89% contained coliforms

Distribution

- 1) ***E. faecalis*** and ***E. faecium*** are primarily of **fecal origin**.
- 2) Classical enterococci also exist on **plants** and **insects** and in **soils**. In general, enterococci on insects and plants may be from animal fecal matter.

Enterococci

Distribution

- 3) Enterococci may also be found in **dust**. They are rather widely distributed, especially in such places as **slaughterhouses** and **curing rooms**, where pork products are handled.

Enterococci

Relationship to Sanitary Quality of Foods

1) Classical enterococci **are better than coliforms** as indicators of food sanitary quality, **especially for frozen foods**.
2) In a study of frozen vegetables, **coliforms were more efficient indicators of sanitation than enterococci prior to freezing**, whereas **enterococci were superior indicators after freezing and storage.**
3) Interest in the enterococci as food safety indicators has clearly decreased, probably because of **the interest in faster and more efficient ways to detect and enumerate *E. coli*.**

Bifidobacteria

a) The **common occurrence of the bifidobacteria in stools** suggests the use of these G(+) anaerobic bacteria as **indicators of fecal pollution, especially of waters**.

b) Some bifidobacteria are employed in the production of fermented milks, yogurt, and other food products, and some are believed to provide some health benefits.

Bifidobacteria

c) The genus *Bifidobacterium* consists of at least 25 species of **catalase-negative**, **nonmotile rods** whose minimum and maximum growth temperature ranges are **25˚ to 28˚C** and **43˚ to 45˚C**, respectively.

d) They grow best in the **pH range 5 to 8** and **produce lactic and acetic acids** as the major end products of their carbohydrate metabolism.

Bifidobacteria

Distribution

- 1) The concentration of bifidobacteria (10^8-10^9/g) has been reported to **be higher than *E. coli*** (10^6-10^7/g) in human feces.
- 2) die-off rates: **bifidobacteria > coliforms > enterococci.**
- 3) They are strict anaerobes, they tend to grow slowly and require several days for results.

Bifidobacteria

Distribution

4) The **close association of bifidobacteria with feces**, their **absence where fecal matter does not occur**, their **lack of growth in water**, and the **specific association of some only with human feces** makes these bacteria attractive as **pollution indicators**.

5) As they are more likely to grow in meat and seafood products than in vegetables, **it is possible that they could serve as indicators for meats and seafood.**

Coliphages /Enterovirus

a) Bacteriophages occur in waters in association with their host bacteria ➔ phages specific for several intestinal pathogens could be measured **as indirect indicators of their host bacterial species**.

b) A coliphage assay procedure for water samples that contain **five or more phages/100 ml** and that can be completed in 4-6 h.

c) There is no way of enumerating all *E. coli* phages or all phages of any other specific bacterium, suggesting the use of **mixed indicators** for best results.

Coliphages /Enterovirus

Utility for Water

1) Coliphages may be uesd as **indicators of enteroviruses**, especially in water. The survival of coliphages in water has been shown to parallel that of human enteric viruses.
2) Because some coliphages have their natural habitat in environmental waters, their numbers **may not correlate directly with fecal pollution**.
3) Human enteric viruses survive better in water than coliforms and are more resistant to destruction by chlorine.

Coliphages /Enterovirus

Utility for Foods

1) High coliphage levels in general reflected products that contained high fecal coliform levels.
2) Coliphages appear to **correlate better with enteroviruses than coliforms**.
3) Coliphages correlated better with *E. coli* and fecal coliforms than total coliforms. Results could be achieved in 4-6 h.
4) Coliphage assays may be suitable either as **an alternative for *E. coli* or coliform determinations** or **as direct indicators for enteroviruses**.

PREDICTIVE MICROBIOLOGY/MICROBIAL MODELING

1) **The presence/absence of indicator organisms** is used **to predict food safety**. If a safety indicator is absent, the product is regarded as being safe relative to the hazard for which the indicator is used.
2) A product can have **extremely low numbers of a safety indicator** and yet not pose a hazard.
3) When low numbers of an indicator or pathogen are present, it is important to know how either will behave in a food product over time.

PREDICTIVE MICROBIOLOGY/MICROBIAL MODELING

4) Microbial modeling or predictive microbiology requires the use of **mathematical models/equations** to predict the growth and/or activity of a microorganism in a food product over time.
5) The effective application of predictive microbiology requires the **selection of appropriate models** to reflect the effect of growth parameters. Computer software packages for predictive microbiology are available from private and commercial sources.