Biofilm Producing Vibrio Species Isolated from Siloso Beach, Singapore: A Preliminary Study

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Abstract

This research aims to preliminarily investigate biofilm production among Vibrio species isolated from Siloso beach, Singapore. 100 µL of sea water sample collected was plated on Congo Red Agar (CRA) using spread plate method. The plates were incubated at 30°C for 24 hours. Forty-six biofilm producing black colonies with dry crystalline consistency were further transferred aseptically into freshly prepared Thiosulfate-Citrate-Bile salts-Sucrose agar (TCBS), a selective medium for isolation of Vibrio spp. All the isolates were found to grow on TCBS after 24 hours incubation at 30°C. Biofilm production in marine bacteria is of significance important to public health. The result of this preliminary study speaks volume of the health-related danger in recreation water bodies.

Introduction

Vibrionaceae, a marine bacteria family are Gram negative, facultative, non spore forming “comma” shaped rod with eight genera at present. They are mostly found in fresh and salt water. Vibrio cholerae is the most studied member of the family. This is as a result of virulence nature of the bacterium in causing cholera. Outbreaks of cholera diseases occur yearly with an estimated rate of almost 5 million cases while death rates is over 100,000 [1]. One of the key factors to these death tolls is short incubation period of 2 hours to few days between the time of ingesting the bacterium via contaminated water or food and disease outbreak. This pathogen can be classified as toxin producer and non-toxin producer [2]. However, non-toxin producers may harbor virulence genes. V. cholerae expresses quite a number of virulence factors needed to establish disease and infection in a host [3]. Biofilm production in V. cholerae is required for attachment to host cells [4, 5]. Biofilms are irreversible congregation of microbial cells attached to one another and surfaces as a result of formation of extracellular polymeric substance-EPS [6, 7, 10]. Production of biofilm in bacteria isolates from both clinical and environmental samples have been described [11, 12]. Silva and Benitez [13], investigated expression of biofilm production alongside other virulence factors in V. cholerae. Interrelationship of biofilm formation in V. cholerae and survival of host defense have reported [2]. In 2004, Heithoff and Mahan revealed signal pathways required for the production of EPS in the bacterium [14]. However, the first stage required in microbial biofilm research is detection of slime and biofilm production. At least three methods have been mostly utilized till date. Congo Red Agar method was developed by [15]. Tube method by [16] and Microplate method by [17]. Public health implication and significance of microbial biofilm formation among clinical and environmental bacterial isolates cannot be underestimated due to substantial increase in resistance to antibiotics. This research therefore aims to investigate biofilm production among Vibrio spp. isolated from marine environment using Congo Red Agar method.

Materials and Methods

Sample collection, isolation of Vibrio spp. and biofilm screening water sample was collected during a day educational trip to Sentosa Island, Singapore in July 2011. This was then transported to the laboratory for processing. 100 µL of water sample was spread on Congo Red Agar following the method of [6] with modifications. CRA consist of 5% w/v sucrose, Brain Heart Infusion Broth 3.7% w/v, Bacto agar 1% w/v and 0.1% w/v Congo red (indicator dye). Plates were incubated at 30°C for 24 hours. Dry but black crystalline colonies were further agar purified and stored. Biofilm positive colonies were streaked on TCBS and incubated at 30°C for 18-24 hours.

Results and Discussion

Forty-six biofilm positive bacteria were isolated after...
24 hours incubation. The colonies exhibited black dry crystalline consistence, a typical cultural characteristics of biofilm producing bacteria [6, 9, 10, 18] as seen in Illustration 1a and b. Biofilm producing bacteria constitute over 60% of diseases causing pathogens [19]. Increase in antibiotics resistance has been attributed to formation of biofilm matrix [17], helps in circumventing host defense mechanism and initiation of various infections [12]. Biofilm production in V. cholerae is required for attachment to host cells [4, 5]. They are irreversibly congegated of microbial cells attached to one another and surfaces as a result of formation of extracellular matrix [6]. All the isolates displayed yellow colorations on TCBS plates, a typical colony characteristic of Vibrio spp. Presence of Vibrio spp. in water body poses danger to public health as the bacteria could cause infections such as cholera in case Vibrio cholerae in human and can also infect marine life inhabitant such fish. Sentosa Island is a popular recreational centre in Singapore for both local and international tourists. Many of the tourists spend most of their in the beach refreshing themselves. From the result of this study, it could be seen that open seas water for bathing or other recreational purposes could pose danger to human health and marine life. In the case of international tourists, it can be a vehicle of transmitting pathogens from one country to another especially if no disease or infection symptoms were noticed before departure of the infected tourist. In the case of this, it can result in socio-economic lost to Singapore due to tourists’ avoidance of the beach.

Conclusion

Hence, precautionary measure should be taken while visiting recreational resorts. Further research work on antimicrobial resistance to commercially available antibiotics, virulence and biochemical characterization of isolated Vibrio spp. are strongly required.

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Illustrations

Illustration 1

1a and b: 24 hours culture on Congo Red Agar

Illustration 2

Pure biofilm positive culture.
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