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# Bacterial isolation, identification and storage

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# CHAPTER I

## Bacterial Isolation, Identification, and Storage

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## PRINCIPLE

Bacterial isolation, purification and identification are the first steps to bacteriological studies. Isolation is done to obtain pure bacterial cultures. Bacteria are usually isolated from fish kidney and spleen; and from the hepatopancreas, lymphoid organ and muscles of shrimp. These tissues are monitor organs that usually harbor the disease-causing bacteria during infection.

To obtain a pure bacterial culture is the first step to bacterial identification. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics, and susceptibility to antimicrobial agents of a particular bacterial strain.

Pure cultures are best obtained by using solid media, by streak plate or pour plate method. Streak plate, if properly done, is the most practical method. In the streak plate method, a loopful of the inoculum is placed near the periphery of the plate with agar medium and spread or streaked on the upper portion of the plate with parallel overlapping strokes. The inoculum is streaked over other portions of the plate so that isolated colonies could be observed in the last streaked area.

The identification of a bacterial pathogen is important in fish diagnosis. Treatment could be implemented only after the causative agent or the bacterium has been identified. Bacterial species differ in morphological, physiological and biochemical characteristics and those can be used when coding or labelling them (Appendix 1.1). Therefore, identification is accomplished by performing several morphological, physiological and biochemical tests. Results of these tests are compared to established taxa or identification schemes (Appendix 1.2).

Bacterial cultures should be preserved for future study. Storing in appropriate medium preserves bacterial cultures. The simplest method is by sub-culturing or by transferring the organism to fresh solid medium that has a minimal nutrient content to prevent bacterial overgrowth. The bacteria are allowed to grow before storing in the refrigerator or are covered with paraffin oil and stored at room temperature in the dark. Another simple method is by deep-freezing of the bacterial culture, stocked in a broth medium with glycerol. Glycerol is added to prevent the dessication of bacterial cells. Bacterial cultures may also be preserved by freeze-drying or lyophilization. In this method, water is removed from the frozen bacterial suspension by sublimation under vacuum.

Bacterial cultures should be properly labeled or coded before storage. It is important to label the tube or vial for storing bacterial cultures with an indelible ink. The label or code should include the reference number and other pertinent information such as source of sample (host animal, location), date of isolation, special properties, identification, name of the person who isolated the organism and the date of preparation of the stock culture.

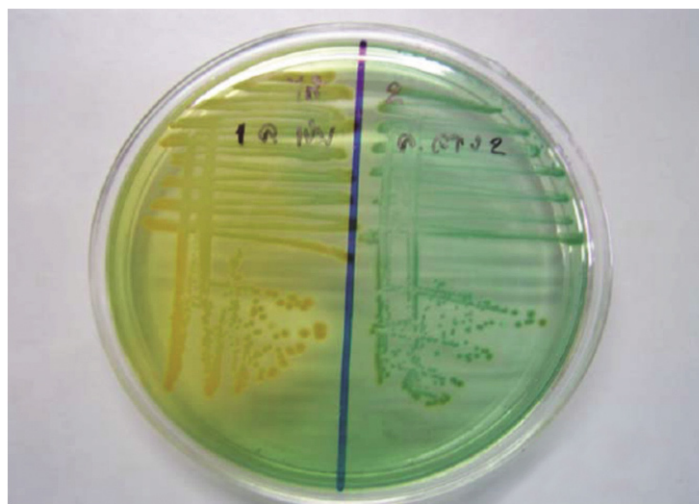
## ISOLATION

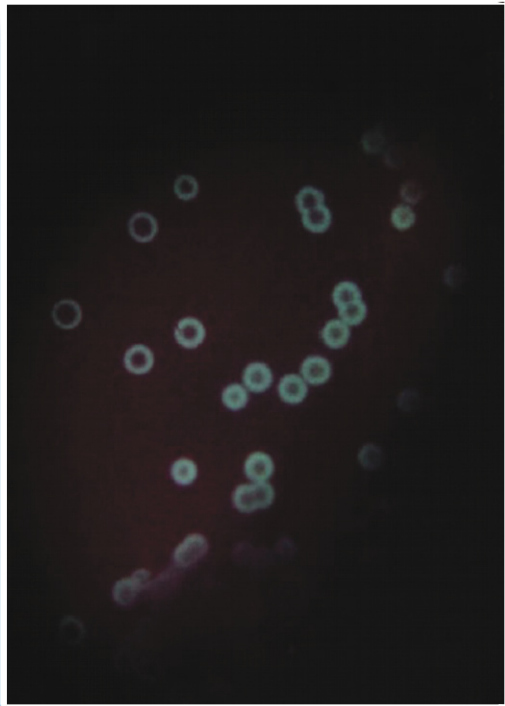
### Media

Bacterial isolation can be done using a general medium, wherein various bacteria can grow, and selective media that allows growth of specific genera. Examples of general media are nutrient agar (NA), tryptic soy agar (TSA), and brain heart infusion agar (BHIA). Examples of selective media are thiosulfate citrate bile sucrose agar (TCBS) for vibrios, and glutamate starch phenol red agar (GSP) for aeromonads and pseudomonads. Media are supplemented with 1-2% sodium chloride (NaCl) if to be used for marine species. Adjust the pH of the culture media to 7.2-7.4 by adding 0.1 N NaOH.

### Streaking

- 1 Using inoculating loop, get samples of shrimp (such as the hepatopancreas and muscle) and fish (kidney, spleen) tissues and streak onto the upper one-fourth portion of an agar plate with parallel overlapping strokes. Use one agar plate for each animal sample. The plate can be divided into half and streaked with two different tissues from the same sample. Be sure to label the plate.
- 2 Flame the loop and allow it to cool. Turn the plate at right angle. Overlap the previous streak once or twice and repeat the streaking process on one-half of the remaining area.
- 3 Repeat procedure 2.
- 4 Incubate plates overnight at 30°C. Photo at right shows a streaked plate after incubation.



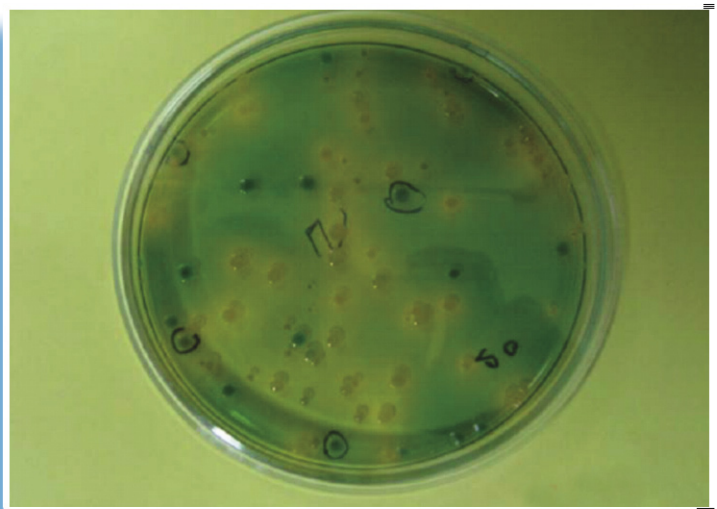


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After incubation for 16-20 hours, check for bacterial growth. Check for luminescence under dark conditions, marking the luminous colonies on the plate with a pentel pen. Isolated colonies should be observed in the last streaked area.

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Select representative bacterial colonies based on the difference in shape, size and color. Mark selected colonies from each plate. Subculture onto trypticase soy agar (TSA) plate and incubate overnight.



## STORAGE

- 1 Observe the colonies on the agar plate to determine the purity of the culture. Pure cultures should show the same colony characteristics and not overlapping.



- 2 Select a pure well-isolated colony. Stab each strain into 2 tubes of 1.2% TSA, label and incubate. These will serve as stock cultures.

- 3 Keep the stock cultures in the lowest compartment of the refrigerator (8-12°C) or at room temperature until use. Do not stock cultures in these conditions for over 6 months.

**4**

Purified bacterial cultures may also be stocked in nutrient broth with 20% glycerol and stored at  $-80^{\circ}\text{C}$  until use. Bacterial cultures may be stocked in this condition for 2 years.

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**NOTE:** *The stocks may be coded or labeled based on the source of the samples, date of collection, color of the colony and ability of the colony to emit light (Appendix 1.1).*

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**IDENTIFICATION**

Bacterial isolates may be identified using conventional methods based on their morphological, biochemical and physiological characteristics. The following are important biochemical tests for the identification of bacterial genera that are important in aquaculture:

1. Gram reaction
2. Oxidase test
3. Motility
4. Oxidation and fermentation test
5. O/129 sensitivity test
6. Sensitivity to novobiocin

The scheme for the identification of bacterial genera that are important in aquaculture is presented in Appendix 1.2.

Further biochemical characterization must be carried out if there is a need to identify up to the species level.

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**NOTE:** *Aseptic techniques must be observed all throughout.*

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## REFERENCES

- Bailey, W.R. and E.G. Scott. 1966. Diagnostic Microbiology, Second Edition. Toppan Company Ltd., Japan, 342 pp.
- Tonguthai, K., S. Chinabut, T. Somsiri, P. Chanratchakol and S. Kanchanakhan. 1999. Diagnostic Procedures for Finfish Diseases. Aquatic Animal Health Research Institute, Bangkok, Thailand.



**APPENDIX 1.1.** Example of important bacterial characteristics for *Vibrio* species, that are taken into consideration when coding or labeling isolated bacteria.

<b>Source of sample</b>	Host animals and geographical location are important informations to use as code of drug resistant bacteria.
<b>Color of the colony on TCBS</b>	<i>Vibrio</i> bacteria normally appear as green or yellow colonies on TCBS medium. Many vibrios that form green colonies are more virulent to shrimp.
<b>Special characteristics</b>	Luminous bacteria emit light in the dark. They are, therefore, easy to collect for special or future studies. Luminous bacteria may be labeled as LB.

**APPENDIX 1.2.** Identification scheme for the identification of bacterial genera that are important in aquaculture (modified from Tonguthai *et al.*, 1999).

