

## **Detection and Determination of Pathogenic *Vibrios* in Foods.**

### **Equipment:**

Refer to lab1. (Equipment, Materials and Glassware).

### **Culture media:**

#### **Culture Medium:**

- Thiosulphate Citrate Bile Salts Sucrose Agar (**TCBS**) .
- Gelatin Phosphate Salt Broth and Agar (**GPS**) .
- Kligler Iron Agar(**KIA**) .
- T1 N1 Agar.

### **Procedure:**

#### **Enrichment**

Weigh 25g sample and transfer to 225ml of **GPS broth**.

Incubate at 35 C for 6 to 8 h.

#### **Plating:**

Prepare dried plates of TCBS and GPS agar medium.

Transfer a loopful of the surface growth of the broth culture to the surface of the two plating medium and streak in a manner that will yield isolated colonies.

Incubate plating medium for 18 to 24 h at 35 C.

#### **Interpretation:**

Typical colonies of *V.cholerae* on TCBS agar are large (2 to 3 mm in diameter) smooth, yellow (occasional slow sucrose fermentors are green), and slightly flattened with opaque centers and translucent peripheries. On GPS agar the colonies

have a cloudy zone around them that becomes more definite after a few minutes of refrigeration. In oblique light, the colonies appear iridescent green to bronze colored and finely granular.

Typical colonies of *V. parahaemolyticus* on TCBS agar appear round, opaque, green or bluish colonies, 2 to 3 mm in diameter.

### **Confirmation:**

Subculture all suspect colonies of *V. cholerae* on to T<sub>1</sub>N<sub>1</sub> agar and incubate at 35 C for 24h. Stab streak a KIA slant with the culture and incubate the KIA slant overnight at 35 C. *V. cholerae* cultures have an alkaline (red) slant and an acid (yellow) butt, no gas and no blackening in the butt.

Also perform the string test on suspect cultures as follows. Emulsify a large inoculum from the T<sub>1</sub>N<sub>1</sub> agar culture in a large drop of 0.5% sodium desoxycholate in 0.85% saline solution. Within 60 seconds, a mucoid mass forms and this material strings when a loopful is lifted (up to 2 to 3cm) from the slide. Further confirmation is by serological reactions.

Stab streak suspect colonies of *Vibrio* on the Triple Sugar Iron (TSI) agar slant and incubate overnight at 35 C. Typical reaction of *V. parahaemolyticus* is an alkaline slant and an acid butt but no gas or H<sub>2</sub>S production

### **Results:**

Test for pathogenic *Vibrios* = Positive/ Negative