Microbial Biofouling: A Possible Solution to Treat Harmful Microorganisms in Ship Ballast Water

Bhagwan Rekadwad and Chandrahasya Khobragade

Abstract Ships carry huge quantities of seawater in a specially designed ballast water tank required to sink in the sea. While loading cargoes, stored ballast water is discharged from ballast water tanks and vice versa. This compulsory exchange of ballast water is taking place for the past many decades. The movement of ballast water results in extensive transport and exchange of billions tons of national water in a global ocean along with many animals, plants, and microorganisms (especially pathogenic microorganisms such as bacteria, fungi, and pathogenic yeast). Ballast water exchange and transport results in invasion of unnecessary species into new environment. They accommodate into a new environment and utilize every available natural resource and become dominant. The complete eradication and removal of dominating pathogenic microorganisms is becoming a close to impossible task. These pathogenic species can be removed or their growth can be suppressed using halophilic antibiotic producers, halophilic bacterial bionts from marine invertebrates, and the use of mobile genome (host-specific bacteriophages/viruses). Only such natural methods will eradicate the pathogenic microorganisms from ballast water tank and will clean it. Moreover, the use of antipathogenic microorganisms checks microbial biofouling/invasion of new species through eco-friendly means.

Keywords Ballast water • Biofouling • Microbial invasion • National water • Open ocean

1 Introduction

Biofouling is defined as the unwanted and undesirable accumulation of a biotic deposit on man-made surfaces such as on the ship external jacket, in ballast water tanks, and in pipes and pipe-like structures. Biofilm-forming microorganisms such as pathogenic and nonpathogenic bacteria, fungi, and algae and biofouling by organisms like hydroids, barnacles, tubeworms, and bivalves on submerged

B. Rekadwad (✉) • C. Khobragade
School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded 431606, India
e-mail: rekadwad@gmail.com

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surfaces and in ship ballast tank pose a serious problem (Oliver 2010; Flemming and IWW 2011). The biofilm-coated submerged surfaces, pipes, and pipe-like parts of a ship in an aquatic milieu have harmful bacteria, microalgae, and pathogenic protozoan species called "disease hive" which can be removed using physical and chemical means. But one can't deny the risk of aquatic microbial invasions in ship ballast water tanks in the form of pathogenic biofilms (Drake et al. 2005). World common harbors are the places where ship has to be ballasted/de-ballasted. Through this routine and compulsory process, almost all ships acquired and contain most similar population of dominated and detrimental biofilm at the base, submerged in ballast tanks, or as deposited cover or sediments in a corner and pipes. This indicates that one ship is sufficient to seed other waters (Baier et al. 2014). Since many decades (nineteenth century), ship has used and exchanged ballast water at the common harbors and during succession. Ship safety and stability is the only reason for the exchange of ballast water at the harbors and in the Open Ocean. The causes of exchange may be one of the following: ballast water required for propulsion of ships, to acquire weight of lost and/or consumed fuel or water, and to sink in the ocean. On the other hand, from the same time period ship already exchanged a huge quantity of National water in shipping routes through their ballast water tanks. This activity of ballast water exchange results in biological invasions of many aquatic biota and pathogenic microbiota, which feeds on aquatic organisms or causes harm to human populations. These include toxic dinoflagellates, *Vibrio cholerae*, and numerous heterotrophic aerobic bacteria (HPC), etc. (Drake et al. 2007). This chapter describes a possible solution to treat and eradicate harmful microorganisms in the ship ballast water tank.

2 Halophilic Antibiotic Producers

Polyextremophilic microbes thrive under new extremes such as high/low temperature, high pressure/salt concentration, low quantity of nutrients, oxygen level below required level, and presence of adverse chemicals, etc., and have to compete with other microorganisms and enemies for their survival (van der Wielen et al. 2005; DasSarma and DasSarma 2012). These microbes secrete antibiotics and antibiotic-like compounds having inhibition potential which is necessary to maintain their constant growth and to fight infections (Kalia 2014a). Halophilic organisms have the potential to produce a variety of antibiotics (Oren 2010; Ghosh et al. 2010; Kalia 2013) and antimicrobial pigments (Venil et al. 2013). The antibiotics and pigments produced by a certain group of halophilic microorganisms such as *Haloarcula, Halobacterium, Halococcus, Haloferax, Halogeometricum, Halorubrum*, and *Haloterrigena* species have potential to kill or inhibit the different pathogenic bacteria (*Bacillus subtilis, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi*), fungi (*Aspergillus niger, Penicillium species*), *and* Yeast (*Candia albicans)*. Three identified strains of halophilic microorganism possess pVC1, pVC2, pVC3, and

megaplasmids (Schwartz 2009). Other plasmids that have plasmids include *C. israelensis* (pH 11, 48 kb size), *Halomonas subglaciescola* (pHS1—70 kb size) (Huma et al. 2011), and *H. elongate* (possesses pMH1 plasmid) (Todkar et al. 2012). The organic extracts of some marine bacteria (Selvin et al. 2009; Aguila-Ramírez et al. 2014; Kalia and Kumar 2015) associated with the sponge *Aplysina gerardogreeni* belong to the genera *Bacillus, Micrococcus, Paracoccus, Pseudobacter, Pseudovibrio, Psychrobacter, Staphylococcus*, and *Terribacillus* showing antifouling (AF) activity (Plouguerné et al. 2008; Chambers et al. 2011; Thabard et al. 2009; Plouguerné et al. 2010; Kumar et al. 2015). These diverse microbes have roles in ballast water treatment.

3 Use of Halophilic Bacterial Bionts from Marine Invertebrates for Ballast Water Treatment

The organisms in marine ecosystem including invertebrates such as bryozoans (moss animals) (Selvin et al. 2009), soft corals, sea fans and sea whips (coelenterates), nudibranchs and sea hares (mollusks), tunicates, echinoderms (starfish, sea cucumbers), and sponges (Selvin et al. 2009) are the biotechnologically as well as pharmacological important target groups having bacterial species (bacterial bionts). These produced compounds have structurally unique and effective secondary metabolites (Kijjoa and Sawangwong 2004). These natural products comprise ranges of chemical classes such as alkaloids, acetogenins, peptides, polyketides, and terpenes having pronounced pharmacological potential (Rodrigues et al. 2004; Wang et al. 2010).

In the limelight of available reports, bacteria isolated from bivalves, corals, and marine sponges of the Indian Ocean region have produced effective antibacterial compounds. It is reported that 100 heterotrophic halophilic bacteria species are isolated from nine sponges, five corals, and one bivalve species (Gnanambal et al. 2005; Anand et al. 2006; Chandran et al. 2009; Donio et al. 2013).

The crude broth (centrifuged broth supernatant) of the 46 isolated bacteria showed inhibitory activity (Kalia et al. 2011, 2014a, b; Kalia 2013) against pathogenic bacteria (*Aerobactor aerogenes, E. coli, Staphylococcus citreus, Proteus vulgaris, S. typhi*, and *Serratio marcescens*). Furthermore, obtained results have been confirmed in the presence of bioactive compounds by partially purified extracts (ethyl acetate extract) of crude supernatant. Surprisingly, highest numbers of bacterial bionts having pharmacologically important products (Tokunaga et al. 2008, 2010) show inhibitory activity against harmful human pathogens. Here, we mention that the bacterial bionts isolated are the richest producers of bioactive compounds. The diversity of marine organisms and the highly competitive, environmental habitats in which access to space and nutrients is limited are responsible for this stunning variety (Velho Pereira and Furtado 2012; Kalia 2014a, b, 2015; Kumar et al. 2013, 2015) and capability to flourish; said adverse habitats can be

used to treat ballast water by incorporating them into the ballast water tank. These microorganisms may be used for scaling up from benchtop to pilot plant scales for the production of antibiotics (Rezanka et al. 2010).

4 Use of Mobile Genome (Host-Specific Bacteriophages/ Virus) to Control Microbial Biofouling

"The host specific mobile genome showing host killing activity is known as Viruses". Viruses are the submicroscopic organisms that specifically attack only their host and timely kill them.

Viruses occupy the twilight zone that separates the "living" from the "non-living." They do not have a cellular organization and contain DNA/RNA either in single-stranded or double-stranded form but never both. They use the host cellular mode of replication system and hijack host synthesis machinery for their multiplication. They utilize host metabolic processes, product for production of new progeny virions either through lytic or lysogenic cycle. They can be classified based on their host such as animal viruses, plant viruses, bacteria viruses, archaeal viruses, algal viruses, and fungal viruses (Rohwer and Breitbart 2005; La Scola et al. 2008).

Ballast water is the source of many invasive animal, plant, algae, and pathogenic bacteria and fungi. These include bacteria (*A. aerogenes, B. subtilis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus, S. citreus, S. marcescens, S. typhi, V. cholera*), fungi (*A. niger*, *Penicillium* species), and yeast (*C. albicans*). Each of these microorganisms is capable of infecting ballast water tanks and causing serious infections in human beings in the place where de-ballasted. Likely, each of these pathogenic organisms has their natural enemies in the form of bacteriophages and viruses (Table 1).

Effective control measures should be adopted to treat ballast water. The mobile genomes play a very important role to treat ballast water pathogens. The concentrated and active viruses can be used to treat ballast water. For this the colder water-soluble gelatin will be used to prepare Gelatin virus balls (GVB) and Liquid virus filled and sealed gelatin capsules (LVFSGC) (Yu et al. 2008). After loading of ballast water into ballast water tank, GVB and LVFSGC can be released into the ballast water tank (s) and will be slowly dispersed. The movement of ship causes uniform mixing and liberation and dispersion of viruses from GVB and LVFSGC will attach to their host. Ultimately, the disease-causing agent may be killed and the pathogenic infection will be removed from the ballast water tank without any harm to the environment (Freshney 2010; Atlas 2010; Bosch et al. 2015). All these processes should be done before being the ballast water discharged. Hence, the GVB and LVFSGC will be used for the release of viruses which systematically kill disease-causing agents.

Table 1 Bacteriophages and viruses of pathogenic bacteria inhabit ballast water

Microorganism	Viruses/Bacteriophages	References
Staphylococcus citreus	CoNS phages	Deghorain and Van Melderen (2012), Xia and Wolz (2014)
Staphylococcus aureus	φ11, φ80, and φ80α of serogroup B, φ81	Mašlaňová et al. (2013)
Proteus vulgaris	Phage 4a, 4b, 34a, 26a,36b, 21b, 21c, Fr2, Fr5	Schmidt and Jeffries (1974)
Serratio marcescens	KSP20, KSP90, KSP100, P2-like phage, T4-type phage, and phiEco32 phage, wIF3, SM701	Petty et al. (2006), Yu et al. (2008), and Matsushita et al. (2009)
Salmonella typhi	E1 ($n = 8$), UVS ($n = 5$), E1, *A* phage, biotype I, øSH19, Vi01-like phage family, Vi-phage-type, DT7a	Lalko and Gunnel (1967), NIID (1998), Goode et al. (2003), Atterbury et al. (2007), Trung et al. (2007), De Lappe et al. (2009), Pickard et al. (2010), Wall et al. (2010), Hooton et al. (2011), Lettini et al. (2014), Srirangara et al. (2015)
Aerobacter aerogenes	phi-mp	Souza et al. (1972)
Escherichia coli	T4 and T4-like phages	Brüssow (2005)
Vibrio cholerae	Vibriophage	Jensen et al. (2006), Bosch et al. (2015)
Klebsiella pneumoniae	Phage SS, *Myoviridae* phage (KP15 and KP27), *Siphoviridae* phage (KP16 and KP36), *Podoviridae phage,* T7-like phages (KP32)	Chhibber et al. (2008), Kęsik-Szeloch et al. (2013)
Pseudomonas aeruginosa	phi6 and phiKMV	Bosch et al. (2015)
Bacillus subtilis	SP5, SP6, SP7, SP8, SP9, SP13, SP3, SP10, PBS1, SP alpha, SP beta	Brodetsky and Romig (1965), Krasowska et al. (2015)
Ostreococcus	Prasinoviruses, OtV-2	Weynberg et al. (2011), Clerissi et al. (2012)
Aspergillus niger	Mycovirus, CSP, Virus-like particle (hepatitis B)	Plüddemann and Van Xyl (2003), Refos et al. (2013)
Penicillium sp.	PsV-S, PsV-F, AfV-S, AfV-F	Lemke and Nash (1974), Border et al. (1972)
Candida albicans	Hybrid phage displaying the Sap epitope VKYTS	Yang et al. (2007)

5 Perspectives

The USA and other countries including India, China, and Africa having Ocean regime proposed and adopted strict International rules to control or to reduce risks of microbial invasions. The current ballast water treatment measures do not have effective control over the ship ballast water microbial invasions. Moreover, additional care must be taken when using microbial anti-biofoulers to treat harmful

infections/microorganisms occupying space in the ballast water tank. It is an acceptable method because the use of halophilic antibiotics and pigment producers, halophilic bacterial bionts, and mobile genome (s) (GVB and LVFSGC) could be the most cost-effective, eco-friendly, and alternative biotherapy that definitely achieves an acceptable hygiene (cleanliness).

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