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Involvement of quorum sensing in the activity of growth-inhibiting bacteria against the toxic dinoflagellate *Alexandrium catenella* (Group I)

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Abstract: Quorum sensing (QS) is a term referring to the language of bacteria, or intercellular communication. In recent years, the details of QS mechanisms have been rapidly elucidated in the field of marine microbiology. We investigated QS in bacteria that inhibit the growth of the paralytic shellfish toxin-producing dinoflagellate *Alexandrium catenella* (Group I, formerly *A. tamarense*) isolated from Akkeshi-ko Estuary and Akkeshi Bay. There were bacterial strains that showed strong growth inhibitory effects against *A. catenella* (Group I) when the colonies of bacterial cells were added to the alga. On the other hand, the same bacterial strain showed no inhibitory activity when added in liquid form (cell suspension) to *A. catenella* (Group I). We explored whether the growth inhibitory activity of these bacterial strains were due to QS or not using the QS inhibitor *β*-cyclodextrin. The algal cultures with the bacterial strains AK12 and AK24 exhibited no growth inhibition in the presence of *β*-cyclodextrin, showing the same results to the control without the bacterial addition. It was considered that these strains inhibited the growth of microalgae through QS using N-acyl-homoserine lactones (AHLs) as autoinducers. The strain AK12 belonged to the genus *Kordia*, while the strain AK24 was closely related to the genus *Vibrio*. This study reinforces the significance of QS as a crucial regulatory factor controlling ecosystem functions of plankton in marine environments. Considering QS will be important to elucidate the interactions between algae and bacteria in the marine environment.

Key words: *Alexandrium catenella* (Group I), N-Acyl homoserine lactone, growth-inhibiting bacteria, quorum sensing, *β*-cyclodextrin

Introduction

Harmful algal blooms (HABs) are spreading around the world. HABs have been observed to be increasing in frequency, duration, and geographic range on a global scale (Hallegraeff 1993). In Japan, the aquaculture industry is thriving, and its history is a continuous battles against HABs (Imai et al. 2021). Factors contributing to the occurrences of HABs include eutrophication of coastal waters (Anderson et al. 2002), transfer of organisms by ballast water (Hallegraeff & Bolch 1991), and climate change (Hallegraeff 2010). Due to the serious socioeconomic impacts caused by HABs, there is a demand for developing measures to prevent them.

Environmental friendliness is fundamentally important for the prevention strategies of HABs (Imai et al. 2021). The use of algicidal bacteria is one of the environmentally friendly approaches for the control of HABs. The population densities of these bacteria have been confirmed to increase in the final stage of red tides (Imai et al. 1998, Kim et al. 1998) and they are considered to be one of the main factors leading to their termination of red tides (Imai 2015). Growth-inhibiting bacteria acting against *Alexandrium catenella* (Group I, formerly *A. tamarense*, Prud'homme van Reine 2017), the causative algae of paralytic shellfish poisoning, have also been identified in natural seawater (Su et al. 2007, 2011, Wang et al. 2010, 2012,

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Bai et al. 2011, Fu et al. 2011). Based on these findings, it is suggested that bacteria may also have the potential for prevention and control of the growth of toxic algae causing shellfish poisoning.

The phenomenon of high-density attachment and colonization of algicidal bacteria was discovered in the biofilm on the surface of seagrasses and seaweeds, and later confirmed to be universal (Imai et al. 2002, 2009, Onishi et al. 2014, Imai 2015, Inaba et al. 2017). In Akkeshi-ko Estuary located in the eastern part of Hokkaido and which has a vast eelgrass bed, cysts of *A. catenella* (Group I) have been reported to berarely observed in the bottom sediments, with no toxin contamination of paralytic shellfish toxins in bivalves occurring (Shimada & Miyazono 2005). It has been reported that cysts are distributed at high densities in Akkeshi Bay, adjacent to Akkeshi-ko Estuary, the cyst distribution correlates with the appearance of vegetative cells. Since *A. catenella* (Group I) is largely absent from Akkeshi-ko Estuary, it would be difficult to complete its life cycle there. We found and isolated bacteria that inhibit the growth of the paralytic shellfish toxin-producing alga, *A. catenella* (Group I), at a high frequency and abundance in the seagrass bed of Akkeshi-ko Estuary during our field exploration of Akkeshi-ko Estuary and Akkeshi Bay (Onishi et al. 2021). The loss of seagrass and seaweed beds is continuing worldwide with coastal development. It has been reported that a decrease in seagrass bed area and an increase in the number of HAB occurrences have occurred simultaneously in Tokyo Bay and the Seto Inland Sea (Imai et al. 2009). Taking these facts into account, the important roles of seagrass and seaweed beds in coastal areas can be further highlighted, since they have the potential to prevent massive phytoplankton growth and maintain ecological balance.

In 1970, it was discovered that the bacterium *Vibrio fischeri* began to luminesce when it exceeded a certain density in culture (Nealson et al. 1970). About 20 years later, a mechanism called quorum sensing (QS) was proposed in which microorganisms express genes in a manner dependent on cell density (Fuqua et al. 1994). It is considered that by conducting experiments taking QS into account, the interrelationships between algae and bacteria in their natural environments can be more precisely understood.

QS has also been suggested to be involved in the elucidation of the mechanism of attack on microalgae by algicidal bacteria (Skerratt et al. 2002). The bacterial strain MS-02-063 belonging to the γ-Proteobacteria, isolated from seawater off the coast of Nagasaki Prefecture, shows algicidal activity against the raphidophyte *Heterosigma akashiwo* (Nakashima et al. 2006). This strain was an indirect-attack bacterium that produces a red pigment called prodigiosin PG-L-1, which kills microalgae, and its production was due to QS. Nakashima et al. (2006) demonstrated this using the reagent β-cyclodextrin, which generates a complex with the QS molecule, including AHLs (acyl-homoserine lactones).

During the co-culture experiments between *A. catenella* (Group I) and growth-inhibiting bacteria, we identified two situations that showed a significant difference in growth inhibition when added to the alga: one situation was cultured using liquid medium (bacterial cells were dispersed in the culture solution), and the other was cultured in colony form (bacterial cells were in contact with each other). It is speculated that the phenomenon of these bacteria exhibiting particularly strong growth-inhibition ability in the colony state is due to QS. In fact, algicidal activity was demonstrated when bacteria cultured in colony form were added to algal cultures, but algicidal activity was not observed when bacteria cultured in liquid medium were added to algal cultures (Imai et al. 2013, Kodama et al. 2017).

In this study, we postulated the possibility that the algicidal mechanism of the *A. catenella* (Group I) growth-inhibiting bacteria isolated in a previous study (Onishi et al. 2021) is mediated by QS. As a result of experiments using β-cyclodextrin, we verified that QS plays a significant role in the growth-inhibiting activity, and we report this finding.

Materials and Methods

Screening of bacteria

Growth-inhibiting bacteria acting against *Alexandrium catenella* (Group I) that were isolated from surface seawater and seagrass leaf samples from Akkeshi-ko Estuary and Akkeshi Bay in eastern Hokkaido, Japan, from April to June 2011 (Onishi et al. 2021) were used in this study. An axenic strain of the toxic dinoflagellate *A. catenella* (Group I) isolated from Osaka Bay (provided by Dr. Keigo Yamamoto, Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture) was maintained in modified SWM-3 medium (Imai et al. 1996) under the following conditions: temperature 15°C, light intensities 100 to 120 μmol photons m^{-2} s^{-1}, photo cycle 14 h light : 10 h dark. The volume of algal culture was diluted to 10^3 cells mL^{-1} in test tubes. In the bacteria-added tests, triplicate experimental treatments were conducted. First, the bacteria were cultured in $ST10^{-1}$ liquid medium (Ishida et al. 1986) and diluted with sterile filtered seawater to a concentration of approximately 10^5 cells mL^{-1}. Then, 0.5 mL of the bacterial culture was added to the algal culture (final concentration of 10^4 cells mL^{-1}). In this case, bacterial cells were present in the culture medium as individually dispersed suspensions and were added directly to the algal culture. In the second treatment bacterial colonies were cultivated on $ST10^{-1}$ agar medium, suspended in filter-sterilized seawater, and then added to algal cultures so that the initial bacterial inoculum density was around 10^4 cells mL^{-1}. Bacterial colonies were allowed to form on $ST10^{-1}$ agar medium, and then mixed in sterilized filtered seawater before adding them to the algal culture to achieve an initial inoculum density of about 10^4 cells mL^{-1}. In this case, the bacterial cells were in a closely packed environ-

ment during the cultivation stage but were dispersed and suspended when added to the algal culture as confirmed by DAPI staining and epifluorescence microscopy. The third treatment involved preparing bacterial colonies on ST10^{-1} agar medium, and then directly adding the colony using a sterilized toothpick to the algal culture. The amount of colony used was the same as possible in the second method. In this case, bacterial cells were added to the algal culture in colony form where the cells were in contact with each other, as confirmed by DAPI staining and epifluorescence microscopy at the time of addition in each experimental group. As a control, we set up an algal culture to which sterile filtered seawater was added. Three replicate tubes were prepared for each experimental condition.

Test of *A. catenella* (Group I) for *β*-cyclodextrin resistance

We examined whether the QS inhibitor *β*-cyclodextrin affected the growth of the dinoflagellate *Alexandrium catenella* (Group I). First, *A. catenella* cultures were diluted with modified SWM-3 medium to a cell density of about 10^3 cells mL^{-1} in test tubes. Culture conditions were the same as described above. *β*-cyclodextrin (FUJIFILM Wako Pure Chemical Corporation, CAS No. 7585-39-9) that underwent sterilization treatment with filtered and sterilized seawater was added to the test tubes containing the inoculated algae, to final concentrations of 1, 10, and 100 μM. Experiments were performed in triplicate for each experimental group. Sterile filtered seawater was added to the control test tubes. We monitored the growth and decline of *A. catenella* (Group I) inculture under conditions conducive to growth by measuring the auto fluorescence using a Turner fluorometer (Brand & Guillard 1981).

Test of bacteria for *β*-cyclodextrin resistance

We investigated whether the QS inhibitor *β*-cyclodextrin affected the growth of bacteria. First, the bacterial strains were inoculated into ST10^{-1} liquid medium and cultured until they had grown sufficiently. The culture was diluted with sterile filtered seawater to a concentration of approximately 10^6 cells mL^{-1}, and 0.1 mL diluted culture was spread onto ST10^{-1} agar plates. Sterile paper disks (8 mm in diameter) were soaked in *β*-cyclodextrin solution at concentrations of 1, 3, 10, 30, and 100 μM, and placed on the agar plates with each bacterial strain streaked on them. For each bacterial strain, one petri dish and one paper disk were used for each concentration. After 2 weeks of incubation, the plates were examined to determine whether zones of inhibition were formed around the paper disks.

Verification of QS mechanism

An experiment was carried out to test whether the bacterial strains had a QS mechanism. First, *A. catenella* (Group I) was diluted with modified SWM-3 medium to a cell density of around 10^3 cells mL^{-1}, and samples were prepared in test tubes. Culture conditions were the same as described above. Algal cultures were prepared in a volume of 4.5 mL in test tubes. Then, *β*-cyclodextrin was added to each tube to final concentrations of 1, 10, and 100 μM. Then, a small amount of each bacterial strain in the colony form grown on agar medium was collected with a sterile toothpick and added to the algal culture. The experiment was conducted in triplicate for each experimental group. Algal cultures with sterile filtered seawater added instead of bacteria were used as controls. An algal experimental culture was prepared without *β*-cyclodextrin (bacterial control) with the addition of colony-form bacteria. The algal cells were cultured according to the algal culture conditions described in the previous section, and the algal fluorescence values were measured using a fluorometer (Turner Designs, Sunnyvale, CA, USA).

Results

Selected bacteria

In the first and second experimental treatments, no growth-inhibition was observed in any of the experimental treatments. When the third experimental method was attempted, two bacterial strains, AK12 (collected on April 27 from Northern part of Akkeshi Bay as free-living bacteria) and AK24 (collected on May 18 from Southern part of Akkeshi Bay as particle-associated bacteria) showed significant growth inhibition compared to the control. Based on these results, these strains were selected for subsequent experiments (Table 1). Bacterial strain AK13 (collected on April 27 from Northern Akkeshi Bay, free-living bacteria) which did not show clear growth inhibition in the same experiment was also used as a comparative control. Further detailed information on each strain is provided in a previous study (Onishi et al. 2021).

Table 1. The bacterial strains used in the present study (Onishi et al. 2021). The closest relatives of 16S rDNA gene sequences were also shown, details of the PCR method are described in the present study. FLB means free-living bacteria, and PAB means particle-associated bacteria.

Strain		Origin				Closely related strain			
Name	DDBJ Accession Number	Date	Area	Station	FIB/PAB/Leaf	Name	DDBJ Accession Number	Classification	Homology
AK12	LC465500	April 27 2011	Akkeshi Bay	St. 2	FLB	*Kordia antarctica* strain IMCC3317	JX456458	*Flabobacteria*	99.64
AK13	LC465501	April 27 2011	Akkeshi Bay	St. 2	FLB	*Kordia* sp. LNM-4	AB758592	*Flabobacteria*	99.47
AK24	LC465512	May 18 2011	Akkeshi Bay	St. 3	PAB	*Vibrio* sp. D4058	DQ480136	*γ-Proteohacteria*	99.66

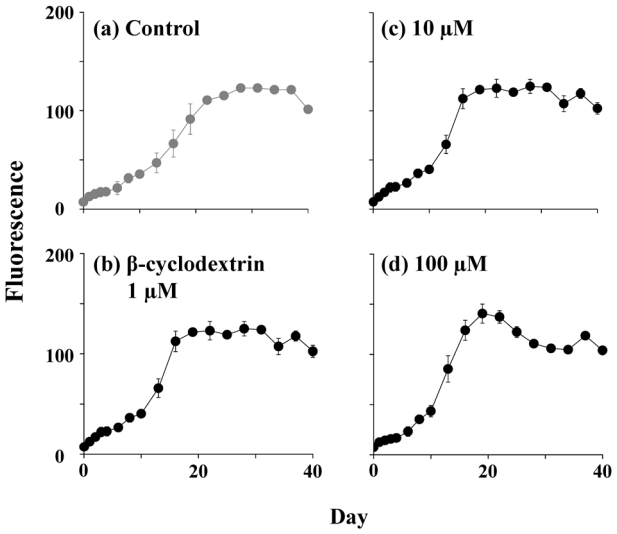

Fig. 1. The effect of *β*-cyclodextrin on the growth of *A. catenella* (Group I) was evaluated by measuring the fluorescence of the algal cells.

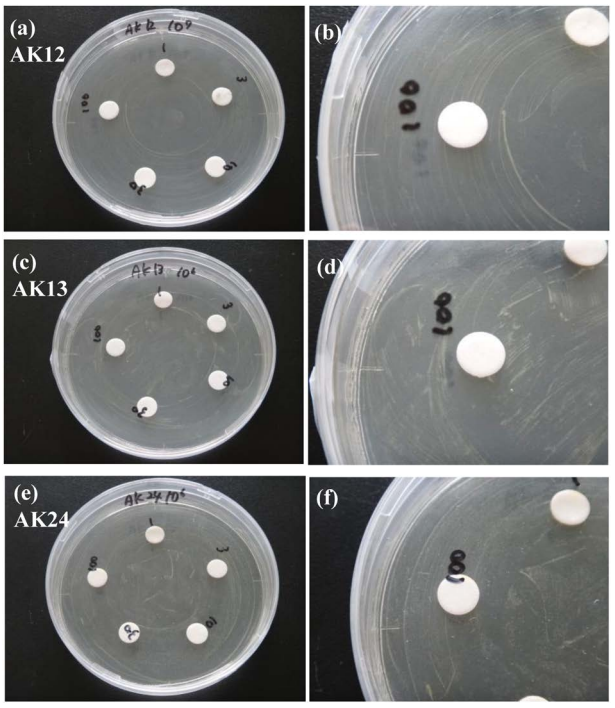

Fig. 2. Effects of *β*-cyclodextrin on growth and survival of the growth-inhibiting bacterial strains AK12 (a, b), AK13 (c, d) and AK24 (e, f) against *A. catenella* (Group I).

β-cyclodextrin tolerance test of algae

Fig. 1 shows the growth of *A. catenella* (Group I) in terms of fluorescence values when *β*-cyclodextrin was added at six different concentrations into the culture medium. In controls without *β*-cyclodextrin, the fluorescence value gradually increased from the initial value of 7.5, reaching a maximum of 127 on day 34, and subsequently decreased (Fig. 1a). In the *β*-cyclodextrin-treated plots, fluorescence values were 7.5–134 (Fig. 1b) in the experiment with 1 μM added, 7.5–133 (Fig. 1c) in the experiment with 10 μM added, and 7.5–137 (Fig. 1d) in the experiment with 100 μM added, respectively. No significant differences were observed among the plots for any of the experiments.

Bacterial *β*-cyclodextrin resistance test

Fig. 2 shows an overall view of a petri dish in which *β*-cyclodextrin-soaked paper disks were placed to spread bacteria, and a close-up view of the area around the paper disks with the highest concentration of *β*-cyclodextrin examined in this study (100 μM). Bacteria grew widely over the entire surface of each petri dish over the experimental range of *β*-cyclodextrin concentrations. They also grew well around paper disks in the experiment with 100 μM *β*-cyclodextrin. No clean zone of inhibition was formed by *β*-cyclodextrin seeping from the paper disk onto the petri dishes. These results show that *β*-cyclodextrin had no negative effects on growth of the bacterial strains or the toxic dinoflagellate *A. catenella* (Group I) and could be used in subsequent experiments.

Verification of QS mechanism

Based on the results of the above two preliminary experiments, the presence or absence of a QS mechanism was verified for bacterial strain AK13 (Fig. 3). First, in algal cultures without bacteria or *β*-cyclodextrin (control), the fluorescence value increased to 5.33–143 during the incubation period, indicating that the algae were reproducing well (Fig. 3a). The maximum was reached on day 31 of incubation, followed by a gradual decrease. In the experiment with AK13, the fluorescence values for bacterial controls (BC, no *β*-cyclodextrin) ranged between 5.33 and 97.3, indicating some growth inhibitory activity (Fig. 3b). In the *β*-cyclodextrin-supplemented experiments, the values were 5.33–92.9 (Fig. 3c), 5.33–99.2 (Fig. 3d), and 5.33–96.2 (Fig. 3e) in the 1 μM, 10 μM, and 100 μM experiments, respectively. Regardless of the *β*-cyclodextrin concentration, the values were almost the same as in BC, and the overall variation was similar. The above results indicate that the AK13 strain was unaffected by *β*-cyclodextrin.

In experiments with AK12, the values for BC (Bacterial control) remained low (5.33–16.2), indicating strong inhibition of *A. catenella* (Group I) growth by this bacterial strain (Fig. 4a). The values for the experiment treated with 1 μM *β*-cyclodextrin were 5.33–130 (Fig. 4b), for the 10 μM experiment were 5.33–129 (Fig. 4c), and for the 100 μM experiment were 5.33–152 (Fig. 4d).

In the experiment with strain AK24, BC ranged between 5.33 and 90.9, and the fluorescence values showed a peak followed by a sharp decrease (Fig. 4e). This indicates some growth inhibitory activity against *A. catenella* (Group I), especially after the peak. The fluorescence values in the experiment treated with 1 μM *β*-cyclodextrin

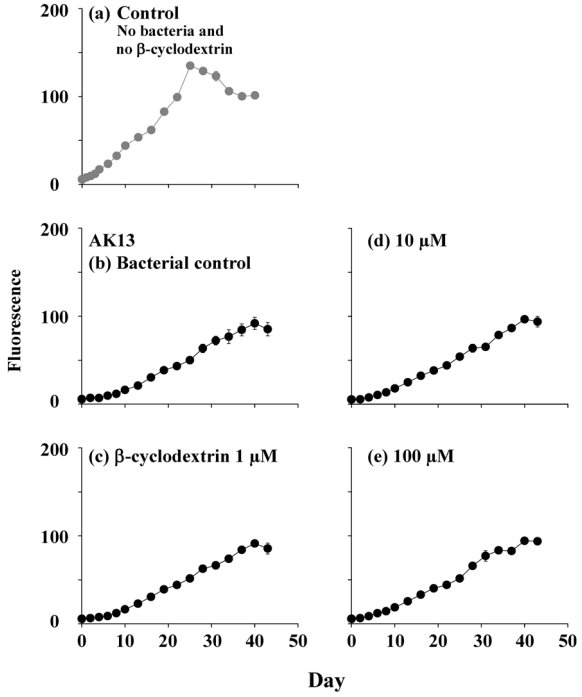

Fig. 3. Effects of the algicidal and growth-inhibiting bacterium strain AK13, with addition of *β*-cyclodextrin, on the growth and survival of *A. catenella* (Group I) were evaluated by measuring the fluorescence of the algae. The bacterial control was set as the treatment with bacteria without *β*-cyclodextrin.

were 5.33–136 (Fig. 4f), 5.33–112 in the 10 μM experiment (Fig. 4g), and 5.33–143 in the 100 μM experiment (Fig. 4h). Unlike strain AK13, both AK12 and AK24 showed higher fluorescence values in the *β*-cyclodextrin-supplemented condition than in the BC condition, which was closer to the control (Fig. 3a).

Discussion

In studies of algicidal bacteria, several reports suggest that their algicidal activities are regulated by QS. The algicidal bacterium *Agrobacterium vitis* O-0805a15 shows some growth inhibitory effect when added as a liquid bacterial culture to the toxic cyanobacterium *Microcystis aeruginosa,* but exhibits strong algicidal activity when colonies are added directly (Imai et al. 2013). Additionally, when bacterial strains isolated from *Zostera marina* were added to the raphidophytes *Heterosigma akashiwo* and *Chattonella antiqua*, the addition of bacterial strains as a culture in liquid medium did not result in algal mortality, however, adding the colonies led to algal mortality (Kodama et al. 2017). In the natural marine environment, it has been reported that highly active algicidal bacteria are particularly found attached to particles (Park et al. 2010). The observed phenomenon in which algicidal activity was exhibited when the bacteria were added to algal cultures in a colony state closely resembles their actual living conditions, where they densely adhere to the surface of particles.

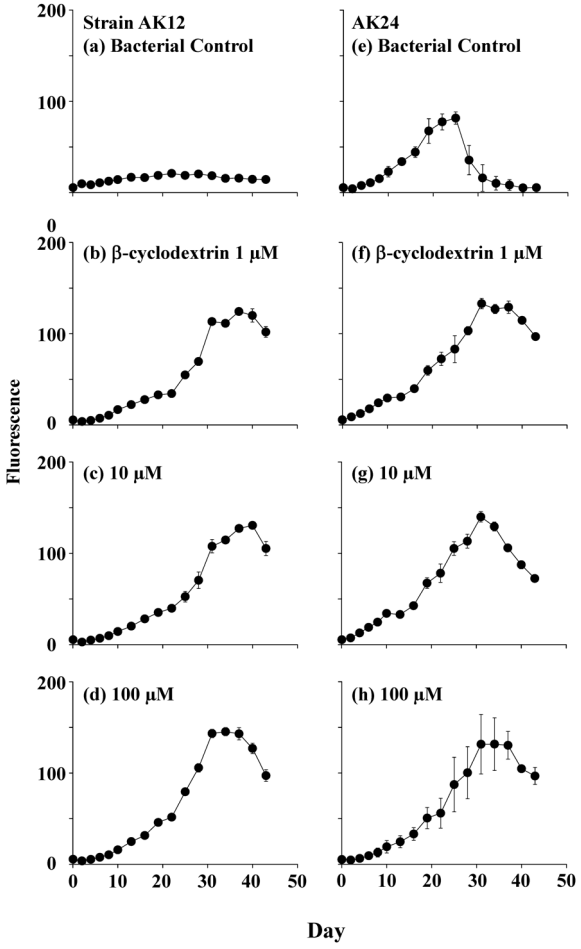

Fig. 4. Effects of the algicidal and growth-inhibiting bacteria strains AK12 and AK24, with addition of *β*-cyclodextrin, on the growth and survival of *A. catenella* (Group I) were evaluated by measuring the fluorescence of the algae. The bacterial controls were set as the treatment with bacteria without *β*-cyclodextrin.

In contrast, the experimental method of adding bacteria as a cell suspension to algae is sometimes inadequate for inducing algicidal activity. It is considered that one of the mechanisms involved in this phenomenon is QS. In this study, the bacterium AK13 exhibited fluorescence values of *A. catenella* (Group 1) approximately two-thirds those of the control at all added concentrations of *β*-cyclodextrin, similar to BC (Fig. 3c, d, e). Therefore, it is considered unlikely that the inhibition of microalgal growth by AK13 was mediated by QS via autoinducers including AHLs. On the other hand, the other two bacterial strains, AK12 and AK24, exhibited behavior closer to the control (Fig. 3a) in the experiments with *β*-cyclodextrin added (Fig. 4b, c, d, f, g, h). These results suggest that strains AK12 and AK24 showed growth inhibition of microalgae via the QS system as autoinducers including AHLs. Alternatively, it is possible that *β*-cyclodextrin encapsulated the algicidal compounds, which may have prevented the algicidal effect from occurring.

Taking a closer look at the two strains, AK12 is closely

related to bacteria in the genus *Kordia*, while AK24 is related to those in the genus *Vibrio* (Table 1). While *Vibrio* spp. are commonly reported as algicidal bacteria, they are also a well-known group of bacteria that perform QS. For example, Cuadrado-Silva et al. (2013) confirmed QS in four *Vibrio* spp. strains isolated from seawater in Santa Marta Bay, Colombia. Golberg et al. (2011) isolated three *Vibrio* spp. strains capable of QS from biofilm on coral surfaces along the coast of Israel. However, with regards to algicidal bacteria within the genus *Vibrio*, there are no previous reports of QS related to algicidal or growth inhibition effects. Thus, our study is the first to demonstrate that *Vibrio*, a major group of algicidal bacteria, inhibit algal growth through QS.

The genus *Kordia*, closely related to the strain AK12, has been reported as an algicidal bacterium. *K. algicida,* has been shown to produce proteases as algicidal substances by QS (Paul & Pohnert 2011). However, it should be noted that this strain is closely related to *Cytophaga latercula* and *Flexibacter tractuosus* NBRC 15980 (Nakagawa 2004). Furthermore, the strain *K. algicida* OT-1 has been reported to be the same strain as *Cytophaga* sp. AA8-2, an algicidal bacterium isolated from a harmful algal bloom of *Heterocapsa circularisquama* in Ago Bay, Mie Prefecture, Japan (Imai et al. 1999, Kondo et al. 1999, Mayali 2007). Many bacterial strains have already been reported in this genus, including those recognized as algicidal bacteria or of being capable of QS, as described above. However, a reassessment of the taxonomic classification of this genus is necessary.

QS is known as a form of bacterial intercellular communication, which is attracting a great deal of attention in various research fields. It has recently been suggested that QS is involved in algicidal and growth inhibition mechanisms against microalgae. In algicidal bacteria, QS has been reported in *Bacillus cereus* and *Planomicrobium* sp. isolated from *Gymnodinium catenatum* in Huon Estuary, Tasmania, Australia (Skerratt et al. 2002), the MS-02-063 strain belonging to the class *γ*-proteobacteria that kills *Heterosigma akashiwo* (Nakashima et al. 2006).

Algicidal bacteria that produce prodigiosin have been reported before. For example, the bacterium *Serratia marcescens* is known to kill the green algae *Coelastrum microporum* and *Bracteacoccus cinnabarinus* using prodigiosin (Darveau & Lynch 1977). The production of prodigiosin in *Serratia* spp. has been shown to be activated by QS, and *N*-Acyl-homoserine lactones (AHLs) act as an auto inducer of Gram-negative bacterial QS signal molecules. The *γ*-proteobacterium *Hahella chejuensis* is known to kill *Cochlodinium polykrikoides* by prodigiosin (Jeong et al. 2005, Kim et al. 2008, Kwon et al. 2010). It is also considered that these algicidal bacterial strains act through a QS-mediated algicidal mechanism.

In recent years, the details of QS mechanisms have been rapidly elucidated in the field of marine microbiology. Hmelo and Van Mooy (2009) identified multiple suspected AHLs in natural seawater and tracked changes in their concentrations using HPLC analysis to estimate their degradation rates. The results showed that AHLs are highly stable in seawater, are hardly degraded by other organisms, and their concentrations decrease only when they lose their activity. Furthermore, the same research team showed that adding particulate organic carbon (POC) containing synthetic AHL to bacterial cultures enhanced bacterial hydrolytic enzyme activity (Hmelo et al. 2011). This suggests a potential means of artificially controlling QS. These studies suggest that QS is an important regulatory factor affecting gene expression and protein synthesis and controlling the ecological functions of plankton in their ecosystems. In the process of exploring mechanisms for preventing and controlling HABs, elucidating the details of QS carried out by algicidal bacteria will be quite an important task in the future. As previously mentioned, we have discovered high densities of algicidal bacteria in biofilms on the surfaces of seagrasses and seaweeds and have demonstrated how these bacteria are constantly distributed at high densities (Onishi et al. 2014, 2021, Imai 2015, Inaba et al. 2017, Imai et al. 2021).

Information on the QS of bacteria in a state attached to the surface of seagrass and seaweed is limited. It was reported that approximately 40% of bacterial strains isolated from biofilms dominated by diatoms on the thallus surface of the seaweed *Fucus vesiculosus* have been reported to interfere with AHL signaling (Romero et al. 2008). The red alga *Delisea pulchra* produces the metabolite halogenated furanone, which inhibits QS by interfering with bacterial AHL receptors and inhibits the formation of bacterial colonies and biofilms on the algal surface (Givskov et al. 1996). Palmitoleic acid (PoA, C16:1Δ9) and myristoleic acid (MoA, C14:1Δ9), two polyunsaturated fatty acids (PUFAs), can inhibit QS (Nicol et al. 2018), and these molecules have been detected at very small amounts (0.5% for PoA) in the leaves of *Z. marina* (Kharlamenko et al. 2001). In *Zostera noltii*, the total content of long-chain polyunsaturated fatty acids (LC-PUFAs) increases significantly with biomass (Duarte et al. 2019), suggesting that the impacts of PUFAs become more significant as seagrass and seaweed grow, and the sizes of seaweed beds and seagrass beds increase. The surfaces of *Z. marina* and other seagrasses are densely inhabited by epiphytic diatoms and dinoflagellates (Nakada et al. 2018). It is plausible that these organisms are able to evade algicidal activity due to the actions of other attached bacteria and interference of QS by PUFA. These functions are likely necessary to maintain stability and balance in the microbial habitat. The interactions among organisms within the biofilms of seagrasses and seaweeds appear to be highly complex. Algicidal bacteria detach from the surfaces of seagrass and seaweed, and float in the seawater, where they attach to detritus particles of organic matter (including microalgae) and exhibit QS abilities. In future studies focusing on QS, it will be quite important to compare and examine the activities of algicidal bacteria attached and living on the leaves of seagrasses

and seaweeds, when they are floating and living on detritus in seawater.

Our study demonstrates that QS plays a significant role in the process of attacking of algae by algicidal and growth-inhibiting bacteria. The method of directly adding small bacterial colonies to microalgae is not commonly used, so it is possible that many isolated bacterial strains have not been recognized as algicidal bacteria. In the future, it will be necessary to consider QS as one of the important processes in the mechanism of algal killing and conduct various experiments accordingly. In addition, it has been reported that gram-negative bacteria use not only AHLs as their autoinducer molecules, but also have the same function with 2-heptyl-3-hydroxy-4-quinolone as their autoinducers (Pesci et al. 1999). Both gram-negative and gram-positive bacteria have been reported to undergo QS via AI-2 (a furanone molecule, Schauder et al. 2001), and it is also believed that there are many other QS systems. It is necessary to expand the research that takes into consideration that more bacteria probably cause algicidal and growth-inhibiting activities through many kinds of QS. This will allow deeper understandings of the mechanisms behind algicidal and growth inhibition effects on HABs by bacteria in the sea.

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