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Revealing the ability of a novel polysaccharide bioflocculant in bioremediation of heavy metals sensed in a *Vibrio* bioluminescence reporter assay☆

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ABSTRACT

A bioflocculant-producing bacterial strain, designated MSI021, was isolated from the marine sponge *Dendrilla nigra* and demonstrated 94% flocculation activity in a kaolin clay suspension. MSI021 was identified as *Bacillus cereus* based on phylogenetic affiliation and biochemical characteristics. The purified extra-cellular bioflocculant was chemically elucidated as a polysaccharide molecule. The polysaccharide bioflocculant was stable under both acidic and alkaline conditions (pH 2.0–10.0) and temperatures up to 100 °C. The purified bioflocculant efficiently nucleated the formation of silver nanoparticles which showed broad spectrum antibacterial activity. The ability of the bioflocculant to remediate heavy metal toxicity was evaluated by measuring the inhibition of bioluminescence expression in *Vibrio harveyi*. Enrichment of heavy metals such as zinc, mercury and copper at concentrations of 1, 2 and 3 mM in culture media showed significant reduction of bioluminescence in *Vibrio*, whereas media enriched with heavy metals and bioflocculant showed dose dependent improvement in the expression of bioluminescence. The assay results demonstrated that the polysaccharide bioflocculant effectively mitigates heavy metal toxicity, thereby improving the expression of bioluminescence in *Vibrio*. This bioluminescence reporter assay can be developed into a high-throughput format to monitor and evaluate of heavy metal toxicity. The findings of this study revealed that a novel polysaccharide bioflocculant produced by a marine *B. cereus* demonstrated strong flocculating performance and was effective in nucleating the formation antibacterial silver nanoparticles and removing heavy metals. These results suggest that the MSI021 polysaccharide bioflocculant can be used to develop greener waste water treatment systems.

1. Introduction

Heavy metal pollution due to industrialization and globalization is an important ecological problem because heavy metals exert deleterious effects on the progressive consumers in interactive networks such as ecological pyramids and on trophic levels, food chains and food webs. Anthropological activities such as the discharge of waste materials from various industries and mining processes have caused heavy metal accumulation in aquatic systems, soil, and air (Chisti, 2004). Heavy metals even at low concentrations now envisaged as deleterious to all life forms and environment through continuous bioaccumulation leads to the level of critical concentration (Liu et al., 2017; Swarnalatha and Nair, 2017). Heavy metals accumulation causes serious lethal effect to all life forms and enter into the food chain through the waste disposal to the water and soil (Farooq et al., 2010). The increased industrial establishments caused heavy metal accumulation in sediments which ultimately leads to bioaccumulation in food chain through water, plants and environment (Zhou et al., 2016; Gąsiorek et al., 2017; Galala and Shehatab, 2017; Liu et al., 2017; Swarnalatha and Nair, 2017). The sources of drinking water supplies including ground water is remaining unsafe for human use due to high levels of heavy metal pollutants (Water

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quality, 2013). Among the various sources of pollutants, heavy metals are being a potential health hazard due to their ubiquitous persistence and non-biodegradable nature (Bourdineaud, 2010). Deleterious effect of heavy metals on physiology and growth of microbial cells was recently reviewed by Liu et al. (2017) and recommended to develop suitable methods to enlarge bioavailability of heavy metals to attain increased bioremediation rate. The impact of heavy metal pollution in human health was well-documented include DNA damage, mutation and altered gene expression, and damages in vital organs such as kidneys, lungs, liver and heart (Thomas et al., 2009; Jomova et al., 2011). Thus, it is important to develop nontoxic and environmentally benign substances to remove toxic heavy metals from natural habitats and drinking water purification systems.

Flocculation is the process involving the formation of floc via the aggregation of colloids and other suspended particles in a suspension with the aid of chemical (flocculant) stimulation. At present, flocculating agents are important in various industries including waste water treatment, drinking water purification, downstream processing and food fermentation (Sathiyanarayanan et al., 2013). Three types of flocculating agents have primarily been reported: inorganic flocculants, synthetic flocculants and natural flocculants (Kurane et al., 1986; Xia et al., 2008). The chemically synthesized flocculants are toxic and causes a serious of health issues and they are not readily degradable (Zhai et al., 2012). Hence bioflocculant gained increased attraction due to environmental friendly and effective removal of suspended solids, heavy metals, dye removal from the contaminated waste waters (Guo, 2015). The chemical composition of a bioflocculant includes polysaccharides, proteins, glycoproteins and nucleic acids. Among these, polysaccharide-based bioflocculants have received significant attention given their prominent efficiency for removal of dyes and heavy metal and pollutant disposal (Deng et al., 2005; Yim et al., 2007; Ozdemir, 2003). Mechanism of heavy metal removal by bioflocculant was envisaged as a formation of floc with heavy metals. The floc formation was depend on ionic groups such as amino and carboxyl in the bioflocculant which facilitate metal-floc interaction. The metal-binding interactions were influenced by various factors include physico-chemical properties of heavy metals, proper positioning with the binding site, ionic strength and tertiary structure of floc (Deng et al., 2005). The industrial and biotechnological use of bioflocculants has been the subject of significant research attention because of their biodegradability and non-toxic nature (Salehizadeh and Shojaosadati, 2001). Mao et al. (2011) extracted a novel biopolymer from *Bacillus cereus* for dye removal and used molasses as a substrate. Bioflocculants can act as stabilizing and reducing agents for silver nanoparticle synthesis (Sathiyanarayanan et al., 2013). The antibacterial activities of silver materials can be harnessed for the development of anti-infective agents (Ulkur et al., 2005), the dissolution of biofilm formation on catheters (Rupp et al., 2004), the elimination of microorganisms on textile fabrics (Yuranova et al., 2003) and as disinfectants in water purification systems. In general, bioflocculants produced by bacteria have demonstrated potential applications in the removal of heavy metals (Lin and Harichund, 2012; Zhang et al., 2012; Subudhi et al., 2016).

Marine ecosystems cover more than 70% of the earth's surface, comprising considerable chemical and biological diversity and representing a limitless resource for food, agro-chemicals, pharmaceuticals, enzymes, molecular probes and many living species. Various species found in marine environments have been recognized as valuable resources for novel bioactive compounds. In particular, marine sponge-associated microorganisms have demonstrated a broad range of biosynthetic capabilities and are able to produce bioactive secondary metabolites (Mehbub et al., 2014; Selvin and Lipton, 2004). In this study, a polysaccharide-based bioflocculant produced by a marine sponge associated bacteria *Bacillus cereus* MSI021 was characterized and evaluated for utility in the synthesis of silver nanoparticles and the removal of heavy metal pollutants. The bioflocculant was thermostable, alkali-tolerant and effective in the removal of heavy metals as assessed using a *Vibrio* bioluminescent reporter assay.

2. Materials and methods

2.1. Isolation of bacteria

Specimens of the marine sponge *Dendrilla nigra* were collected from the southeast coast of India via SCUBA diving (at a depth of 18 m, 25°01 S, 45°06 E). To remove loosely associated microorganisms from the outer and inner surfaces of the sponge specimens, samples were kept in sterilized aged seawater for 2 h. The isolation of sponge-associated bacteria was performed as per the method described in Gandhimathi et al. (2008). Briefly, 1 cm^3 of sponge tissue was excised from the sponge tissue using a sterile scissors, washed several times with sterile seawater and homogenized in phosphate buffered saline. The homogenate was serially diluted in sterile seawater, plated on various media including Zobell marine agar, nutrient agar with 2% NaCl, and starch yeast peptone-seawater agar, and incubated at 28 °C for three days. Based on colony morphology assessment, 44 distinct bacterial strains were isolated and stored at 4 °C for further studies.

2.2. Screening of bioflocculant producers

The isolates were inoculated separately in 50-ml Erlenmeyer flasks containing 10 ml of production medium consist of 8 g/l glucose, 0.4 g/l yeast extract, 1.5 g/l KH_2PO_4, 3 g/l K_2HPO_4, 0.2 g/l $(NH_4)_2SO_4$, 0.5 g/l urea and 1.0 g/l NaCl at pH 7, and then the flasks were incubated at 28 °C for 48 h. After incubation, cell-free supernatants (CFS) were obtained by centrifugation at 10,621 × g for 10 min. The CFS was assayed for flocculant activity as per the method described by Kurane et al. (1994). Briefly, 2.0 ml of CFS was added to 93 ml of kaolin suspension (4 g/l) containing 5 ml of 1% $CaCl_2$ and then the aliquot was vortexed (3 min) and incubated for 5 min at 28 °C. The optical density (OD) of the supernatant of the aliquot was measured at 550 nm. The CFS of uninoculated culture medium was used as a control. Flocculating activity was determined by the following formula:

$$\textit{flocculating activity} = \left\{\frac{A-B}{A}\right\} X\ 100$$

where A is the OD of the control and B is the OD of the sample.

2.3. Identification of bioflocculant producers

The isolate MSI021 was identified based on morphological, biochemical and phylogenetic analysis. DNA isolation was performed as per the method (Enticknap et al., 2006) with suitable modifications. PCR amplification was performed using the primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525 R (5′-AAGGAGGTGWTCCARCC-3′). The amplified product was excised from the gel and purified using a gel extraction kit following the manufacturer's instructions (Qiagen). Phylogenetic trees were constructed with MEGA software version 7.0 (http://www.megasoftware.net) using the maximum parsimony algorithm (1000 bootstrap replicates).