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The Vibrio Latest Tech and Importance in Sea Food

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Introduction

Why to test Vibrio, in sea food and simple and fast and accurate method, when compare with TCBS agar. Shrimp farms can be stressful environments that have high organic matter and experience fluctuations of dissolved oxygen. Changes in the environment in the shrimp farm will influence the number of bacteria and the species present. For example, as the temperature increases and the salinity increases due to evaporation *V. parahaemolyticus* will thrive and become predominate. Shrimp farmers recognize the relevance of *Vibrio* species, and the safety level of heterotrophic bacteria and *Vibrio* to the health of their ponds. Relative counts of bacterial groups are monitored and routine determination of *Vibrio* species are used to help in pond management. Farmers realize that a high prevalence of green colonies on Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) are indicative of diseased ponds. However, not all farmers may be aware of the pitfalls of the TCBS selection system or Now the availability of the CHROMAGAR [A patent product from Paris France]. A innovative chromogenic *Vibrio* detection system.

Vibrio is a genus of Gram-negative bacteria, Find out by ROBERT KOCH possessing a curved-rod (comma) shape, several species of which can cause foodborne infection, usually associated with eating undercooked seafood.All members of the genus are motile and have single polar flagella. All species Produce the Oxidase enzyme and give a positive indole reaction. The genus can be divided Non-Halophilic vibrios & Halophilic *V. cholerae* and other species that are able to grow in media without added salt. The genus and halophilic species such as *V. parahaemolyticus* and *V. vulnificus* that require Salt for growth.

Vibrios grow on ordinary media provided that their requirements for electrolytes are met, and grow best when abundant oxygen is present. Most grow at 30°C but some of the halophilic species grow poorly at 37°C, whereas *V. cholerae, V. parahaemolyticus* and *V. alginolyticus* grow at 42°C. Vibrios have a low tolerance to acid and prefer alkaline conditions (Bacterial growth range pH 6.8–10.2, optimum pH 7.4–9.6). Vibrio is a Facultative anaerobe.

Limitations Of Tcbs Agar

- May not support good growth of all Vibrio.
- Cultures should be visualized right after removal from the incubator as some yellow colonies
- Might revert to green at room temperature.
- Dense plates with a lot of sucrose fermenting colonies might obscure the green colonies because the acid from the sucrose fermenting bacteria can diffuse through the agar.
- There is no correlation between sucrose fermentation and virulence. Not all green colonies are pathogenic and not all yellow colonies are harmless. Detection of the PirA/PirB gene using PCR techniques would indicate the presence of acute hepatopancreatic necrosis disease [APHND] mainly caused by Vibrio Parahaemolyticus. However high numbers of APHND-can still cause disease in shrimp.
- Accompanying sucrose fermenting bacteria can pose a problem with this selection methodology
- CHROMAGAR Vibrio was formulated to be an improved method for the detection of Vibrio parahaemolyticus. This agar medium detects beta-galactosidase using a chromogenic substrate. This detection system eliminated a number of limitations the TCBS Agar had due to issues from the sucrose fermenting selection.

Advantages Of Chromagar Vibrio

- The color changes on CHROMAGAR Vibrio do not change when kept at room temperature.
- Color is not effected by other bacteria.
- CHROMAGAR Vibrio is 2.5% salt compared to TCBS which is 1% salt. The ocean is
- 3.5% salt therefore CHROMAGAR is closer to the natural environment for Vibrio's.
- V. parahaemolyticus turns –Mauve, V.Vulnificus,V.Cholerae-Green blue to Turquoise blue , V- alginolyticus –Colourless growing on and is distinguishable from other non-sucrose fermenting bacteria and Vibrios commonly isolated from the same environment.
- The colour is inside the colony not on the outer surface of the colony, for long storage of the plate, so there will not be any change in the colour of the colony.
- Not required to do autoclave.

Micro organisms	Typical colony colour
V.Parahaemolyticus	Mauve
V.Vulnificus,V.Cholerae	Green blue to turquoise blue
V.Alginolyticus	colourless

- Single biochemical test is recommended ie; Oxidase for confirmatory of Blue and Mauve colonies.
- Not required to perform 12 to 15 biochemical test.

Preparation

- Disperse slowly 74.7 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly. DO NOT HEAT TO MORE THAN 100
- °C. Do Not Autoclave at 121 °C.

Warning 1

If using an autoclave, do so without pressure.

Advice1

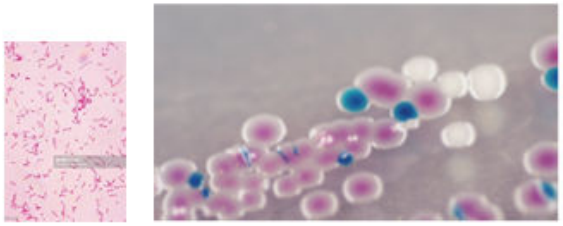
- For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).
- Cool in a water bath at 45-50 °C, swirling or stirring gently.

Advice 2

In case of samples with a high presence of Aeromonas, 50 mg of cefsulodin can be added to the mix once cooled down at 45-50 °C (50 mg/L).

- Pour medium into sterile Petri dishes, Let it dry & gel.
- Store in the dark before use.
- Prepared media plates can be kept for one day at room temperature.
- Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration.

Gram staining looks in microscope comma like structure.



Method	Protocol number	No of days to get the results
IS METHOD	5887 Part 5 -1976	4.6 days
ISO METHOD	21872 Part 1- 2017	4 days
BAM METHOD	8th Edition	4 days
CHROMAGAR	colour technology	2 days

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