

## Isolation and identification of faecal streptococci from fish and fishery products

Streptococci are Gram-positive, non-motile, non-sporeforming, facultatively anaerobic and catalase-negative cocci. They are important human pathogens and grow only in media containing fermentable carbohydrates or enriched with blood or serum. They form circular, translucent to opaque, pinpoint colonies on solid media. The primary habitats of the faecal streptococci are the intestinal tract of human beings, animals and birds and their presence in foods including seafoods indicates faecal contamination of the foods similar to *E. coli*. Contamination of foods with these organisms takes place through the use of contaminated water and unclean workers. Since they are resistant to low and high temperature processing treatments, they are considered as the good indicators of faecal contamination in processed food products.

### Materials required

- K.F. (Kenner Faecal) Agar
- 1% 2,3,5-Triphenyl Tetrazolium Chloride (TTC)
- Physiological Saline
- Blood Agar
- Glucose-Phosphate Medium
- Sorbitol
- Trehalose
- Lactose
- Maltose
- Mannitol
- Dextrin
- Hydrogen peroxide
- Nitrate broth
- Gelatin agar

### Procedure

About 25 g of fish sample is taken aseptically and homogenized with 225 ml of physiological saline. 0.1 ml of appropriate dilution of homogenized sample is spread plated onto KF agar plates and incubated at  $37 \pm 1^\circ\text{C}$  for 48 h. The colonies of faecal streptococci on KF agar appearing as red pinpoint colonies counted and results are expressed as number of colony forming units per g of the sample.

### Calculation

Faecal Streptococci per g = (Number of colonies x dilution factor x 10) / Weight of sample

Typical colonies may be isolated, purified, subcultured onto TSA plates and further subjected to the following biochemical reactions for confirmation. The typical reactions characteristic of faecal streptococci are as follows;

Tests	Reactions	Results
Catalase	No effervescence	Negative
V.P	No change in colour	Negative
Gelatinase	Not liquefied	Negative
Haemolysis (Blood agar)	Clear zone	Positive
Nitrate	Not reduced	Negative
Sorbitol	Fermentative	Positive
Trehalose	Fermentative	Positive
Lactose	Fermentative	Positive
Maltose	Fermentative	Positive
Dextrin	Fermentative	Positive
Mannitol	Fermentative	Positive

### Media Composition

*Kenner Faecal (KF) Streptococcal Agar*

Protease peptone	- 10.0 g
Yeast extract	- 10.0 g
Sodium chloride	- 5.0 g
Sodium glycerophosphate	- 10.0 g
Maltose	- 20.0 g
Lactose	- 1.0 g
Sodium azide	- 0.4 g
Sodium carbonate	- 0.63 g
Agar	- 20.0 g
Distilled water	- 1000.0 ml
Bromocresol purple	- 0.015 g
pH	- 7.2 ± 0.2

Sterilize at 121°C for 15 minutes. Cool to 50°C and add 10 ml of 1% of 2,3,5 – Triphenyl tetrazolium chloride (TTC) and pour onto sterile plates.