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## Dairy Foods Science Notes

### Coliform Bacteria – Indicators in Food & Water

#### Definition & General Description:

Coliform bacteria are most often defined as “aerobic and facultatively anaerobic, gram negative, non-spore-forming rods that ferment lactose with the production of acid and gas within 48 hours at 35°C (32-37°C).” Coliform bacteria are organisms detected by “coliform tests” or selective differential growth media based on this definition:

*Aerobic & facultatively anaerobic:* require oxygen for growth or can grow without oxygen.

- coliform procedures are performed in aerobic or reduced oxygen conditions (e.g., agar overlay)

*Gram negative, non-spore-forming:* stain red with the gram stain, based on cell wall composition

- media/tests use ingredients & conditions that prevent growth of gram positive bacteria & spores

*Ferment lactose with the production of acid and gas:*

- lactose is the only carbon source; media/tests detect acid (e.g., pH color indicators) & gas

A variety of bacteriological media are used to detect coliform in water and foods, including violet red bile agar, m-endo agar/broth, lauryl tryptose broth and brilliant green bile broth. Most contain lactose as the primary fermentable sugar. For some procedures, such as with violet red bile agar, gas production of “presumptive” coliform colonies must be “confirmed” by transfer to a fermentation tube (i.e., brilliant green bile broth with inverted gas tube). Coliform bacteria generally belong to four genera of the Enterobacteriaceae family; *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*. Not all strains of these four genera meet the coliform definition, while a few bacterial strains outside these genera will (e.g., *Aeromonas* spp). *Escherichia coli* is the most well known coliform. *E. coli* is a common resident of the intestines of warm blooded animals and is associated with fecal matter. Not all coliform are associated with the intestinal tract; some strains occur widely in nature (e.g., *Enterobacter* spp. associated with plant material), while some “fecal coliforms” may flourish in diverse environments outside the intestinal tract.

*Fecal Coliforms:* Some strains of coliform bacteria can be further classified as “fecal coliforms,” which are defined as coliform bacteria that can grow at 44.5 – 45.5°C and produce acid and gas from lactose within 48 hrs. Nearly all strains of *E. coli* meet this definition (*E. coli* O157:H7, a pathogenic strain, does not grow and/or produce acid and gas at 44-45.5°C). Other coliforms that meet this definition include strains of *Klebsiella pneumoniae*, *Citrobacter freundii*, and certain *Enterobacter* spp. (*E. agglomerans*, *E. aerogenes* & *E. cloacae*).

#### Indicator Organisms & Coliform Bacteria:

Indicator organisms are organisms that provide insight to the history of a sample or to potential associations with other organisms or conditions (e.g., they can indicate the potential presence of pathogens or spoilage organisms). Coliform bacteria have been used as indicators of unsanitary conditions in water and foods for over a century. This concept originated in the late 1800’s after *E. coli* was found to be ubiquitous to feces, and it’s detection in water was used to “indicate” an increased likelihood that pathogens such as *Salmonella typhi* (typhoid fever) were in the water as well (i.e., an indicator of unsanitary conditions). Indicators have been applied to both food and water safety and quality. For indicator organisms to be of value, they should be:

- a. Easily distinguishable from other microorganisms common to a sample.
- b. Easily detected & enumerated in a relatively short period of time (e.g., rapid tests).
- c. Directly or indirectly association with reduced safety or loss of quality.
- d. Able to survive as well as the associated organism(s) in the water/food being tested.

*Indicators of product safety* are generally associated with common pathogen(s) that originate from similar environments (e.g., intestinal pathogens) and are also able to survive in foods as well as the pathogen.

*Indicators of fecal contamination* should a) be organisms specific to the intestinal tract, b) be present in high enough numbers in feces to be detected easily in water/foods after dilution, c) have high survival rates in the test product and d) be easily detected, even in very low numbers. *E. coli* meets these criteria.

*Indicators of product quality* are generally product specific, depending on the typical spoilage organisms of a food (e.g., molds found in cultured milk products) though general sanitation indicators are often used. One of the most common applications of coliform bacteria as indicator organisms is in their association with hygienic conditions and overall quality, especially concerning heat processed foods. Coliforms at normal levels found in foods are killed by most heat processing conditions (e.g., pasteurization of milk); therefore their presence in a food generally indicates an inadequate heat process or post-processing contamination.

### Coliforms and Foodborne Illness:

Most coliform bacteria are not associated with foodborne illness. Harmless strains of *E. coli* and other coliforms reside in the intestinal tract. However, there are certain coliforms that do cause illness, most of which are strains of *E. coli* (others may include opportunist strains of *Enterobacter sakazakii* and *Citrobacter freundii*):

*Enterohemorrhagic E. coli* (EHEC) – includes O157:H7 as well as others (e.g., O6:NM, O104:H21, O111:NM)

- produce Shiga-like toxins (verotoxins, verocytotoxins), affect primarily the large intestines
- Hemorrhagic Colitis – bloody diarrhea, severe abdominal cramps (nausea, vomiting), fever rare
- Hemolytic Uremic Syndrome (HUS) – toxins in blood, kidney disease; young & old at risk (can be fatal)

*Enteroinvasive E. coli* (EIEC) – multiplies inside intestinal (colon) epithelial cells, spreads to adjacent cells

- no enterotoxins, bloody or non-bloody diarrhea (large amounts) caused by cell damage

*Enteropathogenic E. coli* (EPEC) – adheres to intestinal mucosa, destroying or modifying cells

- no enterotoxins, diarrhea, most common in children under 1 yr old

*Enterotoxigenic E. coli* (ETEC) – attaches to and colonizes small intestines

- traveler's diarrhea in young and adults; sudden, acute, non-bloody, very watery
- may be severe, often fatal in infants in developing countries

### Alternative Testing Procedures:

Methods have been developed based on detecting enzymes that are specific for coliform bacteria and for *E. coli*. These methods have been applied in rapid screening and confirmation procedures for food and water:

- a. Detection of Beta-galactosidase using synthetic substrates (e.g., chromogenic substrate = yellow):
  - Colilert, Colisure, E\*Colite and others used for water testing
- b. Detection of Beta-Glucuronidase ( $\beta$ -GUD-based *E. coli* tests):
  - $\beta$ -GUD commonly produced by *E. coli* (92-99% of strains tested; not produced by O157:H7)
  - Variety of  $\beta$ -D-glucuronic acid based substrates; reaction occurs only if  $\beta$ -GUD *E. coli* is present

Fluorogenic MUG:

4-methylumbelliferyl- $\beta$ -D-glucuronic acid

↓  $\beta$ -GUD

4-methylumbelliferone

(blue fluorescent by-product, UV 365 nm)

X-GLUC:

5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronic acid

↓  $\beta$ -GUD

5-bromo-4-chloro-3-indolyl blue

BLUE COLONIES

### References:

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- FDA/CFSAN Bad Bug Book available at:  
<http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm>