

Enumeration and identification of coagulase-positive *Staphylococcus aureus* from seafoods

Staphylococcus aureus are Gram-positive, non-motile, non-spore forming, facultatively anaerobic, catalase-positive cocci belonging to the family Micrococcaceae. Though ubiquitous in nature, it is commonly present in human beings and animals with about 10 to 30% of healthy people carrying these organisms in the throat and nose. They are among the most resistant of non-spore forming bacteria and have ability to survive low temperatures and also withstand temperature of 60°C for 30 minutes. Since they are normally present in humans, they are considered as indicators of personnel hygiene. Coagulase-positive *S. aureus* are more important as food poisoning organisms and are highly pathogenic to humans. They produce toxin enterotoxin which is stable even at 100°C. The maximum allowable number of *S. aureus* in fish and fishery products is 100 /g.

Materials required

1. Baird Parker (BP) medium
2. Egg yolk emulsion
3. 3.5% Potassium tellurite
4. 0.85% Saline diluent
5. Human or rabbit plasma

Procedure

About 25 g of fish sample is taken aseptically and homogenised with 225 ml of 0.85% saline diluent. 0.1 ml of the inoculum is plated onto the Baird Parker (BP) agar plates and spread using sterile bent glass rods. The plates are incubated at 37°C for 24 to 48 hours. *Staphylococcus aureus* appear as black, convex colonies of 1.0 to 1.5 mm diameter having a narrow white entire margin which is surrounded by a mark of clearing of 2 to 5 mm in width. Colonies may produce wide, opaque zones extending into the cleared medium on longer incubation. Coagulase-negative staphylococci occasionally grow without any clear zone around the colony. This opaque zone is an indication of the presence of coagulase. The positive colonies are counted, calculated and the results are expressed as number of colony forming units per gram of sample.

Calculation

$No. \text{ of } Staphylococcus \text{ aureus/g} = (\text{No. of typical colonies} \times \text{dilution factor} \times 10) / \text{Weight of the sample}$

Suspected typical colonies are picked, purified and streaked onto trypticase soya agar plates, incubated at 37°C for 24 hours and confirmed by microscopic examination and by testing for the presence of coagulase enzyme using slide or tube technique

Media composition

Baird Parker (BP) Medium

Tryptone	-10.0 g
Beef Extract	-5.0 g
Yeast Extract	-1.0 g
Sodium pyruvate	-10.0 g
Glycine	-12.0 g
Lithium chloride	-5.0 g
Agar	-20.0 g
Distilled water	-1000.0 ml
pH	-7.0 ± 0.2

Sterilize at 121°C for 15 minutes. Cool to 50°C and add aseptically 50 ml concentrated egg yolk emulsion and 3 ml sterile 3.5% potassium tellurite solution. Mix well before pouring into plates. Use the plates within 24 hours of preparation. Baird Parker agar medium is a highly selective medium for the isolation of *Staphylococcus aureus* from foods. This selectivity is due to the presence of sodium pyruvate and potassium tellurite. *Staphylococcus aureus* produce two diagnostic characteristics on the opaque medium. Firstly, they may produce clear zones by lypolysis or proteolysis. Secondly, opaque zones may appear within the clear zones, probably caused by lipase or lecithinase. They form black

colonies on this medium, as they reduce potassium tellurite to telluride.

- Egg yolk emulsion
- 3.5% Potassium tellurite
- 0.85% Saline diluent
- Human or rabbit plasma