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Bacterial Identification from Marine Ornamental Fish in Fish Quarantine, Quality Control and Fishery Products Safety Class I Denpasar, Bali

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Abstract. This study aims to identify the types of bacteria that may infect marine ornamental fish. This study was conducted in Fish Quarantine, Quality Control and Fishery Products Safety Class I Denpasar, Bali from January 16, 2017 to February 18, 2017. Bacterial identification was conducted using conventional method, which is biochemical test. The identified bacteria in marine ornamental fish were Vibrio furnissii, Vibrio alginolyticus, and Vibrio parahaemolyticus. The number of contaminated fish was 11 out of 19 marine ornamental fish. The infected fish samples included Pomacentrus, Chromis viridis, Dascyllus carneus, Pseudochromis, Abudefduf sp., Dascyllus trimaculatus, Dascyllus arwanus, and Dascyllus melanurus.

1. Introduction
Indonesia has abundant varieties of marine fish that are potential in the development of non-oil and gas export. Center for Aquaculture Research and Development [1] mentioned that Indonesia is one of the biggest exporters of ornamental fish following Singapore, Malaysia, Japan, Thailand and China. The high potential of ornamental fish marketing, both export and domestic, contributes in bringing in the risk of the entering and spreading of fish pests and diseases. Diseases in fish come emerge as a result of complex interaction between weak hosts, pathogenic organisms and poor environmental quality [2]. One of the pathogenic organisms that cause diseases in fish is bacteria.

Bacteria are prokaryote organisms with high diversity of morphology, ecology and physiology [3]. In general, bacteria infecting fish has the opportunist nature in which they infect stressed fish. The clinical symptoms caused by bacterial attack include abnormal changes such as lesion on the fish’s skin or fins, muscle tissue, and internal organs [4]. Bacterial attack may lead to mass death, causing high economic loss. One of the means to prevent the spread of pathogenic bacteria is through quarantine and test in Fish Quarantine. Fish Quarantine is a state institution in charge for preventing fish pests...
and diseases from entering and spreading from one area to another (PER.25/MEN/2011). The marine ornamental fish commodities to be transported, both export and domestic, should be tested with bacterial identification in order to ascertain the infecting pathogenic bacterial. Afterwards, the identification result will be used as a basis to declare the properness of the fish commodities to be transported.

2. Methodology
2.1. Research method
The study was conducted on 16th January–18th February 2017. This study was conducted in Fish Quarantine, Quality Control and Fishery Products Safety Class I Denpasar, Bali. Data were collected through observation, interviews and active participations. Bacterial identification was conducted using aconventional method, biochemical test. The data was analyzed using descriptive method.

2.2. Materials
The samples of marine ornamental fish were collected from marine ornamental fish farmers in Bali. The media used for bacterial identification consisted of 3% Triptc Soy Agar (TSA), Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar, Triple Sugar Iron Agar (TSIA), Lysine Iron Agar (LIA), Motile Indol Ornithine (MIO), Bassal oxidative-fermentative Bassal (O/F), Gelatine, Urease, and Sugar.

2.3. Research procedure
2.3.1 Bacterial isolation and purification
Bacterial isolation was conducted using streak plate method. The samples of bacteria were isolated from gill organs and were streaked on 3% TSA or TCBS medium. TCBS is a selective medium for identifying Vibrio bacteria. The isolation results were incubated at the temperature of 28°C for 24 hours. Subsequently, bacterial purification was conducted by growing a single bacterial colony from 3% TSA medium to TCBS medium.

2.3.2. Presumptive test
Presumptive test was conducted to determine test microbes’ nature. The presumptive test conducted included catalase test using 3% H₂O₂ addition, Gram test using 3% KOH solution addition, and oxidase test with oxidase test strips.

2.3.3. Biochemical test
The biochemical test conducted included acid fermentation test in TSIA medium, citrate test, citrate test, gelatine test, urea test, Methyl Red Voges-Proskauer test (MR-VP), Sulfide Indole Motility (SIM) test, oxidative fermentative test (OF), nitrate test, lysine test, ornithine test, and sugar testing. The result of biochemical test was identified by referring to Bergey’s Manual of Determinative Bacteriology book [5].

3. Result and discussion
Bacterial identification on marine ornamental fish commodities was conducted from January 16 to February 18, 2017. The result of bacterial identification on 19 samples of marine ornamental fish is presented in Table 1.

| Sample Type    | Amount of Sample | Result | Description       |
|----------------|------------------|--------|-------------------|
| Dascyllus carnea | 2                | Negative 1 Positive 1 | Vibrio furnissii |
| Pomacentrus    | 2                | Negative 0 Positive 2 | Vibrio alginolyticus |
Based on the identification on 19 samples, 10 of them were found positive of containing bacteria. The identified bacteria were *Vibrio furnissii*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus*. Vibrio is categorized into Gram-negative bacteria that is motile and facultative anaerobic. Vibrio are rod-shaped or curved. They can optimally grow at the temperature of 20°-30°C. Vibrio possesses high adaptability toward its surrounding by forming resistance system, reducing cell size during dormant phase, and changing metabolic pathway [6]. Bacterial growth and pathogenicity will increase when environmental quality decreases. Vibrio can be identified through colony formation with yellow and green colours in TCBS medium. The colonies’ different colour in TCBS medium is related to Vibrio species’ ability to ferment sucrose and reduce pH of TCBS medium. Yellow colonies are formed by *Vibrio cholerae* and *Vibrio alginolyticus* [7]. According to Mailoa and Setha [8], Vibrio bacterial colonies’ yellow colour is due to the bacteria’s ability to ferment sucrose and reduce pH of TCBS medium. Reduced pH and fermented sucrose will turn the medium’s green colour into yellow, which turn the bacterial colonies’ colour into yellow as well. Meanwhile, Vibrio colonies that produce green colour are those that are unable to ferment sucrose.

Bacterial characteristics can be seen through biochemical test because each bacterium has different biochemical activities. The result of biochemical test of bacterial isolates is presented in Table 2.

**Table 2. Result of biochemical test of bacterial isolates.**

| Testing            | Vibrio furnissii | Vibrio alginolyticus | Vibrio parahaemolyticus |
|--------------------|------------------|----------------------|-------------------------|
| Colony             | Yellow           | Yellow               | Green                   |
| TSIA Oblique       | Acid             | Acid                 | Alkali                  |
| TSIA Upright       | Acid             | Acid                 | Acid                    |
| TSIA gas           | -                | -                    | -                       |
| Glucose medium     | +                | +                    | +                       |
| Glucose gas        | +                | -                    | -                       |
| OF with paraffin   | +                | +                    | +                       |
| OF without paraffin| +                | +                    | +                       |
| Citrate            | +                | -                    | -                       |
| Nitrate            | +                | +                    | +                       |
| Ornithine          | -                | +                    | +                       |
| Lysine             | +                | +                    | +                       |
| SIM motility       | +                | +                    | +                       |
| SIM indole         | -                | +                    | +                       |
| Gelatin            | +                | +                    | +                       |
| Urea               | -                | -                    | -                       |
4. Conclusion

From the result of the study, it can be concluded that the type of bacterial identified in marine ornamental fish included *Vibrio furnissii*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus*. The amount of fish infected was 11 out of 19 samples. *Vibrio* infection in marine ornamental fish indicates the lack of biosecurity implementation in cultivation system.

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