TIME-EFFICIENT PREPARATION OF CHROMAGAR VIBRIO USING AN IMPROVED HEATING METHOD

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Introduction

Vibrio parahaemolyticus and *Vibrio vulnificus* are major causes of food poisoning, including fatal sepsis, whereas *Vibrio alginolyticus* is associated with significant economic losses in aquaculture. TCBS agar is commonly used for the presumptive identification of *Vibrio* species, but its reliance on sucrose fermentation and the resulting restriction of colony appearance to yellow or green limit species differentiation. CHROMagar Vibrio produces diverse colony morphologies and colors, enabling clearer species identification for those that are difficult to distinguish on TCBS agar. Nevertheless, the conventional manufacturing method requires prolonged heating below 100 ˚C, which is time-consuming and limits its application during rapidly spreading *Vibrio* outbreaks.

In this study, we developed an improved rapid manufacturing method for CHROMagar Vibrio to reduce the identification time of *Vibrio* spp. and assess its effectiveness by comparing it with the conventional method.

Materials and methods

Table 1. Reference type strains used in this study

Strain	Year of isolation	Origin	Region
Vibrio parahaemolyticus	2013	*Litopenaeus vannamei*	Jeju-do
	2020	Sea water	Jeju-do
	2026	*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

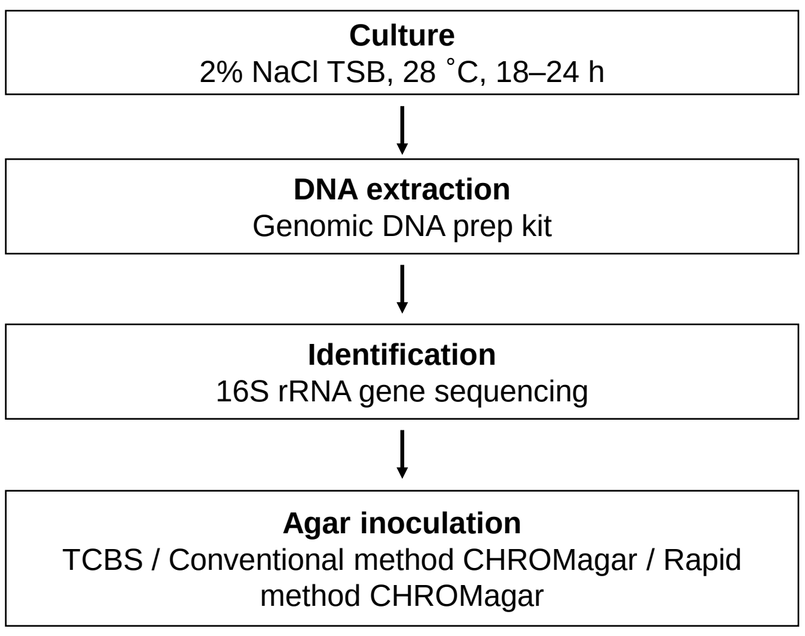


Figure 1. Schematic diagram of strain culture and identification.

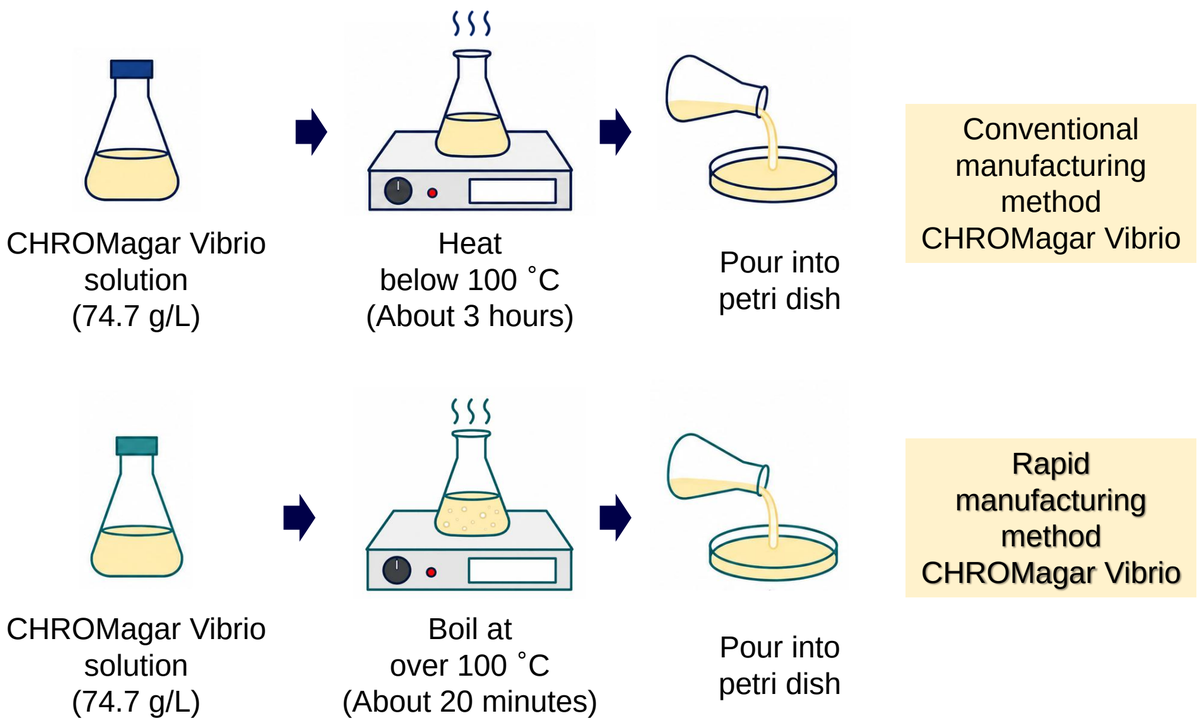


Figure 2. Schematic diagram of the production process of conventional manufacturing method CHROMagar Vibrio and rapid manufacturing method CHROMagar Vibrio.

The experimental strains were predominantly *Vibrio* spp. isolated from Jeju Island. CHROMagar Vibrio was manufactured using both the conventional manufacturing method following the manufacturer's protocol (below 100 ˚C) and the rapid manufacturing method in which the medium was heated above 100 ˚C until boiling. All agar plates were prepared using a tri-petridish to ensure clear strain comparison and prevent cross-contamination. The strains were cultured in TSB, plated onto CHROMagar Vibrio plates prepared by each method, and incubated at 28 ˚C for 24 hours. The resulting colonies were morphologically compared and analyzed.

Results

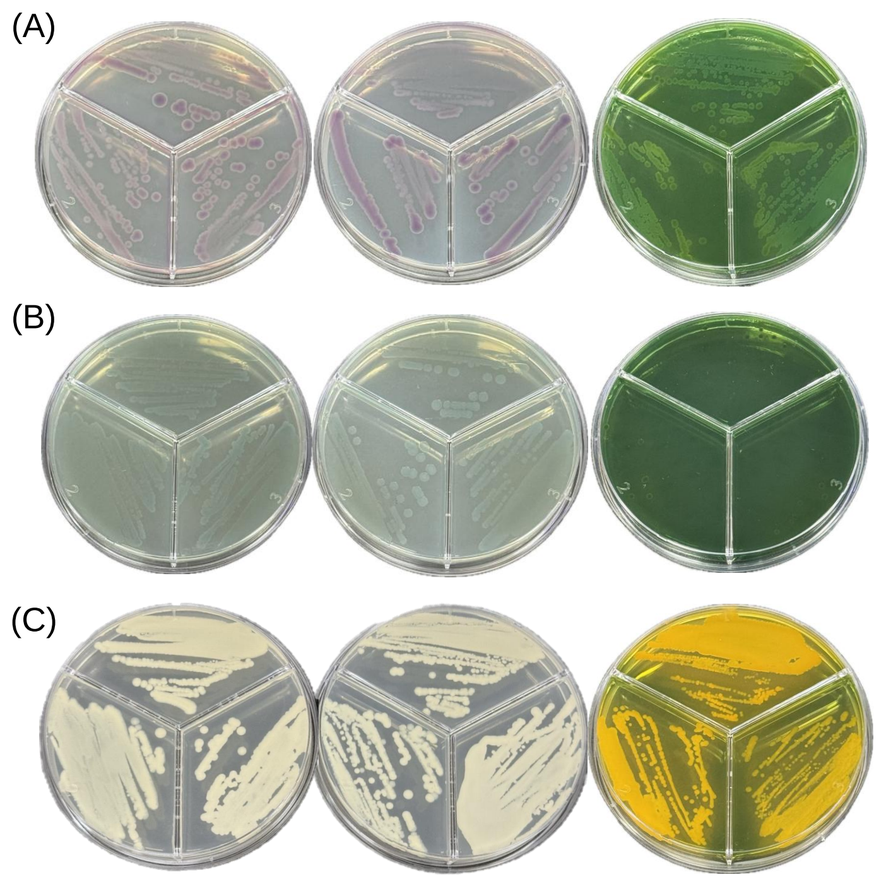


Figure 3. Appearance of *Vibrio* spp. on conventional manufacturing method CHROMagar Vibrio, rapid manufacturing method CHROMagar Vibrio and TCBS agar. (A) *V. parahaemolyticus*, (B) *V. vulnificus*, (C) *V. alginolyticus*. The agar plates, shown in order from left to right, are conventional manufacturing method CHROMagar Vibrio, rapid manufacturing method CHROMagar Vibrio and TCBS agar.

Table 2. Comparison of *Vibrio* spp. colony colors on CHROMagar Vibrio prepared using conventional and rapid manufacturing methods

Species	No. of strains tested	TCBS agar	Conventional method CHROMagar Vibrio	Rapid method CHROMagar Vibrio
Vibrio parahaemolyticus	3	Green	Mauve	Mauve
Vibrio vulnificus	3	Green	Bluish green	Bluish green
Vibrio alginolyticus	3	Yellow	Creamy	Creamy

Key findings

The rapid manufacturing method reduced heating time by approximately 2 h 40 min compared with the conventional method. No visible differences in colony growth or color development were observed between the conventional and rapid methods. The same species-specific colony colors were maintained in *V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus.*

Discussion

Compared with TCBS agar, CHROMagar Vibrio allowed clearer color-based differentiation of the tested *Vibrio* species. In particular, *V. parahaemolyticus* and *V. vulnificus* both appeared green on TCBS agar but showed distinct colors on CHROMagar Vibrio. The similar colony appearance observed between conventional and rapid method CHROMagar Vibrio suggests that the modified heating step did not visibly affect chromogenic performance. These findings support the use of rapid method CHROMagar Vibrio as a practical culture-based screening tool for *Vibrio* spp.

Conclusion

Time-efficient preparation

The rapid manufacturing method shortened CHROMagar Vibrio preparation time from approximately 3 h to 20 min.

Maintained colony characteristics

Species-specific colony colors were maintained in *V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus.*

Potential diagnostic application

This method provides a practical option for faster preliminary differentiation of *Vibrio* spp.

References

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