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Immobilization of algicidal bacterium *Shewanella* sp. IRI-160 and its application to control harmful dinoflagellates

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ABSTRACT

Shewanella sp. IRI-160 is an algicidal bacterium isolated from Delaware Inland Bays. It secretes water-soluble compounds that inhibit the growth of dinoflagellates. Previous research indicated that this bacterium does not have a negative impact on other algal species. In this research, *Shewanella* sp. IRI-160 was immobilized to different porous matrices, including agarose, alginate hydrogel, cellulosic sponge, and polyester foam. The retention of *Shewanella* sp. IRI-160 on or within these matrices was examined at 4 and 25 °C for 12 days. Results indicated that alginate was superior in terms of cell retention, with >99% of *Shewanella* cells retained in the matrix after 12 days. *Shewanella* sp. IRI-160 cells were then immobilized within alginate beads to evaluate algicidal effects on harmful dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum* at bacterial concentrations of 10^6 to 10^8 cells mL^{-1}. The effects on dinoflagellates were compared to non-harmful cryptophyte *Rhodomonas* sp., as well as the effects of free-living bacteria on these species. Results indicated that immobilized *Shewanella* sp. IRI-160 in alginate beads were as effective as the free-living bacteria to control the growth of *K. veneficum* and *P. minimum*, while no negative impacts of immobilized *Shewanella* sp. IRI-160 on the non-harmful control species *Rhodomonas* sp. were observed. Overall, this study suggests that immobilized *Shewanella* sp. IRI-160 may be used as an environmentally friendly approach to prevent or mitigate the blooms of harmful dinoflagellates and provides insight and directions for future studies.

1. Introduction

Harmful algal blooms (HABs) pose a threat to marine organisms and human health worldwide and are continuing to expand globally (Heisler et al., 2008; Glibert et al., 2018). Multiple approaches have been developed to prevent, control and mitigate HABs, including nutrient manipulation (Hagström et al., 2010), clay flocculation (Hagström et al., 2010), sonication (Park et al., 2017), and application of toxic chemicals such as copper sulfate (Anderson, 2009). Despite the effectiveness of these methods, they can be costly or raise concerns about negative effects on other organisms in the environment (Wang et al., 2012; Sun et al., 2018). To address these issues, biological approaches have been developed and applied to control HABs, including methods involving algicidal bacteria (*e.g.* Pokrzywinski et al., 2012; Tilney et al., 2014a; Sun et al., 2018; Hu et al., 2019).

Algicidal bacteria can control HABs by either direct contact or indirect interactions with the target algal species through secretion of algicidal compounds (Meyer et al., 2017). For instance, bacterium *Chitinimonas prasina* LY03 can attach to diatom *Thalassiosira pseudonana* and produce chitinase to degrade algal cell walls, leading to cell lysis and death (Li et al., 2016). Other bacterial species secrete algicidal compounds to control HABs without the requirement of direct contact (reviewed by Sun et al., 2018). For example, bacillamide secreted by bacterium *Bacillus* sp. SY-1 was effective against dinoflagellate *Cochlodinium polykrikoides* (Jeong et al., 2003), and the pigment deinoxanthin, isolated from bacterium *Deinococcus* sp. Y35, had a strong algicidal effect on dinoflagellate *Alexandrium tamarense* (Li et al., 2015). However, some bacteria-derived algicidal compounds have not yet been fully characterized or are difficult to isolate or manufacture. In this case, immobilization of algicidal bacteria within a porous matrix and deployment in areas that experience blooms may be an effective approach to mitigate HABs (*e.g.* Sun et al., 2015; Su et al., 2017).

Immobilization of bacteria onto or within a matrix has been extensively used for a wide range of applications, including alcohol (Wang et al., 2015) and food production (Guzzon et al., 2012), chemical degradation (Joshi and Abed, 2017), and wastewater treatment (Chanthamalee et al., 2013). Recently, immobilized bacteria have been applied to control HABs. For instance, bacterium *Pseudomonas*

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fluorescens HYK0210-SK09 was immobilized into several carriers to control the harmful diatom *Stephanodiscus hantzschii* and showed a higher activity against the target species compared to free-living bacteria (Kang et al., 2007). The advantage of using immobilized algicidal bacteria is the potential for continuous control of HABs without the need for frequent reapplication, as well as the ability to collect the immobilized bacteria for removal when no longer needed. Sun et al. (2016), for example, demonstrated that the algicidal bacterium, *Bacillus methylotrophicus* ZJU, immobilized with the addition of wheat bran and magnetic Fe_3O_4 nanoparticles, can be re-collected efficiently by magnetic means.

In 2005, an algicidal bacterium, *Shewanella* sp. IRI-160 was described by Hare et al. (2005). This bacterium, isolated from Delaware's inland bays, was able to control the growth of dinoflagellates, *Pfiesteria piscicida, Prorocentrum minimum*, and *Lavenderina fissa* (aka *Gyrodinium instriatum*), but had no negative effects on the growth of other algal taxa tested, including diatom, raphidophyte, prasinophyte, and cryptophyte species (Hare et al., 2005). Further study indicated that this bacterium secretes water-soluble algicidal compounds, designated as IRI-160AA, to control the growth of dinoflagellates without the requirement of direct bacteria-algae contact (Pokrzywinski et al., 2012). IRI-160AA exhibited a significantly greater effect on dinoflagellates *Karlodinium veneficum* and *L. fissa* in the exponential phase compared to the stationary phase, suggesting the potential application of this algicide for prevention and control of harmful dinoflagellate blooms during early phases of bloom development (Pokrzywinski et al., 2012). Studies showed that IRI-160AA had negative impacts on nuclear and chromosome structures in dinoflagellates (Pokrzywinski et al., 2017a), as well as on chloroplasts (Pokrzywinski et al., 2017a), PSII and photosynthetic transport chain (Tilney et al., 2014a, 2014b) of photosynthetic dinoflagellates. Cell death was also accompanied by DNA degradation, reactive oxygen species production, cell cycle arrest, and DEVD-ase activity, suggesting a programmed pathway leading to cell death (Pokrzywinski et al., 2017b). Ammonium and several amines were identified in the algicide, each of which may play a role individually or together with other compounds in the algicide to contribute to the algicidal activity (Ternon et al., 2019). Recently, IRI-160AA was tested on organisms at higher trophic levels, including copepods, fish, and shellfish. No negative impacts were observed at concentrations required to control the growth of dinoflagellates (Simons, 2019). This research provided further support for the application of this bacterium and its algicide as an environmentally neutral means to control HABs.

Although the algicidal activity and mechanisms of cell death for dinoflagellates exposed to IRI-160AA as well as its effects on non-target species have been extensively investigated (Hare et al., 2005; Pokrzywinski et al., 2012; Tilney et al., 2014a, 2014b; Pokrzywinski et al., 2017a, 2017b; Grasso, 2018; Simons, 2019; Ternon et al., 2019), there has been no research focused on the direct application of this bacteria to control HABs in the natural environment due to biosafety concerns. In this study, the retention of *Shewanella* sp. IRI-160 within several porous matrices (agarose, alginate hydrogel, cellulosic sponge and polyester foam) and the effects of immobilized bacteria on laboratory cultures of dinoflagellates were investigated. The retention of this bacterium within each matrix was also evaluated at two temperatures, 25 and 4 °C, to assess the effects of temperature for future applications. Furthermore, the algicidal activity of immobilized bacteria at different bacterial densities was determined using laboratory cultures of dinoflagellates, *K. veneficum* and *P. minimum*, and compared with the algicidal activity of free-living bacteria. Overall, the results of this study provide support for the application of immobilized bacterium *Shewanella* sp. IRI-160 to prevent and mitigate dinoflagellate blooms.

2. Material and methods

2.1. Attachment and immobilization of Shewanella sp. IRI-160

2.1.1. Bacterium culture preparation

Cultures of *Shewanella* sp. IRI-160 were prepared as described in Pokrzywinski et al. (2012). Briefly, *Shewanella* sp. IRI-160 was transferred from a single colony into liquid LM medium (Sambrook et al., 1989) and incubated overnight at 25 °C with shaking at 100 rpm. The optical density of bacterial cultures was measured on the following day, and cultures were diluted with liquid LM medium to an OD_{600} of 1.4 before use.

2.1.2. Immobilization and retention of bacteria in alginate beads

The methods of immobilizing *Shewanella* sp. IRI-160 into different matrices were modified from Kang et al. (2007). A 2% (w/v) solution of sterile alginic acid, sodium salt (catalog# 177772500; Thermofisher Acros Organics, Belgium) in MilliQ water was mixed with diluted bacterial culture with a 5:1 (v/v) ratio of alginate: bacterial culture. To make alginate beads, the mixture was extruded from a sterile syringe through sterile silicone tubing into a beaker of cold sterile 0.2 M $CaCl_2$ solution. The solution in the beaker was mixed at low speed during extrusion. The resultant alginate beads were approximately 5 mm in diameter. The beads were washed using sterile f/2 medium (- Si and a salinity of 20; Guillard and Ryther, 1962) twice and kept in f/2 medium before use on the same day. Prior to the start of the experiment, beads were transferred into f/2 medium (total volume of alginate beads: total volume of medium = 1:10) and divided into 2 groups (N = 3). One group was incubated at 4 °C and another group was incubated at 25 °C.

On Days 0, 3, 6, and 12, an aliquot of the medium surrounding the beads from each replicate was collected for cell counts using the method described below. Beads were also removed and dissolved in sterile 1% (w/v) sodium pyrophosphate in MilliQ water to release the bacteria, and cells were counted as below.

2.1.3. Immobilization and retention of bacteria in agarose cubes

Six percent (w/v) sterile low-melting agarose (catalog# S209-500; Fisher Scientific, Pittsburgh, PA) in MilliQ water was heated by microwave, cooled to room temperature and mixed with the bacterial culture at a ratio of 10:1 (v/v). The mixture was poured into sterile Petri plates to a thickness of 5 mm. The mixture in the covered Petri plates was allowed to solidify and then cut into small cubes of 75 mm^3 using sterile scalpels. The cubes were washed and prepared as described above (Section 2.1.2).

On Days 0, 3, 6, and 12, bacterial cells in the surrounding media were counted as below. Cubes from each replicate were then transferred into sodium acetate buffer (pH4.6; 49% 0.2 M sodium acetate [Fisher Scientific], 51% 0.2 M acetic acid [Fisher Scientific]), and heated at 90 °C for 10 min. Bacterial cells released from the agarose matrix were counted as described below.

2.1.4. Immobilization and retention of bacteria in cellulosic sponge and polyester foam

Cellulosic sponge (3 M, Maplewood, MN) and polyester foam (Danner Mfg. Inc., Islandia, NY) were cut into small cubes with volumes of approximately 0.125 cm^3 and 1 cm^3, respectively. Sponge and polyester cubes were autoclaved and each added to the diluted bacterial culture at a ratio of 1:10 (v/v). The mixture was incubated at 25 °C, with shaking at 100 rpm for 3 h, and then incubated at room temperature without shaking overnight. Cellulosic sponge and polyester cubes were washed using sterile f/2 medium twice and kept in f/2 medium before use on the same day. Cubes were washed and the experiment was prepared as described above (Section 2.1.2).

On Days 0, 3, 6, and 12, an aliquot of the medium surrounding the cubes from each replicate was collected for cell counts as described below. Cubes were transferred from each replicate into acetate buffer

(49% 0.2 M sodium acetate, 51% 0.2 M acetic acid, pH4.6) and sonicated using an ultrasonic bath (Fisher Scientific) for 15 min at 25 °C to release the bacteria. The cell suspension was diluted 1:10 with acetate buffer for cell counts.

2.1.5. Free-living bacteria preparation

The bacterial suspension was prepared by centrifuging bacterial culture in LM medium at 6000 rpm for 5 min. The supernatant was discarded and bacteria were washed with f/2 medium, and then re-suspended in f/2 medium before use on the same day. The bacterial suspension in f/2 medium was divided into two groups and incubated at 4 and 25 °C as above ($N = 3$). On Days 0, 3, 6, and 12, one milliliter of free-living bacteria from each treatment was removed and diluted to 1:100 for cell counts as described below.

2.1.6. Bacterial cell counts

Bacterial cell counts were performed using the method described by Ternon et al. (2019) and Wetzel and Likens (2013) with slight modification. Bacteria were fixed with 1.7% formaldehyde (v/v) and stained with sterile 0.1 mg mL^{-1} DAPI (4′,6-diamidino-2-phenylindole, dilactate; ThermoFisher Scientific, Waltham, MA, USA). Stained cells were filtered onto 0.2 μm black polycarbonate filters (Millipore, Bedford, MA, USA). Filters were preserved with mountant solution (ElectronMicroscopy Sciences, Hatfield, PA, USA) on glass slides and cells were counted using a fluorescent microscope (EVOS® FL Auto Imaging System; ThermoFisher Scientific) equipped with a DAPI light cube (excitation: 357/ 44 nm, emission: 447/ 60 nm; ThermoFisher Scientific). Cells were counted at a magnification of 100x; at least 3 fields were counted for each sample. Cell density was calculated as described by Wetzel and Likens (2013):

$$\text{Cells mL}^{-1} = (\text{membrane conversion factor})(\text{avg. number of bacteria per micrometer field})(\text{dilution factor})^{-1}$$

where the membrane conversion factor is the filtration area divided by the area of the micrometer field.

2.2. Algicidal effect of immobilized Shewanella sp. IRI-160 in alginate beads

2.2.1. Algal stock cultures

Stock cultures of *Karlodinium veneficum* (CCMP 2936 [National Center for Marine Algae and Microbiota, https://ncma.bigelow.org/]), *Prorocentrum minimum* (CCMP2233), and control species *Rhodomonas* sp. (CCMP 757; cryptophyte) were cultured in natural seawater at 25 °C with f/2 nutrients (-Si; Guillard and Ryther 1962) and a salinity of 20, with a light intensity of approximately 130 μmol photons m^{-2} s^{-1}. Cultures were kept under a 12 h: 12 h light: dark cycle, and semi-continuously in the exponential growth phase. Stock cultures were diluted to a cell density of 54,000 to 59,000 cells mL^{-1} prior to the start of each experiment.

2.2.2. Bacterial culture preparation

Preliminary results indicated that LM medium may have negative effects on laboratory algal cultures, but that laboratory cultures were able to grow with low concentrations [0.05% (v/v)] of casein amino acids (data not shown). For experiments with algae, *Shewanella* sp. IRI-160 was cultured with sterile 0.05% [v/v] casein amino acids (CAA; Sigma–Aldrich, St. Louis, MO, USA) in f/2 medium (CAA medium) to avoid the negative impacts of bacterial growth medium on algae. A single colony of *Shewanella* sp. IRI-160 from LM plates was transferred to 2 g L^{-1} CAA medium, and the bacterial culture was incubated overnight as described above. The bacterial culture was then diluted 1:4 with f/2 medium and incubated at room temperature without shaking overnight.

2.2.3. Preparation of immobilized bacteria in alginate beads

Sterile 4% (w/v) alginic acid, sodium salt (Thermofisher Acros Organics) in 0.5 g L^{-1} CAA medium was mixed with *Shewanella* sp. IRI-160 culture in a ratio of 1:1 (v/v). The alginate beads were extruded using the same method as above into cold 0.4 mol L^{-1} $CaCl_2$. Control beads were prepared using the same process with the addition of sterile CAA medium but without the addition of bacteria. Alginate beads were stored in 0.5 g L^{-1} CAA medium at 4 °C before use within 2 weeks. Immobilized bacteria cell densities were determined as above before the start of the experiment.

2.2.4. Effects of immobilized bacteria on dinoflagellates

Alginate beads with immobilized bacteria were added to cultures of *K. veneficum, P. minimum*, and *Rhodomonas* sp. in 250 mL polycarbonate flasks to achieve 10^6, 10^7, and 10^8 cells mL^{-1} of bacteria in algal cultures ($N = 3$). Additional beads, without bacteria, were also added to the treatments in the 10^6 and 10^7 cells mL^{-1} groups so that all treatments would have the same number of beads as the 10^8 cells mL^{-1} treatment group. For comparison, free bacteria (not immobilized) were added to separate cultures ($N = 3$) to reach final bacterial concentrations of 10^8 cells mL^{-1}. CAA medium was also added to the cultures in the free-living bacteria treatment group to achieve the same concentrations of CAA medium as in other treatments. Finally, in the control group, control beads were added to cultures ($N = 3$) at the same bead density as in the treatment groups.

Algal cultures were incubated under the same condition as stock cultures. *In vivo* fluorescence was measured at the beginning of the experiment, and then after 1, 2, 4 and 6 days. Specific growth rates (μ) were calculated over 6 days as (Guillard et al. 1973):

$$\mu = \frac{\ln(N2/N1)}{T2 - T1}$$

where N2 is the *in vivo* fluorescence reading of each culture at T2 (Day 6); N1 is the average *in vivo* fluorescence of each species at T1 (Day 0, of the bulk cultures). Algal cell density was also determined by cell counts at the initial time point and on the last day.

Bacteria associated with alginate beads in the control group (contributed from non-axenic algal cultures) and the 10^8 cells mL^{-1} treatment group were counted using the methods described above at the termination of the experiment. Additionally, on the last day, cultures were filtered through 3.0 μm polycarbonate filters (Millipore) and then onto 0.2 μm black polycarbonate filters (Millipore) from each treatment to determine total free-living bacterial cell densities in each culture as described above. For comparison, cell densities of bacteria in each group were divided by average cell densities of controls to calculate the "relative bacterial cell densities". An aliquot of each sample was also filtered through 0.2 μm nylon syringe filters (Corning, Inc., Corning, NY, USA) to measure ammonium concentrations using the procedure described below.

2.2.5. Ammonium concentration

Ammonium concentrations were measured using an API® Ammonia Test Kit (Mars Fishcare Inc., Chalfont, PA, US) modified as described here. Briefly, each of 250 μL hypochlorite solution and salicylate/catalyst solution from the kit were added to 2.5 mL of diluted samples (with sterile MilliQ water), and samples were incubated at room temperature for 10 min for the development of color. Absorbance at 690 nm was measured (NanoDrop 2000 Spectrophotometer; ThermoFisher Scientific) and the concentration of ammonium in each sample was determined by linear regression analysis using a standard curve of ammonium standards (Sigma–Aldrich) ranging from 0 to 200 μM (in MilliQ water).

2.2.6. Statistical analyses

Repeated measures ANOVA was used to test if there was a significant difference in cell densities of *Shewanella* sp. IRI-160

immobilized to each matrix over time, and was also used to test if the densities of free-living *Shewanella* sp. IRI-160 and *Shewanella* sp. IRI-160 released into the medium changed significantly over time ($p < 0.05$). If there was a significant difference, then paired *t*-test was used to analyze the significant difference in cell densities between all pairs of time points ($p < 0.05$). One-way ANOVA was used to test the significant impacts of temperature on the densities of immobilized and free-living *Shewanella* sp. IRI-160, as well as *Shewanella* sp. IRI-160 released to the surrounding medium from each matrix at each time point ($p < 0.05$).

In addition, repeated measures ANOVA was conducted to test the significant difference in *in vivo* fluorescence of each algal species in control and treatment groups over time ($p < 0.05$). One-way ANOVA was used to test the significant difference in *in vivo* fluorescence of each algal species between groups at each time point, as well as the specific growth rates between groups of each species ($p < 0.05$). If a significant difference was detected, then Tukey HSD test was used to analyze the significant difference in *in vivo* fluorescence of each algal species between all pairs of groups at each time point, as well as the specific growth rates of each species between all pairs of groups ($p < 0.05$). One-way ANOVA was also used to test the significant differences in cell densities of each algal species between groups on Day 6, and if a significant difference was detected, then Tukey HSD test was conducted to test the significant difference between all pairs of groups ($p < 0.05$).

A paired *t*-test was used to measure the significant difference in densities of immobilized bacterial cells in alginate beads between Day 0 and Day 6 in each algal culture treated with 10^8 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 ($p < 0.05$); one-sample *t*-test was used to measure if true means of bacterial cell densities in alginate beads in control cultures on Day 6 were 0 (to measure the significant difference between these cell densities and 0; $p < 0.05$). One-way ANOVA was used to test for significant differences in relative densities of free-living bacteria in treatments and controls on Day 6 in each group of algal cultures, as well as absolute and relative ammonium concentrations of these cultures on the same time point ($p < 0.05$); if a significant difference was detected, Tukey HSD test was conducted to test the significant difference between all pairs of groups ($p < 0.05$). All statistical analyses were conducted using R (v. 3.6.0; R Core Team, 2015).

3. Results

3.1. Retention of immobilized Shewanella sp. IRI-160

The distribution of *Shewanella* sp. IRI-160 cells that were in each matrix vs. those in the surrounding medium after 12 days was assessed at 4 and 25 °C (Fig. 1A–D; Table S1). When immobilized in alginate beads at 25 °C, 99.83% of total *Shewanella* sp. IRI-160 cells counted were within the matrix on Day 12, while 0.17% of the cells were not associated with the matrix and were in the surrounding medium (Table S1). At 4 °C, 99.94% of cells counted were in alginate beads on Day 12 and 0.06% of the cells were in the surrounding medium. When immobilized in agarose cubes, 84.09% and 81.05% of cells counted were in the matrix at 25 and 4 °C, respectively. Note that data for *Shewanella* sp. IRI-160 immobilized in agarose cubes at 25 °C on Day 12 were excluded from statistical analyses because one replicate was lost due to contamination. Furthermore, 98.92% and 98.94% of cells counted on Day 12 were in the sponge cubes at 25 and 4 °C, respectively. For polyester, 96.55% and 92.97% of cells counted on Day 12 were immobilized within the polyester cubes at 25 and 4 °C, respectively (Fig. 1, Table S1).

3.1.1. Effects of temperature on immobilized Shewanella sp. IRI-160

The density of immobilized *Shewanella* sp. IRI-160 decreased slightly but significantly over the 12-day period in alginate beads and sponge at 4 °C ($p < 0.05$) but not 25 °C (Fig. 1A and C). No significant changes over time were observed for the density of immobilized

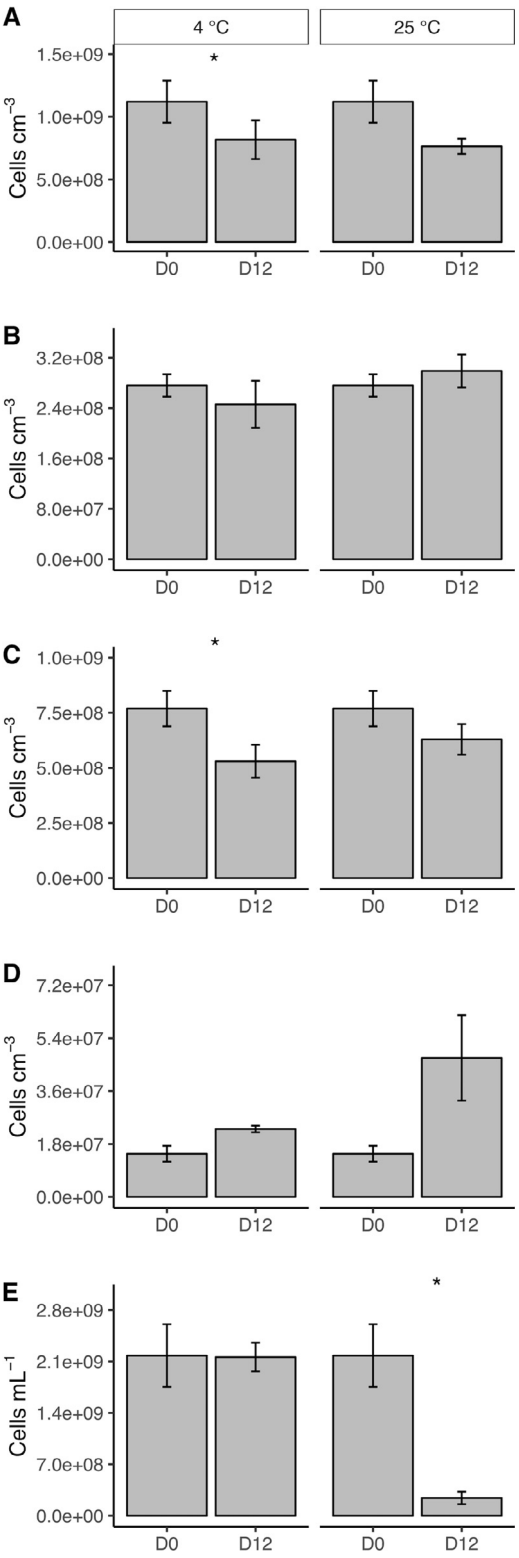

Fig. 1. The cell density of *Shewanella* sp. IRI-160 immobilized in alginate beads (A) or agarose cubes (B), or onto sponge (C) or polyester cubes (D), compared to free-living bacteria that were not immobilized (E) at 4 (left) and 25 °C (right). Results for the initial (D0) and the last day (D12) of experiments are shown. Error bars indicate standard deviations of three replicates, except that only two replicates were used in the D12 data of *Shewanella* sp. IRI-160 immobilized to agarose cubes (B) due to the contamination of one sample. Asterisks "*" indicate significant differences between cell densities of *Shewanella* sp. IRI-160 immobilized to each matrix (A-D) or the free-living cells (E) on D0 and D12 ($p < 0.05$).

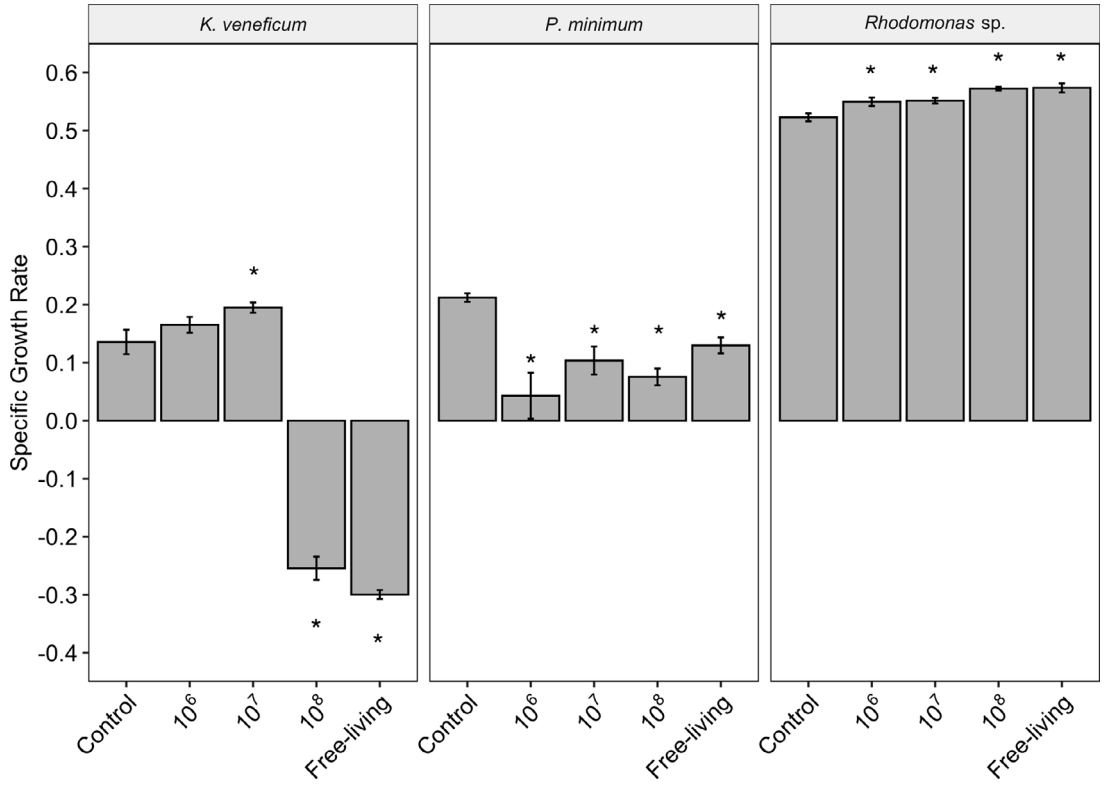

Fig. 2. Specific growth rates of treatments (harmful dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum*, as well as non-harmful control cryptophyte *Rhodomonas* sp. treated with free-living or immobilized *Shewanella* sp. IRI-160 [10^6 to 10^8 cells mL^{-1}]) and controls (treated with blank alginate beads with no bacteria) over 6 days. Asterisks "*" indicate significant differences between specific growth rates of treatments and controls ($p < 0.05$).

Shewanella sp. IRI-160 in agarose cubes or polyester at either temperature ($p > 0.05$; Fig. 1B and D). There was no significant impact of temperature on cell densities of *Shewanella* sp. IRI-160 retained in alginate beads, agarose, or sponge cubes at any time point tested ($p > 0.05$; Fig. 1). Cell densities of *Shewanella* sp. IRI-160 immobilized in polyester cubes at 25 °C, however, were >2-fold higher than those at 4 °C on Day 12 ($p < 0.05$; Fig. 1D).

In contrast to the relatively stable densities of *Shewanella* sp. IRI-160 immobilized to the four matrices, the cell density of *Shewanella* sp. IRI-160 in the free-living treatment group (not immobilized within a matrix) decreased significantly at 25 °C ($p < 0.05$; Fig. 1E), but not at 4 °C. In addition, the cell density of free-living *Shewanella* sp. IRI-160 at 4 °C was significantly higher than at 25 °C on Day 12 ($p < 0.05$).

3.2. Effects of immobilized Shewanella sp. IRI-160 on algae

Shewanella sp. IRI-160 was immobilized in alginate beads, which were then added to achieve densities of 10^6 to 10^8 cells mL^{-1} in cultures of harmful dinoflagellates *K. veneficum* (Fig. 2; Fig. S1A; Table S2) and *P. minimum* (Fig. 2; Fig. S1B; Table S2), as well as the non-harmful cryptophyte *Rhodomonas* sp. (Fig. 2; Fig. S1C; Table S2). The effects of immobilized bacteria on algal growth were then evaluated over 6 days and compared to the effects of free-living *Shewanella* sp. IRI-160 and blank alginate beads with no bacteria (control). Cell densities based on microscopic cell counts were consistent with *in vivo* fluorescence of all species in cultures treated with immobilized or free-living *Shewanella* sp. IRI-160 (data not shown).

3.2.1. Effects of immobilized and free-living Shewanella sp. IRI-160 on harmful dinoflagellate K. veneficum

Growth rates of *K. veneficum* cultures were positive for control cultures as well as the 10^6 and 10^7 cells mL^{-1} immobilized bacteria treatments over 6 days (Fig. 2). There were no significant differences in *K. veneficum* growth rates between the 10^6 cells mL^{-1} immobilized bacteria treatment and controls over the 6-day incubation period ($p > 0.05$). *K. veneficum* growth in the 10^7 cells mL^{-1} immobilized bacteria treatment, however, was 1.44 times greater than the controls. In contrast to other treatments, the specific growth rates of cultures treated with 10^8 cells mL^{-1} immobilized and free-living *Shewanella* sp. IRI-160 were negative and significantly lower than other treatments and controls over the 6-day incubation period ($p < 0.05$); *in vivo* fluorescence of these two treatments were also significantly lower than other groups at all time points tested ($p < 0.05$, Fig. S1A). The *in vivo* fluorescence of *K. veneficum* cultures treated with 10^8 cells mL^{-1} immobilized bacteria was not significantly different from treatments with free-living bacteria at any time point tested ($p > 0.05$, Fig. S1A), while the overall growth rate of *K. veneficum* in the 10^8 cells mL^{-1} immobilized bacteria treatment was slightly but significantly higher compared to the free-living bacteria treatment (by 1.18 times; $p < 0.05$).

3.2.2. Effects of immobilized and free-living Shewanella sp. IRI-160 on harmful dinoflagellate P. minimum

In contrast to *K. veneficum*, cultures of *P. minimum* had positive specific growth rates in all treatments and controls over 6 days (Fig. 2). However, the specific growth rate of *P. minimum* control cultures was significantly higher than the 10^6, 10^7, 10^8 cells mL^{-1} immobilized, and free-living bacteria treatments ($p < 0.05$). In addition, the specific growth rate of *P. minimum* in the free-living bacteria treatment was significantly higher than the 10^6 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 treatments ($p < 0.05$), while no significant difference was observed between all other groups ($p > 0.05$). Similar to *K. veneficum*, significant differences in *in vivo* fluorescence of *P. minimum* cultures were observed between controls and the 10^8 cells mL^{-1} or free-living bacteria treatments on Day 1 ($p < 0.05$; Fig. S1B; Table S2).

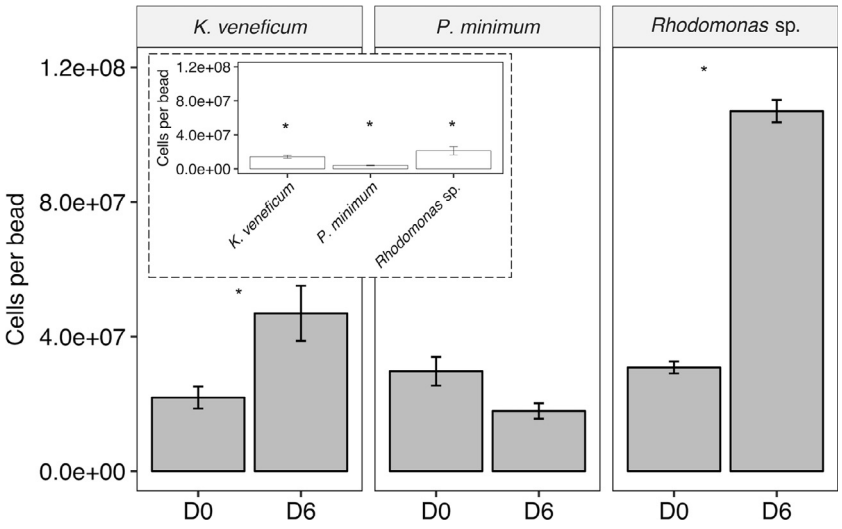

Fig. 3. *Shewanella* sp. IRI-160 density (cells per bead) in alginate beads in algal cultures on Day 0 (D0) and Day 6 (D6) for the 10^8 cells mL^{-1} immobilized bacteria treatments. Insert: Bacterial density (cells per bead) on D 6 in blank alginate beads added to non-axenic control cultures. Error bars indicate standard deviations of three replicates. Asterisks "*" indicate significant differences ($p < 0.05$) between bacterial cell abundance per bead on D0 and D6 (gray bars), or between bacterial cell abundance per bead in control cultures (insert) on D6 (white bars) compared to D0 (not shown).

3.2.3. Effects of immobilized and free-living Shewanella sp. IRI-160 on non-harmful cryptophyte Rhodomonas sp.

The specific growth rates of the cryptophyte *Rhodomonas* sp. were slightly but significantly higher in all treatments compared to controls over the 6-day incubation period ($p < 0.05$), with the highest values observed for cultures treated with the highest density of *Shewanella* sp. IRI-160 (Fig. 2). No significant differences in specific growth rates were observed between the free-living and 10^8 cells mL^{-1} immobilized bacteria treatments, or between the treatments with 10^6 and 10^7 cells mL^{-1} immobilized bacteria.

3.2.4. Immobilized and free-living bacterial densities in algal cultures

There was a significant 2.14-fold increase in the density of immobilized bacteria ($p < 0.05$) in the 10^8 cells mL^{-1} bacteria treatment by Day 6 in cultures of *K. veneficum*, and a 3.49-fold increase in the density of immobilized bacteria in cultures of *Rhodomonas* sp., but no significant increase in the density of immobilized bacteria in the same treatments of *P. minimum* ($p > 0.05$; Fig. 3). In addition, the bacterial abundance in alginate beads without bacteria in the non-axenic control cultures of *K. veneficum, P. minimum*, and *Rhodomonas* sp. ranged from 4.15×10^6 to 2.13×10^7 cells per bead on Day 6 (Fig. 3, insert).

There were significant differences between cell densities of free-living bacteria in controls and treatments of *K. veneficum* and *P. minimum* on Day 6 ($p < 0.05$), while there was no significant difference in *Rhodomonas* sp. cultures ($p > 0.05$; Fig. 4). In *K. veneficum* cultures, the cell densities of free-living bacteria in controls were significantly higher than treatments, where free-living bacteria densities decreased with an increase in immobilized bacteria treatment ($p < 0.05$). For *P. minimum*, the total abundance of free-living bacteria in controls was significantly higher than the free-living *Shewanella* sp. IRI-160 treatment ($p < 0.05$), but not significantly different from the abundance of free-living bacteria in the 10^8, 10^7, and 10^6 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 treatments.

3.2.5. Ammonium concentration

On Day 6, ammonium concentrations in algal cultures ranged from 98.2 to 565 μM (Fig. S2). Ammonium concentrations in *K. veneficum* cultures treated with free-living *Shewanella* sp. IRI-160 or with 10^8 cells mL^{-1} immobilized bacteria were significantly higher than controls as well as the 10^6 and 10^7 cells mL^{-1} immobilized bacteria treatments ($p < 0.05$; Fig. 5; Fig. S2) . The ammonium concentration in free-living bacteria treatments was significantly higher than the cultures treated with 10^8 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 ($p < 0.05$). There were no significant differences in ammonium concentrations between other pairs of groups ($p > 0.05$). Similarly, ammonium concentrations in cultures of *P. minimum* treated with free-living *Shewanella* sp. IRI-160 were significantly higher than controls and other treatments ($p < 0.05$), while there was no significant difference between other pairs of groups ($p > 0.05$; Fig. 5; Fig. S2). There was no significant difference in ammonium concentrations between controls and treatments of *Rhodomonas* sp. ($p > 0.05$).

4. Discussion

Previous research indicated that bacterium *Shewanella* sp. IRI-160 and its water-soluble algicide IRI-160AA were able to control the growth of dinoflagellates with no negative impacts on cell densities of other phytoplankton species (Hare et al., 2005; Pokrzywinski et al., 2012, 2017a, 2017b; Tilney et al., 2014a, 2014b; Ternon et al., 2019) or organisms at higher trophic levels tested (Simons, 2019). Here, *Shewanella* sp. IRI-160 was immobilized using different porous matrices, including alginate hydrogel beads, as well as agarose, sponge, and polyester cubes. The retention of *Shewanella* sp. IRI-160 in each matrix was evaluated for a course of 12 days; the abundance of immobilized bacteria within each matrix was compared to the abundance of bacteria released into the surrounding medium, as well as to the density of bacteria that were not immobilized. To investigate the effect of temperature on immobilized *Shewanella* sp. IRI-160, each experiment was performed at 4 and 25 °C. Alginate hydrogel beads were selected for further experiments due to its high retention of *Shewanella* sp. IRI-160, as well as its low-cost, nontoxic, and biodegradable characteristics (reviewed by Lapointe and Barbeau, 2020). *Shewanella* sp. IRI-160 was immobilized in alginate hydrogel beads at three concentrations to evaluate the ability of immobilized *Shewanella* sp. IRI-160 to control the growth of harmful dinoflagellates.

Results of this research indicated that there was no or only a slight decrease in cell densities of free-living and immobilized *Shewanella* sp. IRI-160 at 4 °C (Fig. 1), consistent with previous studies demonstrating that growth at low temperatures (~4 °C) is a hallmark of the *Shewanella* genus (Hau and Gralnick, 2007). Its survival at low temperatures may be related to changes in morphology as well as the production of alternative proteins and lipids (Abboud et al., 2005). Additionally, there was a dramatic and significant decrease in cell densities of free-living *Shewanella* sp. IRI-160 at 25 °C (Fig. 1E), while the density of immobilized bacteria did not decrease significantly at the same temperature in any matrices tested (Fig. 1A–D), suggesting immobilization to matrices may provide advantages to these bacteria and protect them from environmental conditions where their free-living counterparts may not survive. As reviewed by Wells et al. (2015), warmer temperatures are favorable for dinoflagellate blooms; and have been

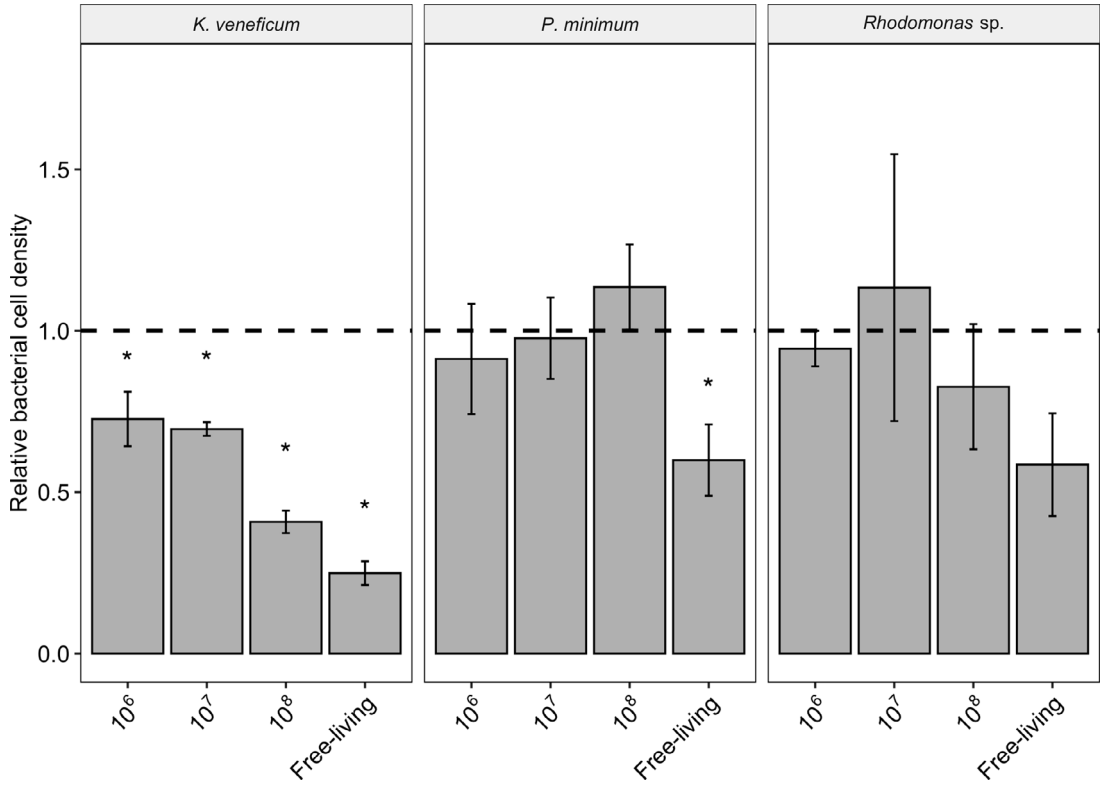

Fig. 4. Total bacterial cell densities in the medium of each treatment relative to non-axenic control cultures (dashed line) on Day 6 for dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum*, and cryptophyte *Rhodomonas* sp.. Samples were incubated with *Shewanella* sp. IRI-160 immobilized in alginate beads (10^6 to 10^8 cells mL^{-1}) or free-living *Shewanella* sp. IRI-160 at 10^8 cells mL^{-1}. Control algal cultures received blank beads only (with no bacteria). Error bars indicate standard deviations of three replicates. Asterisks "*" indicate significant differences in relative cell densities of total bacteria in the medium compared to controls on Day 6 ($p < 0.05$).

associated with their higher annual growth rate and longer duration of bloom seasons (Gobler et al., 2017). The decrease of cell densities of free-living *Shewanella* sp. IRI-160 at 25 °C may limit its ability to control the growth of dinoflagellates in the environment, while immobilization to matrices may contribute to its long-lasting performance in the field.

Over the entire experiment period, the majority of *Shewanella* sp. IRI-160 cells were retained in each of the matrices rather than released into the surrounding medium (Table S1; Fig. 1). On the last day of the experiment, more than 80% of *Shewanella* sp. IRI-160 cells were embedded in each matrix, with the greatest retention (99.94%) in alginate beads. As reviewed by Żur et al. (2016), microbes are mainly found to be associated with surfaces in nature. The attachment behaviors of bacteria may come with important benefits, including better accessibility to nutrients, as well as protection from predators and adverse environmental conditions (Roberts et al., 2006; Ahmed and Holmström, 2015; Madigan et al., 2017). Studies have revealed a congregational behavior of bacteria within the genus *Shewanella*, that they attach to and accumulate cells surrounding insoluble iron and manganese oxides and reduce these solid-phase electron acceptors using specialized outer membrane proteins (Lower et al., 2001; Tiedje, 2002; Fredrickson et al., 2008; Harris et al., 2018). To prepare immobilized bacteria in this study, *Shewanella* sp. IRI-160 was cultured in a nutrient-enriched medium and mixed with each matrix. This may have resulted in a slow release of nutrients and contributed to the matrix-bacteria association.

Alginate is a natural polymer that is characterized as low-cost, nontoxic, and highly biodegradable (reviewed by Lapointe and Barbeau, 2020). In addition to its application in immobilization of other algicidal bacteria to control HABs (Kang et al., 2007; Sun et al., 2015; Zhang et al., 2018), alginate hydrogel has been developed as edible films for food packaging (Bierhalz et al., 2012; Galus and Lenart, 2013), carriers for drug remote release in human bodies (Anugrah et al., 2019), and applied to neural tissue engineering (Homaeigohar et al., 2019). In this research, the impacts of immobilized *Shewanella* sp. IRI-160 on harmful dinoflagellates were investigated using bacteria embedded in alginate hydrogel beads (Fig. 2; Fig. S1; Table S2). Results of this study indicated a rapid, dose-dependent response of harmful dinoflagellates to immobilized bacteria, while no negative effects by immobilized bacteria were observed on the non-harmful control species *Rhodomonas* sp. (though a slight decrease of *in vivo* fluorescence was observed in this species treated with free-living bacteria on Day 1; Fig. S1C; Table S2). The non-negative and even slightly positive effects of immobilized *Shewanella* sp. IRI-160 on non-dinoflagellate species observed in this research were consistent with previous research using cell-free filtrate IRI-160AA. For instance, the cell density of *Rhodomonas* sp. in laboratory culture experiments (Tilney et al., 2014b) and species abundance of diatom *Cyclotella* sp. in microcosm experiments (Tilney et al., 2014a) increased when treated with algicide IRI-160AA, while this algicide was effective in controlling the growth of targeted dinoflagellates in both studies.

In this research, the growth of harmful dinoflagellates *K. veneficum* and *P. minimum* were both suppressed by the addition of immobilized and free-living *Shewanella* sp. IRI-160 at 10^8 cells mL^{-1} (Fig. 2), and as early as Day 1 (24 h exposure; Fig. S1A-B; Table S2). Their cell densities did not recover by the end of the experiment in these treatments. These results are consistent with previous research indicating a rapid response of dinoflagellates to cell suspension (Pokrzywinski et al., 2012) or cell-free filtrate (Pokrzywinski et al., 2012, 2017b) of *Shewanella* sp. IRI-160. For instance, Pokrzywinski et al. (2017b) indicated that cell densities of *K. veneficum* and *P. minimum* declined significantly after 2 h of exposure to cell filtrate of *Shewanella* sp. IRI-160. Other research by Pokrzywinski et al. (2012) reported that the cell density of *K. veneficum*

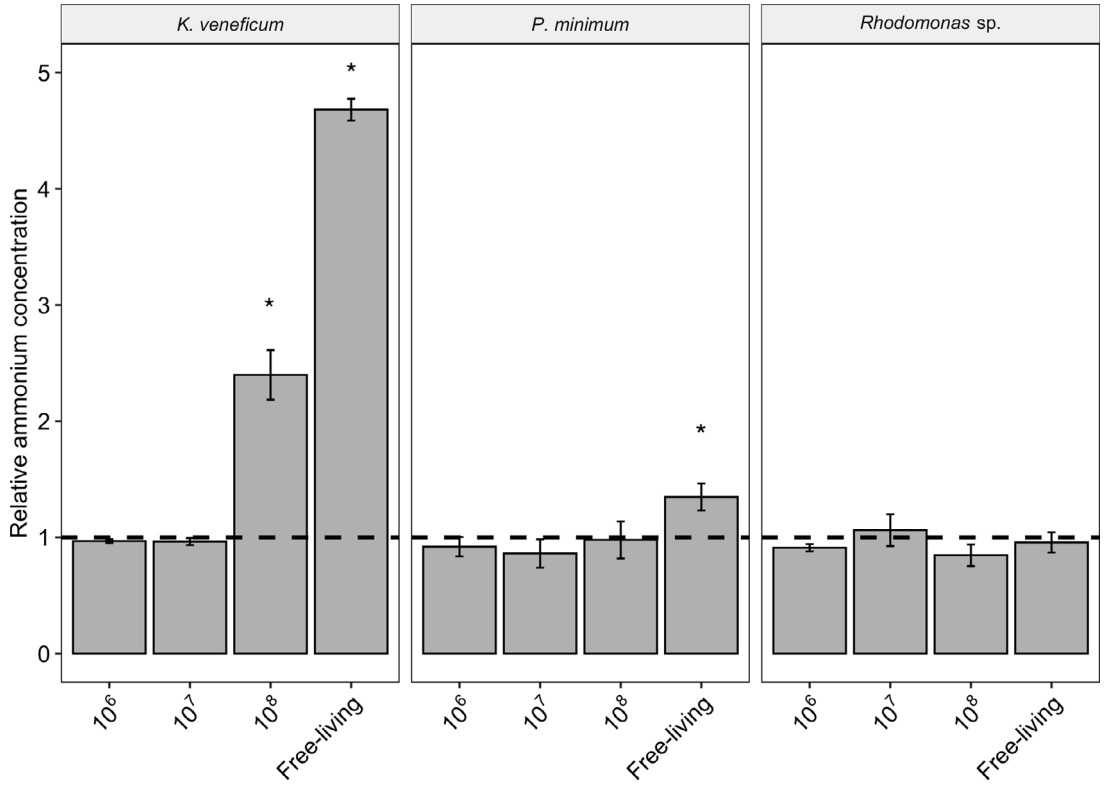

Fig. 5. Ammonium concentrations in each treatment relative to controls (black dashed line) in cultures of dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum*, as well as cryptophyte *Rhodomonas* sp., on Day 6. Samples were incubated with free-living *Shewanella* sp. IRI-160 (10^8 cells mL^{-1}) or *Shewanella* sp. IRI-160 immobilized in alginate beads (10^6 to 10^8 cells mL^{-1}), compared to the control algal cultures incubated with blank beads only. Error bars indicate standard deviations of three replicates. Asterisk "*" indicates a significant difference between relative ammonium concentration in the indicated group and control cultures ($p < 0.05$).

dropped to 20 −40% of controls after 24 h treated with cell suspension or cell-free filtrate of *Shewanella* sp. IRI-160.

Interestingly, although the growth of *K. veneficum* was inhibited by the addition of 10^7 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 on Day 1, cultures in this treatment recovered by the end of the experiment, surpassing controls on Day 6 and leading to higher specific growth rates of this treatment compared to controls over the entire experiment period (Fig. 2; Fig. S1A; Table S2). A growth recovery was also observed in *P. minimum* treated with 10^8 cells mL^{-1} immobilized *Shewanella* sp. IRI-160 on Day 4 (Fig. S1B; Table S2). This suggests a dynamic effect of immobilized *Shewanella* sp. IRI-160 on dinoflagellate species, potentially involving the complex suite of algicidal compounds produced by *Shewanella* sp. IRI-160 (Ternon et al., 2019; also see discussions below) in which one or more of these compounds may play a role in stimulating the growth of dinoflagellates, especially at a lower concentration (*e.g.* ammonium; Ternon et al., 2019). Furthermore, results of this research imply that some portion of the dinoflagellate population may be resistant or may recover from exposure in laboratory culture experiments. However, a different response in the field may be expected. As demonstrated by Tilney et al. (2014a) in natural community microcosm experiments, other phytoplankton species may outcompete dinoflagellates in communities treated with algicide IRI-160AA and/or the algicide may stimulate protistan grazers (*e.g.* ciliates), leading to the overall decrease of dinoflagellate abundance in natural microbial communities.

Additionally, results of this investigation demonstrated greater algicidal activity by immobilized *Shewanella* sp. IRI-160 against *K. veneficum* compared to *P. minimum* at the highest concentration of 10^8 cells mL^{-1} (Fig. 2; Fig. S1A-B; Table S2). The specific growth rates of *K. veneficum* treated with 10^8 cells mL^{-1} immobilized and free-living bacteria were negative over 6 days, while the specific growth rates of *P. minimum* were positive in the same treatments (Fig. 2). Furthermore, during the entire experiment period, *in vivo* fluorescence of *K. veneficum* treated with 10^8 cells mL^{-1} immobilized bacteria was less than 16% of that of controls, while *in vivo* fluorescence of the same treatments of *P. minimum* did not fall below 44% of controls (Fig. S1; Table S2). Immobilized *Shewanella* sp. IRI-160 at lower concentrations (10^6 and 10^7 cells mL^{-1}), however, had negative impacts on *P. minimum* but not *K. veneficum*. Overall, this indicates a varied response of dinoflagellates to immobilized *Shewanella* sp. IRI-160 at different densities, as well as a species-specific response of dinoflagellates to these immobilized bacteria. The higher algicidal activity of *Shewanella* sp. IRI-160 against *K. veneficum* compared to *P. minimum* was consistent with previous research indicating non-thecate dinoflagellates (*e.g. K. veneficum, L. fissa,* and *Karenia brevis*) were more susceptible to the algicide produced by *Shewanella* sp. IRI-160 compared to thecate dinoflagellates (*e.g. P. minimum, Alexandrium tamarense,* and *Oxyrrhis marina*) (Pokrzywinski et al., 2012; Tilney et al., 2014b). Species-specific responses to algicidal bacteria have also been described in other research. Cells of diatom *Skeletonema costatum*, for instance, were lysed within hours by the treatment of algicidal bacterium *Kordia algicida*, while diatom *Chaetoceros didymus* was not affected by the same treatment (Bigalke and Pohnert, 2019).

Kang et al. (2007) reported that the algicidal activity of bacterium *Pseudomonas fluorescens* HYK0210-SK09 against diatom *Stephanodiscus hantzschii* was lower when immobilized to alginate beads compared to the bacteria immobilized to polyester and cellulose sponge, or the free-living bacteria. Sun et al. (2015) also demonstrated that immobilization to alginate beads lowered the activity of algicidal bacterium *Alcaligenes aquatilis* F8 against cyanobacterium *Microcystis aeruginosa*. In the research presented here, however, there was only a slight difference (by 1.18 times) in specific rates between *K. veneficum* treated with free-

living bacteria and bacteria immobilized in alginate beads at the same density, and no difference was observed between the same treatments of *P. minimum* (Fig. 2). This may be due to distinct algicidal mechanisms of these bacteria (*e.g. Shewanella* sp. IRI-160 vs. *P. fluorescens* HYK0210-SK09), or due to characteristics of the algicidal compounds produced by each species of bacteria. As noted by Kang et al. (2007), immobilization to alginate beads may physically separate algicidal bacteria from and limit their opportunities for direct contact with their target. Other research by Jung et al. (2008) demonstrated that attachment of *P. fluorescens* HYK0210-SK09 to diatoms was required for cell lysis, while studies indicated direct contact was not required by *Shewanella* sp. IRI-160 to control dinoflagellate growth (Pokrzywinski et al., 2012; Ternon et al., 2019). Furthermore, the algicidal compounds produced by *Shewanella* sp. IRI-160 are likely to be small polar and water-soluble (Pokrzywinski et al., 2012; Ternon et al., 2019), so that the dispersion of these compounds may not be limited by the alginate matrix.

In this study, the bacterial cell abundance per bead increased when added to cultures of *Rhodomonas* sp. and *K. veneficum* (Fig. 3). The increase in immobilized bacterial densities in these two cultures can be partially attributed to the infiltration of beads by bacteria from non-axenic algal cultures, revealed by the cell density of immobilized bacteria in control cultures with blank alginate beads (Fig. 3 insert). However, when compared to the controls with blank beads, the higher increased bacterial density in beads with immobilized *Shewanella* sp. IRI-160 suggested there might be more complex processes involved, including bacteria-bacteria and/or algae-bacteria interactions. The varied growth response of immobilized bacteria in algal cultures also suggested these interactions may be species-specific, and involve different bacterial communities associated with cultures of individual algal species (Koedooder et al., 2019; Piampiano et al., 2019). These species-specific interactions were also evident in the background (free-living) bacterial densities in non-axenic algal cultures on Day 6; cultures of *K. veneficum* treated with immobilized and free-living *Shewanella* sp. IRI-160 as well as *P. minimum* treated with free-living *Shewanella* sp. IRI-160 had lower free-living bacterial densities compared to the controls, even though *Shewanella* sp. IRI-160 was added to these cultures on Day 0. In contrast, no difference was observed in free-living bacterial densities of *Rhodomonas* sp. cultures between treatments and controls (Fig. 4). Hare et al. (2005) noted the change in the bacterial community in laboratory cultures of *Pfiesteria piscicida* after adding free-living *Shewanella* sp. IRI-160. Tilney et al. (2014a) also demonstrated a change in prokaryotic community composition by the addition of algicide IRI-160AA to environmental samples collected during a bloom of *L. fissa*. Microbial interactions involving *Shewanella* spp. have been reported in other literature (Horta et al., 2014; Rachanamol et al., 2014; Gong et al., 2015), including the antibiotic activity of *Shewanella algae* isolated from a marine sponge (Rachanamol et al., 2014). However, it is not clear if changes in the bacterial community noted in Hare et al. (2005) and Tilney et al. (2014a) were in response to dinoflagellate cell death or if there were direct impacts on the bacterial community by addition of *Shewanella* sp. IRI-160, or both.

In aquatic environments, ammonium is thought to be the preferred inorganic nitrogen source for phytoplankton due to the low energy cost for assimilation. At high concentrations, however, it can be toxic and suppress the growth of algal species (Glibert et al., 2016). As reviewed by Collos and Harrison (2014), studies on tolerance of phytoplankton to high concentrations of ammonium suggested dinoflagellates to be the least tolerant among all algal species reviewed, including dinoflagellates, chlorophytes, diatoms, raphidophytes, and prymnesiophytes. Previous research on algicide IRI-160AA identified ammonium as one of the active algicidal compounds produced by *Shewanella* sp. IRI-160 (Ternon et al., 2019). A synergistic effect was observed by ammonium and n-butylamine, both present in *Shewanella* algicide IRI-160AA, against dinoflagellates *L. fissa* and *P. minimum*, where the combination of ammonium and n-butylamine yielded higher algicidal effects than each compound alone (Ternon et al., 2019). This synergistic algicidal effect was not observed on *Rhodomonas* sp. (Ternon et al., 2019). The involvement of ammonium in the algicidal effects of bacteria was also observed in other studies (Meyer et al., 2017). For instance, a toxic peptide (toxin P) secreted by bacterium *Vibrio shiloi* inhibited the photosynthesis of zooxanthellae in the presence of ammonium (Banin et al., 2001). The authors noted that toxin P may be able to facilitate the uptake of ammonium, which in turn disrupted the cellular pH gradient and photosynthesis.

In the study presented here, ammonium concentrations ranging from 98.2 to 565 μM were observed in treatments and controls (Fig. S2). Except for *K. veneficum* cultures treated with free-living and 10^8 cells mL^{-1} immobilized *Shewanella* sp. IRI-160, these concentrations were in the range for optimal growth of dinoflagellates [110 ± 77 μM; reviewed by Collos and Harrison (2014)]. Within each species group (Fig. 5), significantly higher concentrations of ammonium were observed in *K. veneficum* treated with 10^8 cells mL^{-1} immobilized or free-living *Shewanella* sp. IRI-160 compared to other treatment groups. Results of experiments with *P. minimum* also showed that cultures with free-living *Shewanella* treatments had a higher ammonium concentration than controls as well as the immobilized bacteria treatments. No significant differences were observed in ammonium concentrations of controls and treatments for *Rhodomonas* sp. cultures. To be noted, the source of ammonium in these cultures was unknown, but may be due to remineralization of dissolved organic matter released by dead and dying dinoflagellate cells in *K. veneficum* and *P. minimum* cultures. Overall, this supports previous work suggesting that ammonium may play an important role in the algicidal effects of *Shewanella* sp. IRI-160 on dinoflagellates (Ternon et al., 2019), while other essential compounds within the bacterial exudate may also be required for algicidal activity. Elucidating the role of these other compounds in the algicidal effects of *Shewanella* sp. IRI-160 requires future study.

5. Conclusion

Results of this research demonstrated good retention of *Shewanella* sp. IRI-160 in all matrices tested (alginate beads, agarose, sponge, and polyester cubes) at both 25 and 4 °C and indicated that association to a solid matrix may provide advantages for and protect this bacterium at warmer temperatures. Application of this technology may provide better long-term control compared to the dispersal of free-living *Shewanella* since dinoflagellate blooms often occur at higher temperatures. *Shewanella* sp. IRI-160 immobilized in alginate beads demonstrated rapid negative effects on harmful dinoflagellates *K. veneficum* and *P. minimum*, while no negative impacts were observed on non-harmful control cryptophyte *Rhodomonas* sp.. There was no or just a slight difference in algicidal activities between immobilized and free-living *Shewanella* sp. IRI-160 when added at the same cell density, suggesting that diffusion of algicidal compounds produced by this bacterium is not inhibited by the matrix. In addition to impacts on dinoflagellates, the results of this research indicated potential interactions of *Shewanella* sp. IRI-160 or the algicidal products of this bacterium with other microbes in laboratory cultures. Ammonium, identified as a component in the algicidal filtrate (Ternon et al., 2019), may also play a role in restructuring the bacterial community.

Overall, the results of this research revealed that immobilized *Shewanella* sp. IRI-160 may serve as an environmentally friendly means to control HABs and that immobilization of *Shewanella* sp. IRI-160 in biodegradable material such as alginate hydrogel may provide additional advantages without releasing harmful contaminants to the environment while retaining its effectiveness. Future research efforts may focus on controlled experiments evaluating field applications of immobilized *Shewanella* sp. IRI-160 in areas that are at risk of harmful dinoflagellate blooms.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2020.101798.

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