Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(3):236-240

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Isolation and screening of gut microflora from *Siganus guttatus* (Bloch, 1787) for potential anti-vibrio agents

Victor Marco Emmanuel N. Ferriols*, Marciel G. Siladan, Allan N. Failaman and Rex Ferdinand M. Traifalgar

Institute of Aquaculture – College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines

---

ABSTRACT

Bacteria from the gastrointestinal tract of the rabbitfish Siganus guttatus (Bloch, 1787) were isolated and screened for their potential as anti-vibrio agents. A total of four isolates based on colony dominance and morphology were selected and tested against Vibrio harveyi in cross-streak and co-culture tests. Although no inhibition was observed for all isolates in the cross-streak tests, a significant reduction was observed in the population of V. harveyi in co-culture with all isolates. Reduction in V. harveyi population ranging from 63.9% - 100% were observed within 24 hours of inoculation. This study showed the potential of bacteria isolated from the gut of S. guttatus to act as a possible control agents against V. harveyi especially in cultures of shrimps and prawns wherein S. guttatus could be raised in tandem to produce "green water".

Keywords: *Siganus guttatus*, gut microflora, anti-vibrio

INTRODUCTION

In recent years, the use of "green water" technology as a biocontrol measure to prevent Luminous Vibriosis in shrimp farms has gained much acceptance [3]. This technology has been the subject of many studies ranging from refining protocols in achieving "green water" to elucidating the many factors involved in preventing the outbreak of Luminous Vibriosis.

Although this technology initially focused on the use of Tilapia to produce the desired green water, studies have shown that other fish species such as milkfish, rabbitfish, and seabass produced similar results [12]. Most of these studies have pointed to the role of fish mucus and the various microalgal species present in the green water in inhibiting the growth of *Vibrio* species in culture systems [8]. The role of other bacterial species present in green water in the inhibition of *Vibrio* species has also been proposed through the concept of competitive exclusion.

The presence of Vibrio-inhibiting bacteria has been associated with green water technology although the possible sources of such bacteria have not been elucidated thoroughly in previous studies. One potential source of such vibrio-inhibiting bacteria is the gastrointestinal tract of the fish used in green water technology. Lactic acid bacteria

for instance, which has shown great potential as a probiont in aquaculture systems, has been reported to be present in both freshwater and marine fish indicating that the fish gut may be a natural reservoir for such bacteria[1];[2].

This study aims to isolate and screen microflora from the gut of the rabbitfish *Siganus guttatus* to identify potential bacteria that could inhibit the growth of *Vibrio harveyi*. This could help in elucidating one of the various mechanisms involved in the inhibition of luminous vibriosis in green water technology using *Siganus guttatus* as a biocontrol agent. Moreover, this study could also help in identifying bacteria which could later be used as a probiont in shrimp culture systems.

MATERIALS AND METHODS

Bacterial Isolation

Naturally occurring bacteria in the gut of mature Siganus guttatus were isolated following the methods of Buntin *et al.* [1]. In summary, the gastrointestinal tract of mature S. guttatus were aseptically removed and weighed. Equal amounts of gut tissue were then homogenized and diluted to achieve dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Each dilution was applied to nutrient agar with modified salinity through the spread plate method and incubated for 24 hours at 37 C in order to obtain bacterial isolates from the gastrointestinal tract. Individual colonies of bacteria were then subjected to further purification methods utilizing nutrient agar. Potential bacteria with anti-vibrio properties were selected based on the relative dominance of colonies with the same morphology through visual assessment.

Bacterial Screening

Identification of the anti-Vibrio properties of the isolated gut bacteria were tested on *Vibrio harveyi* obtained from the bacterial collection of the Southeast Asian Fisheries Development Center-AQD in Tigbauan, Iloilo. Initial screening tests involved cross streaking of the isolated bacteria against streaks of *Vibrio harveyi* [6]. Bacteria identified with potential zones of inhibition from the cross streaking test were then further subjected to co-culture tests by inoculating tubes of nutrient broth (modified salinity) with both *Vibrio harveyi* and gut isolates at an initial density of 10^3 CFU ml^{-1}. Prior to co-culture, *V. harveyi* and bacterial isolates were cultured in nutrient broth with 30% NaCl for 24 hours. The bacterial suspensions were quantified using a spectrophotometer at 630nm against a set of McFarland standards. Required volumes of each bacterial suspension were then calculated and measured in order to reach final densities of 10^3 CFU ml^{-1} in the co-culture vessels. Although all isolates did not exhibit any zone of inhibition against *V. harveyi*, all were still subjected to the co-culture test.

Aliquots of the co-culture media were spread on both nutrient agar and TCBS to determine the inhibition of *Vibrio harveyi* after 6, 12, 24, and 36 hours of culture at 37 C (serial dilutions were employed when colony counts were too dense to enumerate). Inhibition of *Vibrio harveyi* was measured as the ratio of colonies of *Vibrio harveyi* formed in TCBS agar over the total bacterial count in NA.

Statistical Analysis

Results of the co-culture test were subjected to statistical analysis using Analysis of Variance (ANOVA) coupled with Duncan's Multiple Range Test in order to determine if the inhibitory action of the various gut isolates were significantly different. Three replicates per isolate were used in order to attain sufficient data for the statistical analysis.

RESULTS AND DISCUSSION

Bacterial Isolation and Purification

Isolation of gut bacteria on nutrient agar (30% NaCl) indicated a gut population level of 4.3 – 5.1 x 10^4 CFU/g (wet weight) of the intestines of *S. guttatus*. Four bacterial isolates were selected using visual assessment of dominant colonies based on their general morphology. Two of the isolates came from the fore gut (A1 and A3) while two more isolates came from the aft gut (B and C). Further purification of the isolates was done on nutrient agar with 30% NaCl (w/v). These isolates exhibited good growth on NA forming visible colonies within the first 24 hours of inoculation. Further culture of these isolates on TCBS agar revealed that none of the gut bacteria were of the genus Vibrio.

Bacterial Screening and Testing

Results of the cross streaking test showed no signs of inhibition by the gut bacterial isolates against *V. harveyi*. No clear zones of inhibition were observed for all bacterial isolates although a considerable colonization effect was observed in all off the isolates as exhibited by the larger areas colonized by the cross streaks and the appearance of isolate colonies on the *V. harveyi* streaks (Figure 1). These were similar to some observations of Purivirojkul *et al.* [9] regarding several strains of *Bacillus* sp. isolated from the gut of *Peneaus monodon*. Although no inhibitory effects were observed, the potential of the isolates in controlling *V. harveyi* could still be possible through rapid colonization of the culture environment. All the isolates were further subjected to co-culture tests with *V. harveyi* to test this theory.

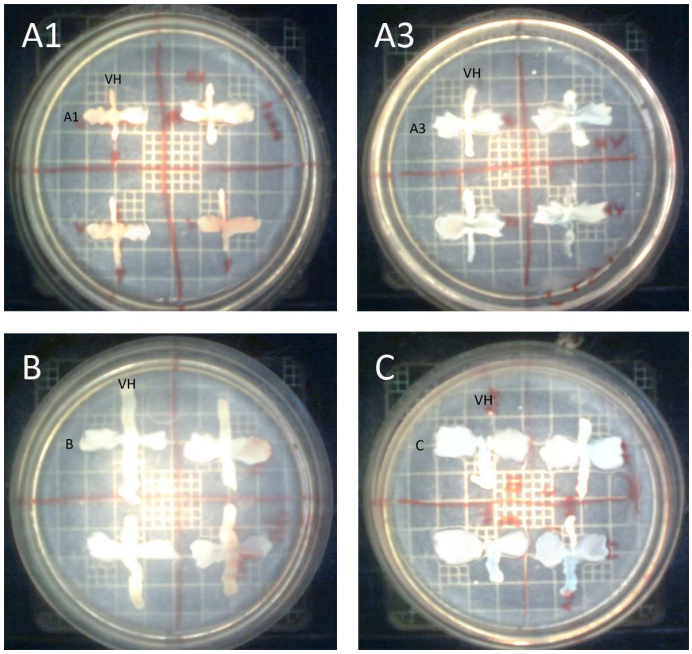


Figure 1: Cross streak tests show no considerable inhibitory effects for all isolates although noticeable colonization effects were observed. Vertical streaks are *V. harveyi* (VH) while horizontal streaks are those of the isolates (A1, A3, B, and C).

Figure 2: Ratio of the population of isolate A1 against *V. harveyi* in co-culture over 36 hours

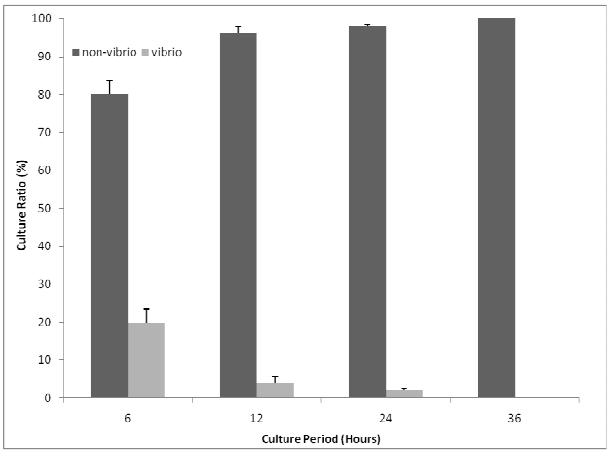
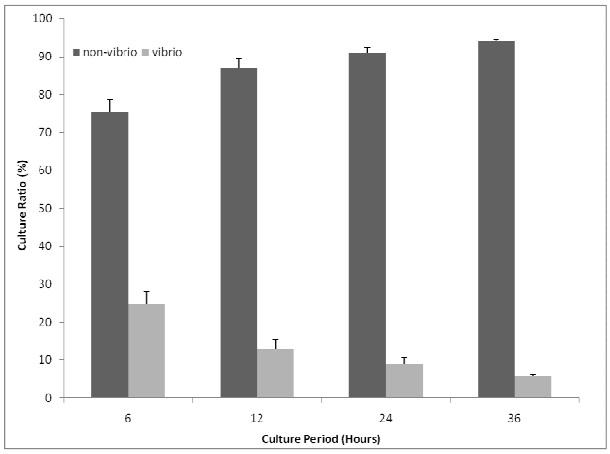


Figure 3: Ratio of the population of isolate A3 against *V. harveyi* in co-culture over 36 hours



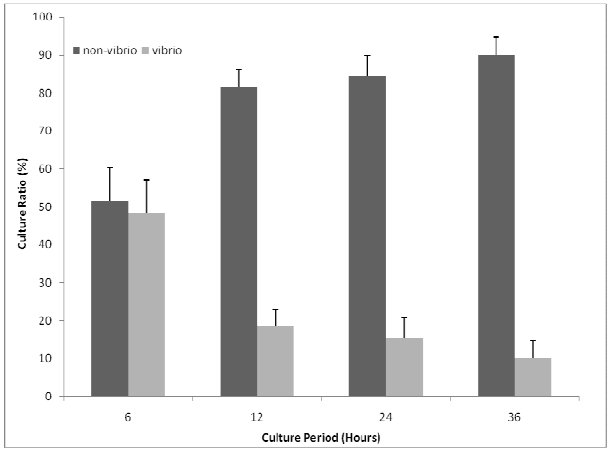


Figure 4: Ratio of the population of isolate B against *V. harveyi* in co-culture over 36 hours

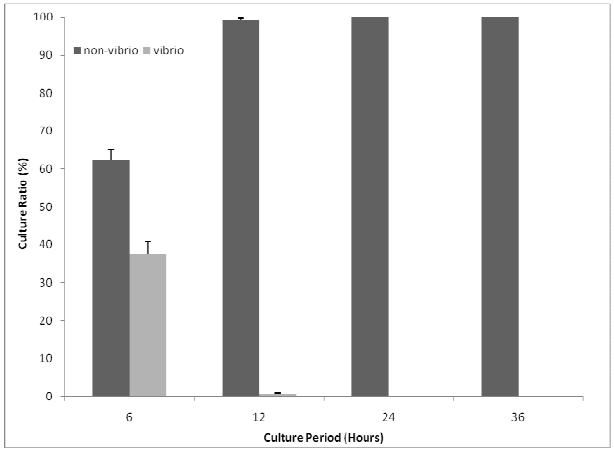


Figure 5: Ratio of the population of isolate C against *V. harveyi* in co-culture over 36 hours

Results of the co-culture tests show a significant reduction in the differential plate counts *for V. harveyi* against all bacterial isolates. Significant reductions of *V. harveyi* ranging from 69.3 – 100% of the bacterial culture were observed within 36 hours of inoculation (Figures 2-5). Among all the bacterial isolates, A1 and C showed the greatest reducing effect on the population of *V. harveyi* with reductions of up to 100% within 36 and 24 hours respectively altough these were not significantly different from isolates A3 and B. These observations indicate the potential of all the isolates to act as potential *Vibrio* control agents through rapid colonization of culture environments.

Results of this study are similar to those of Rico-Mora et al. [10] wherein a selected bacterial strain prevented the establishment of a *Vibrio alginolyticus* strain in cultures of the diatom *Skeletonema costatum*. In the study, no *in vitro* inhibitory effects were observed suggesting that the mode of action in the control of *V. alginolyticus* was through the competitive consumption of available nutrients in the environment. Verschuere *et al.* [13] suggested that the microbial community in aquatic environments is generally dominated by heterotrophic bacteria that compete for organic substrates as sources of energy and carbon. The ability of one bacteria to outcompete another species may lie in its capacity to utilize available nutrients in the water more efficiently and rapidly than others.

Aside from competing for available nutrients in the environment, another mode of action for bacterial strains to prevent the colonization of other populations is through competition for adhesion sites in the environment. Several species probiotc bacteria effect their beneficial roles through this mechanism. The adhesion of such strains of bacteria in the intestinal walls of aquatic organisms for instance prevent further colonization by other pathogenic bacteria. In the external environment of fish, such bacteria could also compete for adhesion sites on various substrates such as detritus and sediments [7].

As an integral part of microbial communities in aquatic ecosystems, bacteria could also have a complementary role in green water technology systems. The presence of co-existent bacteria in samples of green water indicated that there was a mutual relationship between these bacteria and microalgal strains present in the water although it was not established which of the two microorganisms produced extracellular substances for the suppression of *Vibrio* species [11]. The presence of these strains of bacteria could nonetheless play a significant role in competing for available nutrients and space in the culture environment.

As one of the potential sources of bacteria in the environment, the gastrointestinal tract of cultured organisms becomes a good candidate in screening for the presence of bacteria with probiotic characteristics [4]. The dominance of these strains in the intestines of fish indicates their viability to proliferate in the aquatic environment [5]. As results of this study show, gut isolates from *S. guttatus* not only survived well in nutrient substrates that simulate the external environment of the fish gut but also dominated bacterial populations thus controlling the presence the pathogenic bacteria *V. harveyi* through competitive exclusion.

CONCLUSION

This study showed the potential of bacteria isolated from the gut of S. guttatus to act as a possible control agents against *V. harveyi*. Although no inhibitory effects were observed in the cross streak tests, the control mechanism of the gut isolates could come from the more efficient and rapid competition for available nutrients and space in the culture environment. This was exhibited in the colonization effect observed in the cross streak test and the reduction in *V. harveyi* populations in the co-culture tests. All bacterial isolates exhibited these characteristics with isolates A1 and C showing the fastest rates in controlling *V. harveyi* in co-culture. The ability of these isolates to proliferate on NA and nutrient broth (amended with 30% NaCl) also indicates the capacity of these bacteria to survive outside the gastrointestinal tract of the fish. This could hint at their possible contribution in controlling the presence of *Vibrio* species in green water systems that utilize *S. guttatus*.

Further studies are recommended in order to elucidate on the actual mechanisms involved in the ability of these isolates in controlling *V. harveyi* and other potential pathogens. Identification and characterization of the bacterial isolates through molecular techniques will also help in determining their role and presence in green water technology systems and their potential as putative probionts in other aquaculture systems.

REFERENCES

[1] Buntin N, Chanthachum S and Hongpattarakere T, *Songklanakarin J Sci Technol*, **2008**, 30:141-148.
[2] Colwell R and Liston J, *Pacific Science*, **1962**, 16:264-269.
[3] Corre VL, Janeo RL, Caipang CMA and Calpe AT, *Philippine Council for Aquatic and Marine Research and Development – Department of Science and Technology Currents*, **1999**, Volume 1. No. 2. 32 pp.
[4] Deepa S, Bharathidasan R and Panneerselvam A, *Adv Appl Sci Res*, **2012**, 3(2):895-899.
[5] Ghosh S, Sinha A and Sahu C, *Bamidgeh*, **2007**, 59(3): 127-132.
[6] Lertcanawanichakul M and Sawangnop S, *Walailak J Sci & Tech* **2008**, 5(2): 161-171.
[7] Moriarty DJW, *Aquaculture*, **1998**, 164:351-358.
[8] Nagashima YA, Sendo K, Shimakura K, Shiomi T, Kobayashi T, Kimura B and Fujii T, *Journal of Fish Biology*, **2001**, (58) 6: 1761-1765.
[9] Purivirojkul W and Areechon N, *Kasetsart J. (Nat. Sci.)*, 2007, 41: 12 –132.
[10] Rico-Mora R, Voltolina D and Villaescusa-Celaya JA, *Aquacult Eng*, **1998**, 19:1–6.
[11] Sakata T, Yoshikawa T, Maeda K and Del Castillo C, *Mem. Fac. Fish. Kagoshima Univ*, **2005**, 55: 35-43.
[12] Tendencia E, dela Peña M and Choresca C, *Aquaculture*, **2006**, 260: 1-4.
[13] Verschuere L, Rombaut G, Sorgeloos P and Verstraete W, *Microbiology and Molecular Biology Reviews*, **2000**, 64:655-671.