# EVALUATION OF CHROMAGAR VIBRIO FOR THE DIFFERENTIATION OF FISH-DERIVED *Vibrio* SPECIES

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## 1. Introduction

Pathogenic bacteria present in marine environments cause disease outbreaks and mortality in farmed fish in domestic aquaculture systems, driven by factors such as rising water temperatures and the increasing prevalence of antimicrobial-resistant bacteria (Jeong *et al*., 2018; Kim *et al*., 2019). In addition, the increase in average sea surface temperature associated with global warming has led to higher detection frequencies of these pathogens and an increased incidence of related diseases (Go *et al*., 2013). Among them, *Vibrio* spp. are Gram-negative rod-shaped bacteria widely distributed in marine environments, with more than 100 species reported to date (Baker-Austin *et al*., 2018).

Thiosulphate-citrate-bile salt sucrose (TCBS) agar is widely used for the detection of *Vibrio* spp., allowing differentiation based on colony color changes resulting from sucrose fermentation (Roberts *et al*., 1982). However, its selectivity is limited, and the detection rate of *Vibrio* spp. on TCBS agar may be lower than that on non-selective media.

CHROMagar Vibrio, a selective medium similar to TCBS agar, enables the differentiation of certain *Vibrio* species through chromogenic substrates. However, previous studies have primarily focused on *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio alginolyticus*, and *Vibrio cholerae*, and its applicability to a broader range of fish-derived *Vibrio* species has not been sufficiently evaluated.

Therefore, this study aimed to analyze colony color development of eight species of fish-derived *Vibrio* on CHROMagar Vibrio and to evaluate its potential for bacterial identification through comparison with TCBS agar.

## 2. Materials & Methods

Table 1. Bacterial strains used in this study

| Strain | Year of isolation | Origin | Region |
|---|---|---|---|
| *Vibrio parahaemolyticus* | 2020 | Sea water | Jeju-do |
| | 2020 | Sea water | Jeju-do |
| | 2013 | *Litopenaeus vannamei* | Jeju-do |
| *Vibrio vulnificus* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Vibrio alginolyticus* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Vibrio harveyi* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Photobacterium damselae* (subsp. *damselae*) | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Vibrio gigantis* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Vibrio scophthalmi* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2022 | *Paralichthys olivaceus* | Jeju-do |
| *Vibrio alfacsensis* | 2022 | *Paralichthys olivaceus* | Jeju-do |
| | 2021 | Sea water | Wan-do |
| | 2021 | Sea water | Wan-do |
| *Streptococcus parauberis* | 2025 | *Paralichthys olivaceus* | Jeju-do |
| | 2025 | *Paralichthys olivaceus* | Jeju-do |
| | 2025 | *Paralichthys olivaceus* | Jeju-do |

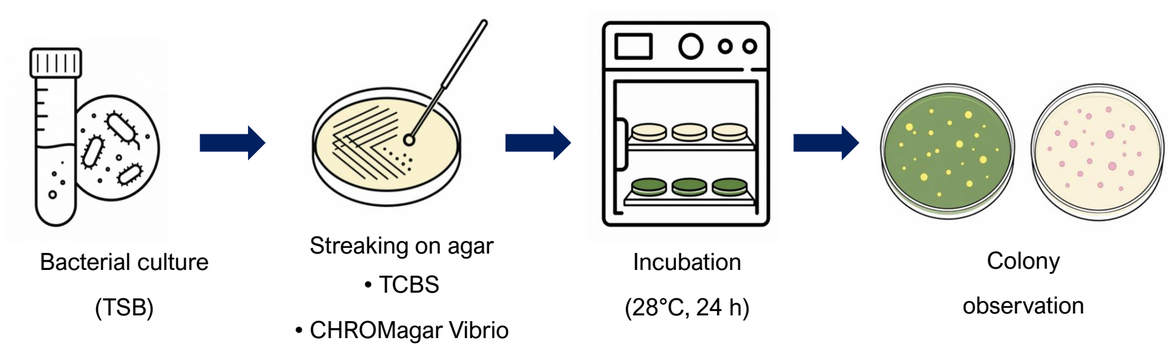



Figure 1. Schematic diagram of the experimental workflow.

## 3. Results

Table 2. Colony colors of various bacteria grown on TCBS agar and CHROMagar Vibrio

| Species | No. of strains tested | TCBS agar | CHROMagar Vibrio |
|---|---|---|---|
| | | Color of colony | Color of colony |
| *V. parahaemolyticus* | 3 | Green | Mauve |
| *V. vulnificus* | 3 | Green | Bluish green |
| *V. alginolyticus* | 3 | Yellow | Creamy |
| *V. harveyi* | 3 | Yellow | Light pink |
| *P. damselae* | 3 | Green | Ivory |
| *V. gigantis* | 3 | Green | Ivory (center pink) |
| *V. scophthalmi* | 3 | Green | Light purple |
| *V. alfacsensis* | 3 | Green | Blue |
| *S. parauberis* | 3 | NG* | NG |

*NG, no growth.

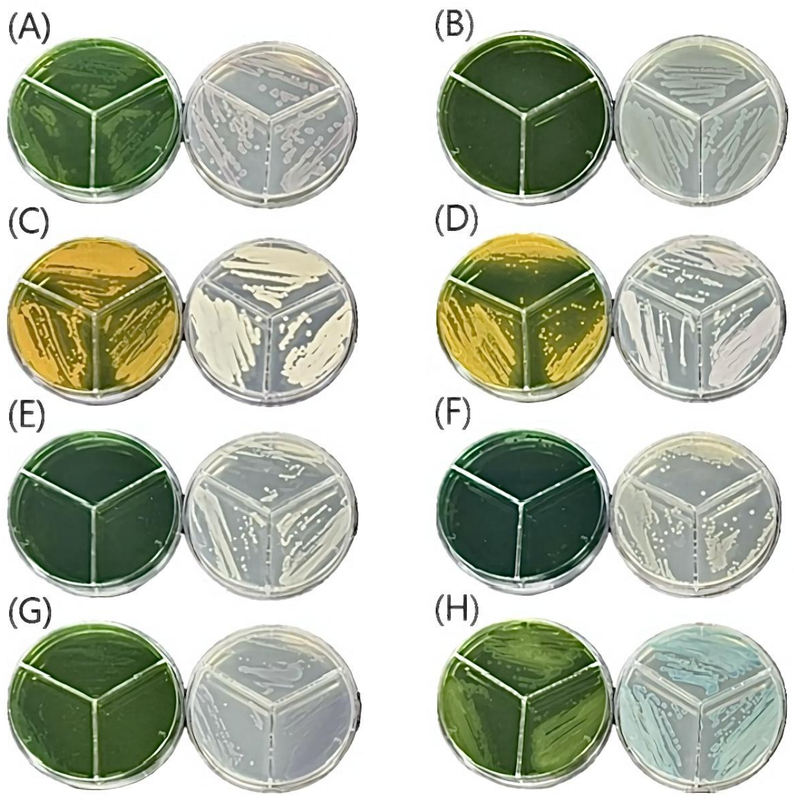



Figure 2. Appearance of *Vibrio* spp. on TCBS agar and CHROMagar Vibrio.
(A) *V. parahaemolyticus*, (B) *V. vulnificus,* (C) *V. alginolyticus*, (D) *V. harveyi*, (E) *P. damselae*, (F) *V. gigantis*, (G) *V. scophthalmi*, (H) *V. alfacsensis.* The agar plates, shown in order from left to right, are TCBS agar and CHROMagar Vibrio.

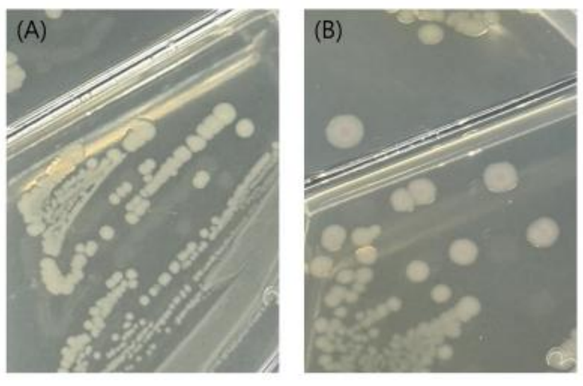



Figure 3. Appearance of *P. damselae* and *V. gigantis* on CHROMagar Vibrio. (A) *P. damselae*, (B) *V. gigantis.*

## 4. Discussion

As a result, colony colors of the eight species on TCBS agar appeared only as yellow or green, making precise species-level differentiation difficult. CHROMagar Vibrio produced distinguishable colony colors for each strain, including mauve for *V. parahaemolyticus*, bluish green for *V. vulnificus*, creamy for *V. alginolyticus*, light pink for *V. harveyi*, ivory for *P. damselae*, ivory with a pink center for *V. gigantis*, light purple for *V. scophthalmi*, and blue for *V. alfacsensis*. In particular, *P. damselae* and *V. gigantis* formed visually similar ivory-colored colonies; however, *V. gigantis* exhibited a pink coloration in the central region of the colony, enabling it to be distinguished from *P. damselae*. This difference is likely due to the degradation of chromogenic substrates by β-galactosidase in *Vibrio* spp.

The broader range of colony color expression observed on CHROMagar Vibrio may improve the preliminary differentiation of fish-derived *Vibrio* spp. compared to TCBS agar.

## 5. Conclution

In this study, eight species of *Vibrio* spp., mostly collected from aquaculture farms in Jeju between 2013 and 2025, were cultured on the selective media TCBS agar and CHROMagar Vibrio to compare colony color development.

Unlike TCBS agar, which produces dichotomous colony color patterns, CHROMagar Vibrio, which forms a wide range of colony colors, may serve as a useful tool for more effective differentiation of diverse *Vibrio* spp. isolated from farmed fish.

Therefore, the use of CHROMagar Vibrio, which enables diverse colony color development, may facilitate the rapid identification of *Vibrio* spp., allowing timely responses to disease outbreaks. Such rapid diagnosis of vibriosis is expected to reduce economic losses caused by disease spread and mass mortality in aquaculture, and may also contribute to mitigating public health concerns, including antimicrobial resistance.

## References

Baker-Austin, C., Oliver, J.D., & Alam, M.: *Vibrio* spp. infections. Nat Rev Dis Primers 4, 1–19, 2018.

Go, Y.J., & Jang, J.S.: Relationships of Pathogenic Vibrios and Environmental Factors Affecting their Occurrence in the Seawater of Incheon Coastal Area. The Korean Journal of Food And Nutrition, 26(3), 414–420, 2013.

Hara-Kudo, Y., Nishina, T., Nakagawa, H., Konuma, H., Hasegawa, J., & Kumagai, S.: Improved method for detection of *Vibrio parahaemolyticus* in seafood. Appl Environ Microbiol 67, 2001.

Jeong, Y.I., Myung, G.E., Choi, E.J., Soh, S.M., Park, G.J., & Son, T.J.: Distribution of Pathogenic Vibrios in the Aquatic Environment Adjacent to Coastal Areas of South Korea and Analysis of the Environmental Factors Affecting Their Occurrence. Journal of Environmental Health Sciences, 2018;44:133-142, 2018.

Kim, Y.J., Seo, J.S., Park, J.O., Jeong, A.R., & Lee, J.H.: Monitoring of aquatic medicine managements in South Korea. Journal of Fish Pathology, 32(1), 37–43, 2019.

Roberts, N., Siebeling, R., & Kaper, J.: Vibrios in the Louisiana gulf coast environment. Microb Ecol 8, 299–312, 1982.