Usage Of Gelatin-Virus Balls And Liquid Virus Filled Gelatin Capsules To Control Coral Reef Diseases: Model For Phage Therapy

Bhagwan N. Rekadwad, PhD
Chandrahasya N. Khobragade, PhD
School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, India

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Abstract

Coral reefs are very sensitive to environmental pollution. Coral reefs frequently get infected by various bacteria, fungi, marine algae and protozoa. The diseases include Bacterial Infections (BI), Fungal Infections (FI), Black Band Disease (BBD), Black Overgrowing Cyanophyta (BOC), Black Aggressive Band (BAB), Lethal Orange Disease (LOD), Skeleton Eroding Band (SED), PEYssonnelia (PEY), PNEophyllum (PNE) and White Syndromes (WS). Here in we have proposed a proposed model in which cold water soluble gelatin will be used to prepare Gelatin virus balls (GVB) and Liquid virus filled and sealed gelatin capsules (LVFSGC). GVB and LVFSGC will be prepared as per standard protocol in the form of paintballs and capsules. Above mentioned infecting agents of Coral reefs will be used as inoculum for production their production on the pilot scale. These produced infecting agents will be added with specific viruses of infecting agent (host-specific viruses). After the lysis of cell (naturally/artificially), lysate containg host-specific viruses will be used as infecting viruses to the Coral reef infecting agents. This lysate will be used for preparation of GVB and LVFSGC. These paintballs and capsules contain host-specific viruses can be made to release on a surface of sea water and dispersed on affected coral reefs zone naturally by Sea water current/waves. The dispersed viruses from GVB and LVFSGC will attach to their host. Ultimately, the disease-causing agent may be killed and the coral reef infection will be removed from sea water without any harm to the environment. GVB and LVFSGC will be used for the release of viruses against disease-causing agents. The GVB and LVFSGC will systematically kill and save the coral reefs.

Keywords: Corals; Cold water soluble gelatin; Phages; Sea Bed; Viral DNA, biological pollutants

Introduction

Coral reefs provide coastal protection, supply food and natural products useful in pharmaceutical and cosmetic industries and provide foreign currency to the country by attracting the tourists from the rest of the world. Thus, Coral reefs play a key role in the economy of many countries having coastal region. Coral reefs have hundreds of species in a natural marine ecosystem (Kelman et al., 2006; Charpy et al., 2012. Saxena, 2015; Rekadwad and Khobragade, 2015). The demand of coral reefs increasing steadily due to their useful natural products. As the economy of any country grows, the adverse impacts slowly get imprinted on the ecosystem (Hunter et al., 1995; Haapkyla et al., 2007; Olsen et al., 2015). The marine ecosystem nowadays gets contaminated by various types of pollutants. Damage caused by both the physicochemical and biological pollutants to some extent can be controlled by adopting preventive measures (Nicolet et al., 2013). On the other hand, those host-specific biological pollutants are very harmful than any other pathogens which include bacteria (Vidaver et al., 1973; Comeau et al., 2005; Kulakov et al., 2009), fungi (Barrero-Canosa et al., 2013; Mann et al., 2014) and marine algae (James, 2012). Few species of protozoa also cause harm to coral reefs. All these pathogens also have their specific infection-causing agents called host-specific viruses. They systematically kill their host by utilizing host synthesizing machinery without any harm to useful biota and will not be a cause for the negative change in native ocean environment (Pratte, 2014).

In the present study, an attempt has been made to design a virtual experiment to save coral reefs from the variety of infections. Host-specific viruses encased in Gelatin virus balls and Liquid virus filled and sealed gelatin capsules may kill specifically the host's infecting organisms.

Material and methods

Isolation of organisms infecting coral reefs and cultivation of viruses in laboratory

Infected Coral reef species is a good source of infection-causing organisms and can be isolated using the specific medium. Nutrient broth will be used for isolation of bacteria, potato dextrose broth, and malt medium will be used for isolation of fungi, Bold's basic medium and Bristol's medium will be used for cultivation of algae while protozoa will be cultivated in serum-free and serum containing media for the isolation and enrichment purposes (Atlas et al., 2010; Freshney, et al., 2005). In enrichment medium, host-specific viruses will be added. For example, bacteriophage phi6 and phiKMV-like viruses will be added for Pseudomonas, *Vibrio* phage will be added for *Vibrio*, cyanophage, algal viruses will be added for *Chlorella* and *Ostreococcus* etc. These viruses will attack their host. When cell burst, the

new progeny of viruses will be released in the medium. The released viruses again infect the new host cell and the cycle will be repeated. Thus, culture medium has a huge number of cultivated viruses (Bosch et al., 2015).

Isolation of viruses

The enriched media prepared as above will be used for isolation of viruses. After incubation, enriched broths/culture media will be cold lysed. The enriched cultures added with lytic enzymes such as trypsin and collagenase are allowed to stand for 1-2 hr in shaking incubator (at 100 rpm) at 4 ^{o}C. From these treated cultures, cell debris will be removed by centrifugation. The clear supernatant will be filter sterilized using bacterial filters. This filter sterilized supernatant directly will be used as virus suspension (Matsushita et al., 1995).

Preparation of Gelatin Virus Balls and Liquid virus filled and sealed gelatin capsules

Virus suspension will be used to prepare Gelatin virus balls (GVB)/ Liquid virus filled and sealed gelatin capsules (LVFSGC).

Preparation of GVB

GVB will be prepared using cold water soluble gelatin. Briefly preparation will be done by drying and aqueous solution containing virus suspension, gelatin and a hydrolyzed corn starch having dextrose, an acid (fumaric, citric, malic, adipic, ascorbic, tartaric, succinic, and phosphoric acids), and surface active agent containing a polysorbates, hydroxylated lecithin, acetylated monoglycerides, succinylated monoglycerides, ethoxylated mono- and diglycerides, sodium stearoyl 2-lactylate. These surface active agents render less drying period and more easily dispersible in cold water. GVB could be prepared (each has weight 100 g or as per requirement) by drying at reduced pressure (50 to 100 millimeters of mercury) within a relatively narrow temperature range to 37-70 ^{o}C. These GVB dried at elevated temperature (80-90 ^{o}C) for 5-10 minutes.

Preparation of LVFSGC

Similarly, the cold water soluble gelatin will be used to prepare Liquid virus filled and sealed gelatin capsules (LVFSGC) containing virus suspension. Also, LVFSGC will be prepared by using gelatin paintballs, surface and non-porous gelatin capsules coated with Eudragit L100. Prepared GVB and LVFSGC can be used for field experimentation to control coral reef infections (Brown et al., 1986; Cole et al., 1999; Guo et al., 2002).

Liberation of Gelatin Virus Balls (GVB) and Liquid Virus Filled and Sealed Gelatin Capsules (LVFSGC) on the Sea water surface

The area to be treated is marked by Global Positioning System (GPS). The total area is again divided into equal squares (5 meters each side). If required the area may be increased or reduced depending on experimental requirements and size of GVB and LVFSGC. After mapping the area, more than one GVB or LVFSGC or both may be dropped as per requirement (Fig. 1-2). These GVB and LVFSGC will be passed through Sea water bed by gravitational force to the Sea bottom. This allows the release of viruses in the Sea water.

Results and discussion

Action of Viruses on Coral Reef pathogens

Immediately after the release of Gelatin virus balls (GVB) and Liquid virus filled and sealed gelatin capsules (LVFSGC) will be dissolved in Seawater. This results in the release of viruses in Sea water (Fig. 3).

The released host-specific viruses attach to their host host-specific receptors and cause the infection in their host. The viruses will grow and multiply in an infection-causing organism. The progeny viruses will be released periodically when each host cell lyses and again cause the infection to the new host cell. Therefore, the host will be killed systematically and Coral reef infection will be recovered. The recovery of Coral reefs may be directly proportional to the elimination of infection.

Specifications of data

Subject area	*Biology*
More specific subject area	*Microbiology, Marine Microbiology and Biotechnology*
Type of data	*Figure*
How data was acquired	*Constructive model*
Data format	*Raw*
Experimental factors	*Not applicable*
Experimental features	*Virtual model developed for treatment of coral reef diseases*
Data source location	*Swami Ramanand Teerth Marathwada University, Nanded-431606, Maharashtra, India*
Data accessibility	*Within this article*

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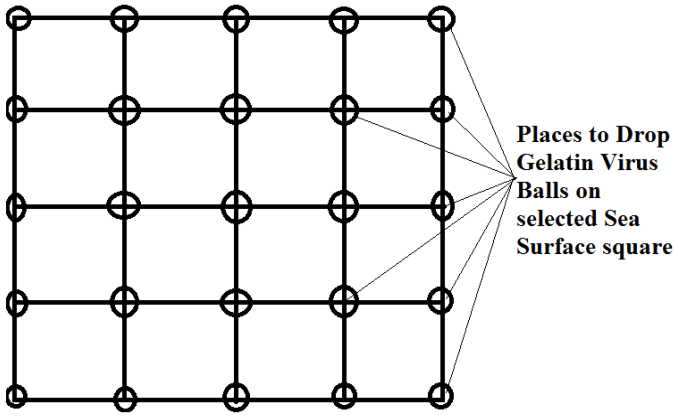

Fig.1 Global Positioning System (GPS) mapped area for release of GVB and LVFSGC on Sea Surface.

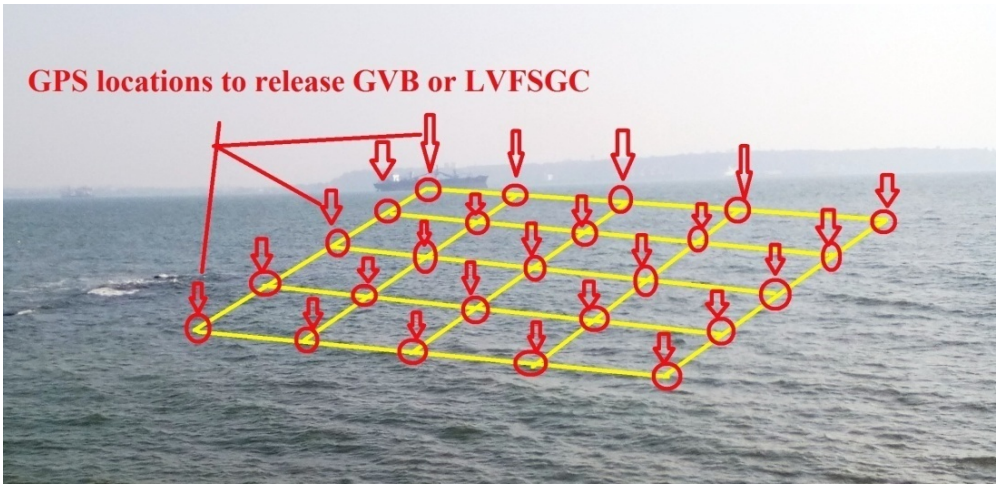

Fig. 2 Liberation of GVB or LVFSGC on GPS mapped Sea surface.

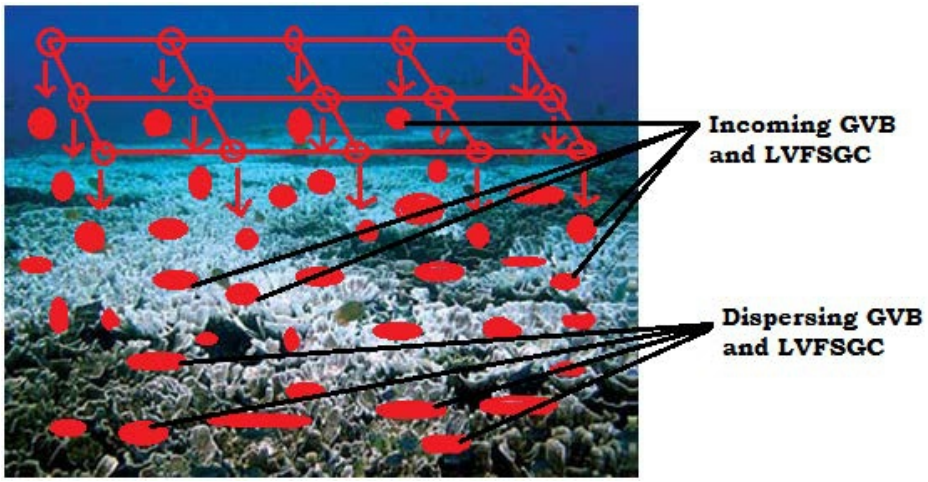

Fig. 3 Dispersion of released GVB or LVFSGC beneath Sea surface (modified and adapted from Raymundo et al. 2008)