9.Isolation of *Vibrio cholera /V. parahaemolyticus*

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Introduction

Seafood is a nutritious food that constitutes one of the desirable components of a healthy diet. Nevertheless, there is health risks associated with the consumption of seafood. One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favorable conditions. In contrast to most other foodborne pathogens, Vibrio spp. has the aquatic habitat as their natural niche. As a result, vibrios are most commonly associated with seafood as natural contaminants. Foodborne infections with Vibrio spp. are common in Asia. Most of these foodborne infections are caused by *V. parahaemolyticus* and *V. cholerae*, and to a lesser extent by *V. vulnificus.*

Vibrio cholerae

V. cholerae, a Gram-negative motile rod causes massive cholera outbreaks. Cholera is a global threat to public health and it was estimated that between 2008 and 2012 cholera caused an annual average of 2.9 million cases, and 95,000 deaths, worldwide Particular serogroups (O1 and O139) of this bacterium are responsible for cholera epidemics and pandemics. Human infection with *V. cholerae* begins with ingestion of contaminated food or water containing the bacterium.

V. cholerae colonizes the small intestine and secretes cholera enterotoxin (CT) into the host cells resulting in rapid efflux of chloride ions and water into the lumen of the intestine, leading to profuse diarrhea and severe dehydration. *V. cholerae* is commonly associated with chitin-containing zooplankton, particularly copepods and chironomids. Recent evidence supports the hypothesis that fish and water birds may also be intermediate reservoirs and vectors of *V. cholerae*.

Vibrio parahaemolyitcus

Vibrio parahaemolyticus was first discovered by Tsunesaburo Fujino in 1950 as a causative agent of food borne disease following a large outbreak in Japan which recorded 272 illnesses with 20 deaths after consumption of *shirasu*. Virulent *V. parahaemolyticus* strains are transmitted by consumption of raw or undercooked seafood

causing acute gastroenteritis. Since its discovery, *V. parahaemolyticus* has been found to be responsible for 20–30% of food poisoning cases in Japan and seafood borne diseases in many Asian countries. *V. parahaemolyticus* was also recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States. The worldwide prevalence of *V. parahaemolyticus* gastroenteritis cases stresses the need for understanding of the virulence factors involved and their effects on humans.

Enrichment and plating for Vibrio spp.

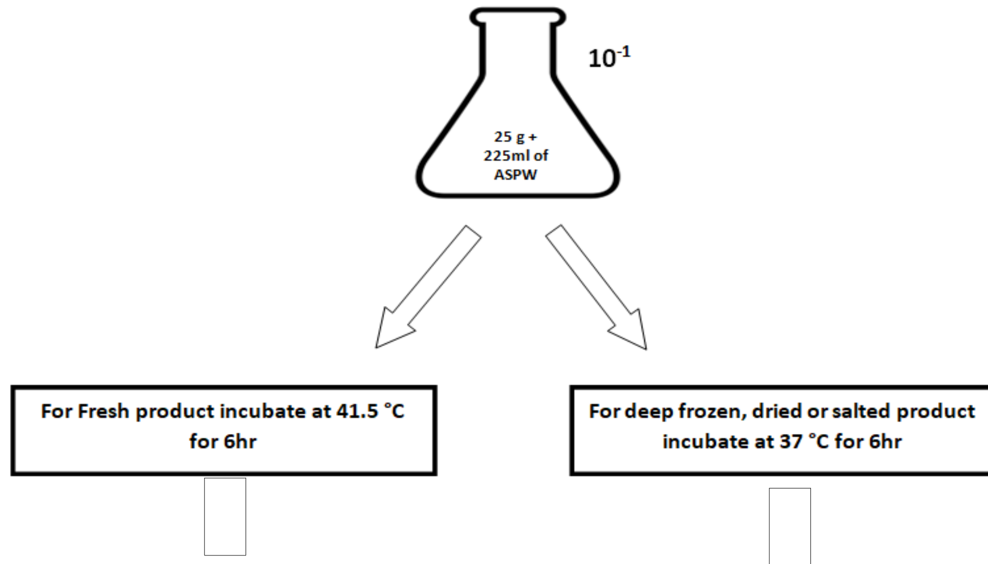
- Weigh 25 g of sample into a sterile stomacher bag (capacity approximately 500 ml).

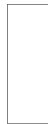
  Add 225 ml ASPW alkaline saline peptone water to jar. Thoroughly mix the sample or blend 2 min at high speed.

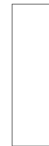
- Incubate ASPW at 37°C for 6h for deep frozen, dried or salted product and for fresh product incubate at 41.5 °C for 18h.
- Inoculation of the second enrichment medium 10 ml ASPW with the 1ml cultures obtained in primary enrichment medium .Incubation of inoculated enrichment medium at 41.5 °C for 18 h and/or 37 °C for 18 h For deep frozen, dried or salted product .
- Prepare dried plates of TCBS agar.
- Transfer a 3-mm loopful from the surface pellicle of ASPW culture to the surface of a dried TCBS plate, and streak in a manner that will yield isolated colonies incubate TCBS for 24 h at 37°C.
- On TCBS agar *V. parahaemolyticus*, and *V. cholerae* exhibit different typical colony morphologies:
- Typical colonies of *V. parahaemolyticus* and are smooth, green (negative sucrose) and of 2 mm to 3 mm in diameter;
- Typical colonies of *V. cholerae* are smooth, yellow (positive sucrose) and of 1 mm to 2 mm in diameter.
- For confirmation, subculture from each selective medium at least one well isolated colony considered to be typical or similar to each of the potentially pathogenic Vibrio spp.
- Inoculate the colonies selected onto the surface of plates of saline nutrient agar (SNA) or suitable medium of the laboratory's choice to obtain isolated colonies. Incubate at 37 °C for 24 h .

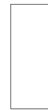
- Confirmed by biochemical test.

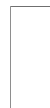
Detection of *Vibrio Cholerae / VibrioParahaemolyticus*











Test	***Vibrio Cholerae***	***Vibrio Parahaemolyticus***
Oxidase	**+**	**+**
LDC	**+**	**+**
ADH		
ONPG Hydrolysis	**+**	
Production of indole	**+**	**+**
Growth in peptone water with		
0% NaCl	**+**	
6% NaCl		**+**
10% NaCl		

Table: 1 Differentiation of Pathogenic *Vibrio species*:

		V. alginolyticus	*V. cholerae*	*V. fluvialis*	*V. furnissii*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*A. hydrophilia* **	*P. shigelloides* **
TCBS agar		Y	Y	Y	Y	NG	Y	G	G	G	Y	G
mCPC agar		NG	P	NG	NG	NG	NG	NG	NG	Y	NG	NG
CC agar		NG	P	NG	NG	NG	NG	NG	NG	Y	NG	NG
AGS		KA	Ka	KK	KK	Ka	KK	KA	KA	KA	KK	nd
Oxidase		+	+	+	+	+	–	+	+	+	+	+
Arginine dihydrolase		–	–	+	+	–	+	–	–	–	+	+
Ornithine decarboxylase		+	+	–	–	–	–	+	+	+	–	+
Lysine decarboxylase		+	+	–	–	–	+	+	+	+	V	+
Growth in (w/v):	0% NaCl	–	+	–	–	–	–	+	–	–	+	+
	3% NaCl	+	+	+	+	+	+	+	+	+	+	+
	6% NaCl	+	–	+	+	+	+	–	+	+	+	–
	8% NaCl	+	–	V	+	–	V	-	+	-	-	-
	10% NaCl	+	–	–	–	–	–	–	–	–	–	–
Growth at 42°C		+	+	V	–	nd	V	+	+	+	V	+
Acid from:	Sucrose	+	+	+	+	–	+	–	–	–	V	–
	D-Cellobiose	–	–	+	–	–	–	–	V	+	+	–
	Lactose	–	–	–	–	–	–	–	–	+	V	–
	Arabinose	–	–	+	+	+	–	–	+	–	V	–
	D-Mannose	+	+	+	+	+	+	+	+	+	V	–
	D-Mannitol	+	+	+	+	–	+	+	+	V	+	–

		V. alginolyticus	V. cholerae	V. fluvialis	V. furnissii	V. hollisae	V. metschnikovii	V. mimicus	V. parahaemolyticus	V. vulnificus	A. hydrophila **	P. shigelloides **
	ONPG	−	+	+	+	−	+	+	−	+	+	−
	Voges-Proskauer	+	V	−	−	−	+	−	−	−	+	−
Sensi-tivity to:	10 µg O/129	R	S	R	R	nd	S	S	R	S	R	S
	150 µg O/129	S	S	S	S	nd	S	S	S	S	R	S
	Gelatinase	+	+	+	+	−	+	+	+	+	+	−
	Urease	−	−	−	−	−	−	−	V	−	−	−

* Adapted from Elliot *et al.* (31)

** *Aeromonas hydrophila, Plesiomonas shigelloides*

Abbreviations: TCBS, thiosulfate-citrate-bile salts-sucrose; mCPC, modified cellobiose-polymyxin B-colistin; AGS, arginine-glucose slant; Y = yellow NG = no or poor growth S = susceptible nd = not done
G = green V = variable among strains R = resistant P = purple, V = variable, KK = Slant alkaline / Butt alkaline KA = Slant alkaline /Butt acidic, Ka = Slant alkaline/ Butt slightly acidic

10. ISOLATION METHOD OF *LISTERIA MONOCYTOGENES*

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- **Protocol**

1. Weigh 25 g of sample into a sterile stomacher bag (capacity approximately 500 ml).
2. Add 225 ml (HFB-Half Fraser Broth) thoroughly mix, blend or stomach and incubate for 24 h at 30° C.