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Screening of the Bacterial Consortium Related to the Microcystin-LR Degradation Isolated From the Marine Area, Isahaya-Bay, and the Freshwater Area, Isahaya-Shin-ike-Pond, Japan

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Abstract

Eutrophication is still a severe global problem, and cyanobacterial microcystins (MCs) have been detected in sea areas such as harbors and coastal regions in addition to freshwater areas. Therefore, it is necessary to clarify the fate of MCs in sea areas. In Japan, Isahaya-Shin-ike-Pond, a freshwater area divided by the dike from Isahaya-bay, Nagasaki-prefecture, has eutrophicated, and cyanobacterial blooms have occurred sometimes. Water in Isahaya-Shin-ike-Pond is regularly discharged to Isahaya-bay to control the water levels in Isahaya-Shin-ike-Pond. Some researchers in Japan detected MCs from the sediment sample of Isahaya-bay; thus, MCs discharged from Isahaya-Shin-ike-Pond could be threatening the environment in Isahaya-bay. In this regard, some bacterial consortiums were isolated from Isahaya-Shin-ike-Pond and Isahaya-bay, which might degrade Microcystin-LR (MC-LR), the highly toxic representative MCs. In this report, we screened and determined the MCs degradation activity in these bacterial consortiums. As a result, we found that all bacterial consortiums degrade MC-LR. Furthermore, the HPLC chromatogram had the peak of substance-like intermediate of MC-LR decomposition. Consortium collected from approximately 4,900 m offshore from the dike degraded 97% of MC-LR. Although the bacterial communities in these consortiums differed, *Bacillus* sp. was in both consortiums collected from Isahaya-Shin-ike-Pond and Isahaya-bay. Because some strain of *Bacillus* sp. has the potential for MCs degradation, it might play an essential role in these bacterial consortiums. These results indicate that the bacteria degrade MCs even though MCs are discharged from the Isahaya-Shin-ike-Pond to Isahaya-bay.

Keywords: Isahaya-Bay, Isahaya-Shin-ike-Pond, Microcystins, Bacterial consortium, Biological degradation

1. Introduction

In closed-water bodies, the abnormal growth of toxic cyanobacterial blooms caused by nutrient influence is still a global problem. Various species are included in these toxic cyanobacteria, and the *Microcystis* genus tends to become a dominant species quickly. These cyanobacteria can produce a high toxicity cyclic heptapeptides, Microcystins (MCs) (Campos & Vasconcelos, 2010; Arman & Clarke, 2021). Thus, many researchers have investigated degrading bacteria of MCs, decomposition processes, and related environmental factors (Massey & Yang, 2020). All these results are for freshwater environments.

On the other hand, the detection of MCs in sea areas such as the harbors and coastal areas of the Baltic Sea, San Francisco Bay has been reported in recent years (Preece et al., 2017, Peacock et al., 2018, Chernova et al., 2019). Moreover, Preece et al. (2017) refer to the report about the case of Isahaya-bay, Nagasaki prefecture, Japan. Isahaya-Shin-ike-Pond in Nagasaki Prefecture is an artificial lake constructed by the reclamation dike dividing the original inner bay, Isahaya-bay (Figure1). Freshwater is regularly drained into Isahaya-bay to control the surface level in Isahaya-Shin-ike-Pond. In recent years, several reports pointed out cyanobacterial blooms in the Isahaya-Shin-ike-Pond and mentioned the accumulation of MCs in sediment and benthic animals (Umehara et al., 2012, 2015, 2017, Takahashi et al., 2014; Takahashi, 2019, 2020). However, the Ministry of Agriculture, Forestry and Fisheries of Japan [MAFF], which holds the jurisdiction over, determined no MCs in the bottom sediment of Isahaya-bay (MAFF, 2013 a). Thus, there are two

conflicting reports. About this conflict, Ruike et al. (2020) verified the persistence of MC-LR in actual sediment and surface water collected from Isahaya-bay under temperature control, and they reported the MC-LR disappeared for 30 days and no long-term persistence in sediment. In addition, the temperature increases shortened the term of MC-LR disappearance; thus, some specific bacteria might degrade MC-LR. Ruike et al. (2020) also reported the possibility that the biodegraded MCs were detected by the method used in the previous study, and achieved the isolation of the bacterial consortium, which might degrade MC-LR from Isahaya-Shin-ike-Pond and Isahaya-bay. Since the degradation characteristics of MCs by microorganisms in the sea are almost unknown, the isolation of a bacterial consortium involved in MC degradation is highly novel, and more detailed studies have been desired. However, the MC degradation capability of the above isolated bacterial consortium has still to be determined because Ruike et al. (2020) only reported the possibility of MC-LR degradation.

Based on the above points, this study aimed to screen and determine the MC-LR degradation capability and bacterial communities of the consortium collected by Ruike et al. (2020).

2. Method

2.1 Bacterial Consortium

The previous study successfully collected the bacterial consortium cultivated (Ruike et al., 2020). These bacterial consortia were obtained by MC-LR enrichment culture using sediment and surface samples collected from Isahaya-Shin-ike-Pond and Isahaya-Bay. According to MAFF (2013), the sampling points S21, S24, and S25 of Isahaya-Shin-ike-Pond and four points of B3, B4, B5, and B6 of Isahaya-bay (Figure 1). Each consortium is abbreviated as C-S21, C-S24, C-S25, C-B3, C-B4, C-B5, and C-B6 in this report. C-S21, C-S24, and C-S25 means consortium collected from Isahaya-Shin-ike-Pond, and C-B3, C-B4, C-B5, and C-B6 from Isahaya-bay. The chloride ion concentration of the surface water in Isahaya-bay was 17,300 to 17,600 mg l^{-1} when each sample was collected.

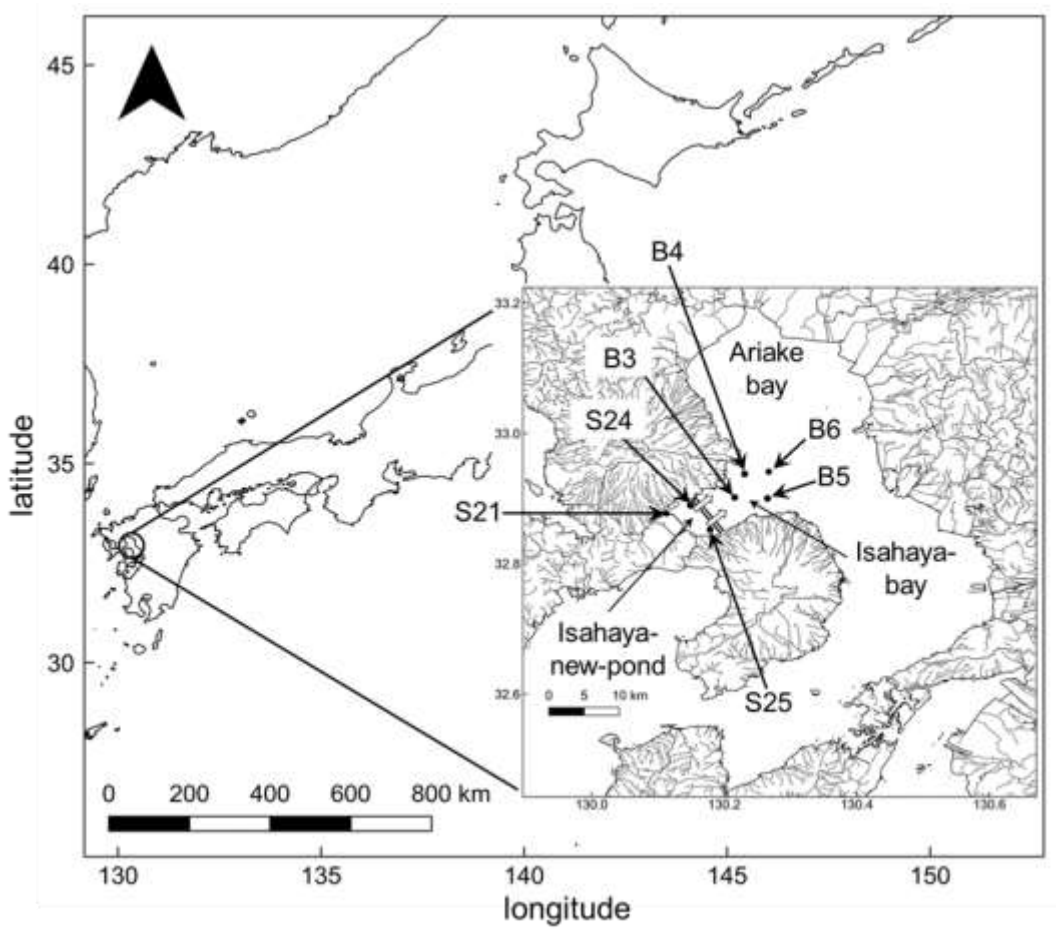

Figure 1. The point of source samples collected from "Isahaya-Shin-ike Pond" and "Isahaya-bay" to acquire the bacterial consortium related to MCs degradation based on MAFF (2013 b)

Double line represents the reclamation dike dividing between Isahaya-Shin-ike-Pond and Isahaya-bay. White arrows represent the north and south gate that discharge the fresh water of Isahaya-Shin-ike-Pond into the Isahaya-bay according to the water-level difference between inside and outside of the reclamation dike.

2.2 MC-LR Solution for the Experiment

MC-LR for the biodegradation experiment was extracted from the mass cultivation of *Microcystis aeruginosa* NIES-298 strain. *M. aeruginosa* NIES-298 strain was purchased from the Microbial Culture Collection at the National Institute for Environmental Studies, Japan. This strain was cultivated in 10 L of M-11 media (Na_2CO_3: 30 mg l^{-1}, $MgSO_4 \cdot 7\ H_2O$: 75 mg l^{-1}, $CaCl_2 \cdot 2\ H_2O$: 40 mg l^{-1}, $NaNO_3$: 100 mg l^{-1}, $Na_2EDTA \cdot 2\ H_2O$: 1 mg l^{-1}, $FeSO_4 \cdot 7\ H_2O$: 1 mg l^{-1}, K_2HPO_4: 10 mg l^{-1}, pH 8.0, describe as Yagi et al., 1979, Hagiwara et al., 1984). After cultivation, the culture solution was treated with 5% of acetic acid solution and super-sonicated (20 kHz, 15 min). Then, crude extract, including MC-LR, was treated with filtration, and the solid-phase extraction removed the foreign substances in this filtrate. The solid-phase extraction devices were the ODS cartridge (Presep-C C-18 (ODS), FUJIFILM Wako Pure Chemical Corporation) and Silica cartridge (Presep Silica Gel Type L Filter, FUJIFILM Wako Pure Chemical Corporation). First, the ODS cartridge was activated with 5 ml of methanol twice and 20 ml of Milli-Q water twice, then the above filtrate was passed through the ODS cartridge at a rate of less than 5 ml min^{-1}. Next, the ODS cartridge was cleaned with 20 ml of 5% methanol twice to remove contaminants, then eluted with 3 ml of methanol. Next, the Silica cartridge was activated with 10 ml of methanol, and the eluate from the ODS cartridge was passed through at a rate of less than 5 ml min^{-1}. Then, 20 ml of methanol was passed through twice to remove contaminants and was eluted with 20 ml of 70% methanol. Finally, the residual materials after the evaporation of the elute from the Silica cartridge were dissolved with an ultrapure water. This solution, mainly including MC-LR (MC-LR extract), was used for the experiment. All methanol used in this study was HPLC grade.

2.3 Screening Test of the MC-LR Degradation in Each Consortium

2 ml of actual surface water collected from Isahaya-Shin-ike-Pond and Isahaya-bay, 1 ml of MC-LR extract, and the glucose at a final concentration of 0.5 g l^{-1} were charged into the test tube and autoclaved (121 ℃, 20 min). Then, 8 ml of each culture solution of the bacterial consortium was added to the test tube, sealed by a cap with an air exchange part, and shaken at 160-165 rpm at 30 ℃. All operation was on a clean bench.

1 ml of sample was collected from each test tube at 0, 29, and 105 hours later. The sample at 0 hours is to check the initial concentration of MC-LR. The experimental period was determined based on the pre-cultivation test before this study. Collected samples were filtered and treated with the ODS cartridge. The method of the ODS cartridge was the same as mentioned above. After the evaporation of the elute from the ODS cartridge, the residue was dissolved in 1 ml of HPLC mobile-phase solution and filtered through a PTFE filter (Pore size = 0.20 μm, Millex-LG, Merck Millipore). As a result of HPLC analysis of this filtrate, initial MC-LR concentration in each test tube was described below; 146 μg l^{-1} in C-S21, 276 μg l^{-1} in C-S24, 113 μg l^{-1} in C-S25, 253 μg l^{-1} in C-B3, 295 μg l^{-1} in C-B4, 211 μg l^{-1} in C-B5, 186 μg l^{-1} in C-B6.

2.4 Next Generation Sequence Analysis of Bacterial Consortium

A bacterial consortium with a high MC-LR degradation rate was selected for the DNA metabarcording analysis. The bacterial communities were analyzed using Miseq and MiSeq Reagent Kit v.3 (Illumina, Inc.). First, samples were combined with the lysis Solution F (in the ISOFECAL, Nippon Gene Co., Ltd.), and after bead beating (2 mins, 1,500 rpm) with Shake Master Neo, it was allowed to stand at 65 ℃ for 10 mins. After centrifugation at 12,000×G for 2 mins, the supernatant containing DNA was collected. Then, DNA was purified using MPure 12 and MPure Bacterial DNA Extraction Kit (MP Bio). A library for analysis was prepared by the 2-step tailed PCR method. The first PCR amplified the V3-V4 region (341F-805R) of the bacterial 16S rRNA gene, and the second PCR added the index sequences and adapter sequences for Miseq. After analysis by Miseq, the primer effects, chimeric, and noise sequences were removed with the FASTX Toolkit (Ver. 0.0.14) and the DADA2 plugin of Qiime 2 (Ver. 2021.4). After that, an annotation search of OTUs based on 97% was performed using Greengene (Ver. 13_8) as a database. DNA extraction, sequencing, and various analysis were outsourced to Bioengineering Lab. Co., Ltd.

2.5 HPLC Analysis

MC-LR quantitative analysis was performed by the HPLC system (Shimadzu 20A series, Shimadzu Corporation). The column was a reversed-phase ODS column (STR ODS-II, 150×6.0 mm, Particle size=5 μm, Shinwa Chemical Industries Ltd.), the column oven temperature was 40 ℃, and the mobile phase for HPLC analysis was 55% methanol with 0.05 M phosphorus acid buffer (pH 3.0). UV/Vis Spectrometer was used as the HPLC detector, and the flow rate was 1.2 ml min^{-1}. The wavelength of the absorption was 238 nm. Each standard reagent was purchased from FUJIFILM Wako Pure Chemicals Corporation. Methanol used was HPLC grade.

3. Results

3.1 Degradation Characteristics in the Bacterial Consortium

The results of the MC-LR degradation experiments with the bacterial consortium are shown in Figure 2. The MC-LR existence rate based on the initial concentration in all bacterial consortiums decreased dramatically and reached 0% after 105 hours. These results mean that a bacterial group involved in MC-LR degradation is in Isahaya-Shin-ike-Pond. In addition, the MC-LR existence rate also decreased in the bacterial consortium collected from Isahaya-bay, and MC-LR was not detected after 105 hours in consortium C-B3, especially. These results indicate a group of bacteria related to MC-LR degradation is in Isahaya-bay. On the other hand, MC-LR was detected in C-B4, C-B5, and C-B6 from Isahaya-bay, even 105 hours after the end of the test. However, a decreasing trend of the MC-LR existence rate throughout the experiment was observed in C-B4 (Figure 2 (B)). Moreover, in the consortium C-B3, which has the lowest MC-LR abundance after 29 hours, a new peak appeared at a retention time earlier than MC-LR on the HPLC chromatogram compared with it at the start (Figure 3). This new peak is presumed to be an intermediate biological decomposition product of MC-LR.

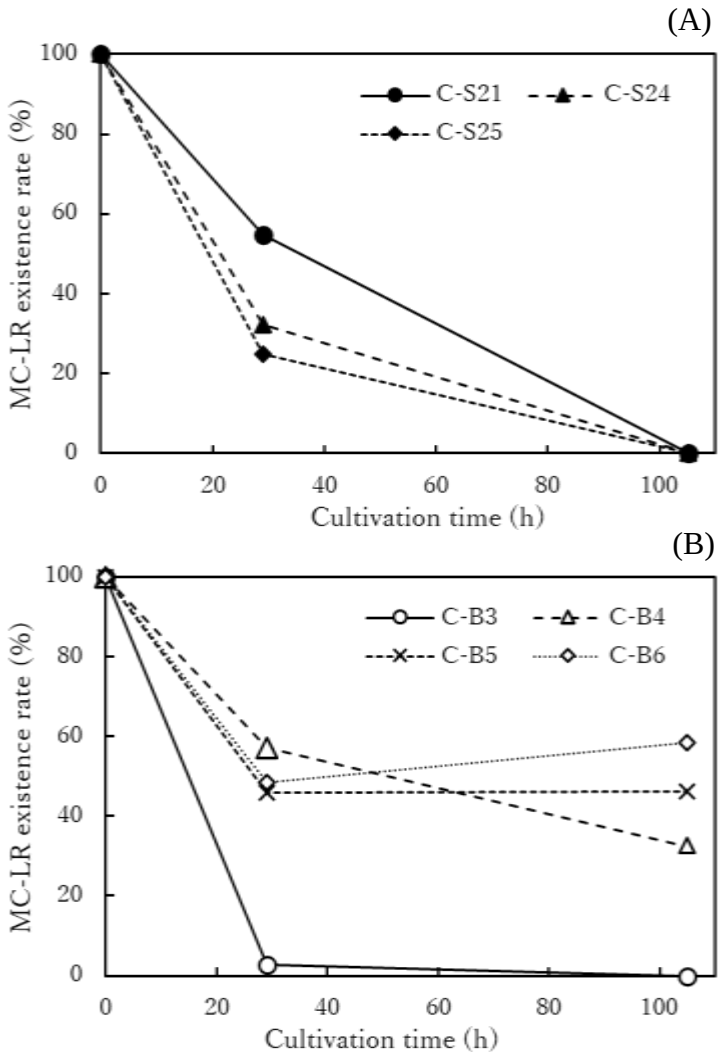

Figure 2. MC-LR existence rate in each bacterial consortium

A: Consortiums acquired from the Isahaya-Shin-ike-Pond, B: Consortiums acquired from the Isahaya-bay.

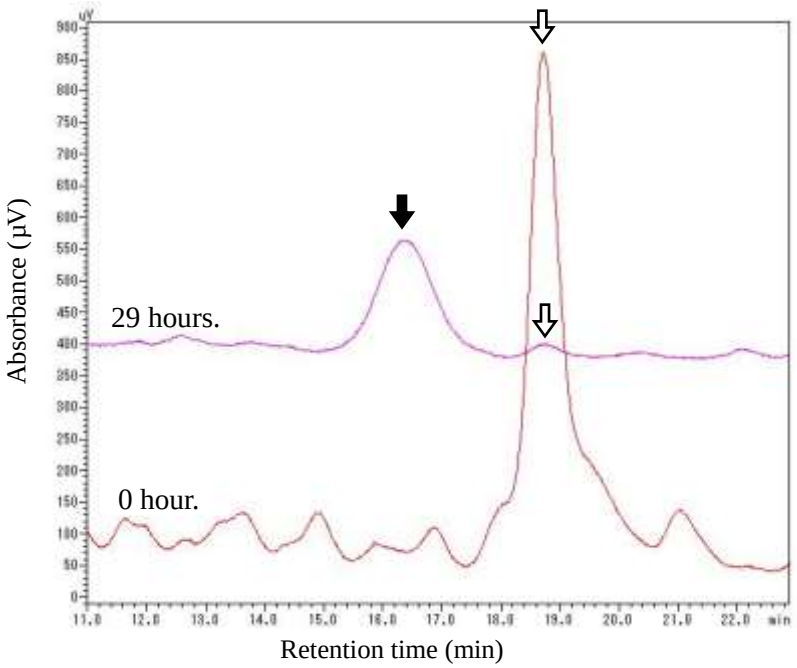

Figure 3. HPLC chromatogram of MC-LR analysis in Consortium C-B3

Red line: 0 hour (initial), purple line: 29 hours later, the white arrow represents the MC-LR peak, the black arrow represents the new peak other than MC-LR after 29 hours.

3.2 Bacterial Communities in the Consortium

Based on the MC-LR decomposition experiment, DNA metabarcoding analysis was performed for consortium C-S25 from Isahaya-Shin-ike-Pond and consortium C-B3 from Isahaya-bay. Figure 4 shows bacterial components in C-S25 and C-B3. Other in Figure 4 consists of OTUs (Operational taxonomic units) less than 97% of sequence homology in the database. As shown in Figure 4, the consortium C-S25 collected from freshwater consists of *Clostridiaceae*, *Paenibacillaceae*, *Peptococccaceae*, *Bacillaceae,* and *Lachnospiraceae*. At the genus level in C-S25, *Clostridium* spp. (52.5%), *Paenibacillus* spp. (22.9%), *Desulfosporosinus* spp. (10.7%) and *Bacillus* spp. (4.9%) was mainly detected. On the other hand, the consortium C-B3 derived from seawater mainly consists of *Kiloniellaceae*, *Bacillaceae*, *Planctomycetaceae*, and *Simkaniaseae* (Figure 4). In C-B3, *Thalassospira* spp. (60%) and *Bacillus* spp. (7.0%), *Simkania* spp. (1.3%) were detected. Although the bacterial communities were significantly different between consortiums C-S25 and C-B3, *Bacillus* spp. was detected in both consortiums (Figure 4).

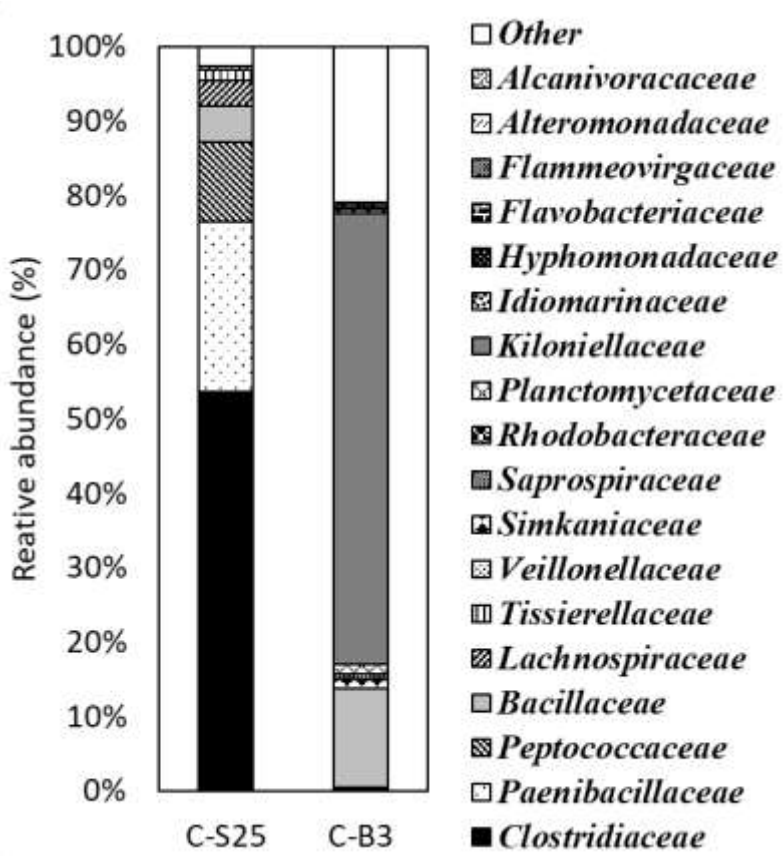

Figure 4. Bacterial communities in each bacterial consortium based on a family

4. Discussion

4.1 Variation of the MC-LR Degradation in Each Consortium and Its Factor

MCs are known to be resistant to decomposition by pH, heat, and general proteases, and several previous reports about biodegradation experiments confirmed no degradation in control series (Harada et al., 1996, Maruyama et al., 2004, Okano et al., 2006). In addition, Mazur & Pliński (2001) investigated the stability of MC-RR and MC-LR in the practical salinity range of 0-24 PSU (Practical Salinity Unit) using sodium chloride, and no decomposition was observed. In this study, we examined the stability of MC-LR in artificial seawater in advance, and no decrease in concentration was detected. Moreover, a new chromatogram peak, like an intermediate decomposition product, was detected during the experiment (Figure 3). Therefore, a decrease in the MC-LR existence rate in Figure 2 is due to biodegradation. This result means that MC-LR degradation bacteria exist in the freshwater, Isahaya-Shin-ike-Pond, and the seawater, Isahaya-bay. Mazur & Pliński (2001) also confirmed MC-LR decomposition in seawater collected from the Gulf of Gdańsk on the Baltic coast. Thus, it is suggested that MCs-degrading bacteria have already inhabited sea areas where they experience the MCs exposure. However, differences in MC-LR degradation were observed between each consortium (Figure 2). To compare the degradation potential of MC-LR between each consortium, the degradation ratio and speed after 29 hours were calculated (Table 1). From Table 1, C-S24 showed the highest MC-LR degradation ratio in the consortium, and this was derived from the S-24 point in Isahaya-Shin-ike-Pond, which was the hot point of *M. aeruginosa* occurrences in the summer (Kasuya & Shitama, 2014). In the consortium from Isahaya-bay, C-B3 showed the highest degradation ratio, and approximately 97% of the added MC-LR was decomposed in 29 hours (Table 1).

On the other hand, the MC-LR degradation ratio and rate of C-B4, C-B5, and C-B6 are lower than that of C-B3, and the original samples of these consortia were collected from points far from the dike. Regarding this point, the 29-hour degradation rate of MC-LR in each consortium was compared with the horizontal distance from the reclamation dike to the sampling point of the original sample in each consortium. As a result, the degradation rate decreased as the horizontal distance from the reclamation dike to the collecting point increased (Figure 5, $r = -0.98$, $p < 0.05$). This relationship suggests that the abundance of the bacteria related to MC-LR degradation is fewer and biodegradation activity is lower,

farther from the reclamation dike and closer to the Ariake Sea.

Table 1. Degradation rate and speed of MC-LR for 29 hours by the bacterial consortium

29 hours degradation characteristics	From Isahaya-bay				From Isahaya-Shin-ike-Pond		
	C-B3	C-B4	C-B5	C-B6	C-S21	C-S24	C-S25
Ratio (%)	97	43	54	52	45	68	75
Rate ($\mu g\ l^{-1}\ h^{-1}$)	8.5	4.4	4.0	3.3	2.3	6.5	2.9

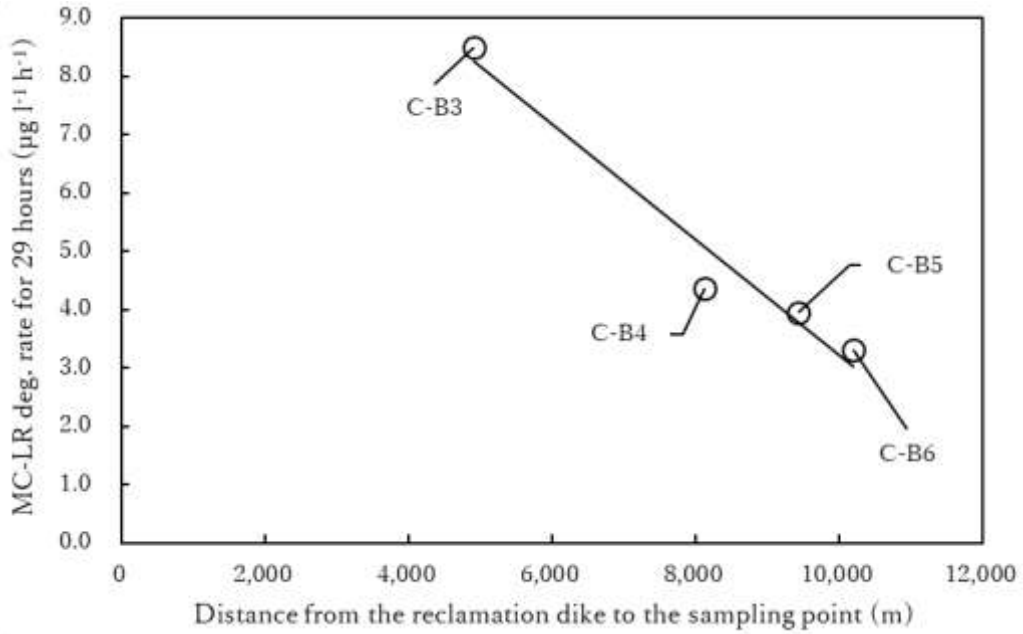

Figure 5. Relationship between a distance from the reclamation dike to the sampling point and MC-LR degradation rate for 29 hours in each consortium

4.2 Prospective Species of MC-LR Degradation in the Consortium

Various MCs-degrading bacteria have been isolated from freshwater bodies (Massey & Yang, 2020). On the other hand, there are almost no reports of MCs-degrading bacteria in seawater. This study investigated the degradation characteristics of a consortium of bacteria that could contribute to MCs degradation in seawater and found that all consortiums collected from Isahaya-bay had degradation activity (Figure 2 (B)). Moreover, the substance presumed to be an intermediate decomposition product was detected in C-B3 (Figure 3). The retention time of this substance was earlier than that of MC-LR, which was similar to the case of MC-LR degradation by *Sphingomonas* sp. ACM-3962 strain (Bourne et al., 1996). In addition, the degradation rate in Table 2 is close to that of *Spingopyxis* sp. TT 25, *Paucibacter toxinivorans*, *Burkholderia* sp., and *Trichaptum abietinum* reported previously (Park, 2014). However, the bacterial community analysis detected no genes related to the above bacteria. In other bacteria, *Bacillus* sp. was detected in freshwater-derived C-S25 and seawater-derived C-B3 consortiums. Some researchers reported that some *Bacillus* sp. strains have an MC-RR and MC-LR degradation potential (Alamri, 2010, 2012; Hu et al., 2012; Kansole et al., 2016). In particular, Alamri (2010, 2012) confirmed the decomposition activity of MC-RR by *Bacillus* spp. in the presence of NB medium (Bacto peptone + Meat extract). Because this experiment also added glucose as a carbon source, it is possible that some species of *Bacillus* sp. degraded MC-LR. Furthermore, the ratio of *Bacillus* spp. in consortium C-B3 was higher than that in C-S25, and the degradation rate of C-B3 was also higher than that of C-S25. Thus, *Bacillus* spp. might be a leading role player for MC-LR degrading in this study. However, the DNA sequences of *Bacillus* spp. in C-B3 differed from that in C-S25. This mismatch suggests the possibility that the bacterial species related to MCs degradation differ between Isahaya-Shin-ike-Pond and Isahaya-bay, and the microbial communities related to MCs-degrading vary depending on the presence or absence of seawater. This is new knowledge that adapted microbial communities to each environment

contribute to MCs degradation.

5. Conclusion

This study focused on two water areas facing each other across the reclamation dike, the freshwater in Isahaya-Shin-ike-Pond and the seawater in Isahaya-bay. We investigated the effect of biodegradation of MC-LR by a consortium of bacteria collected from the actual water area. Regardless of the freshwater or seawater environment, the results revealed that the bacterial consortiums collected from both Isahaya-Shin-ike-Pond and Isahaya-bay have MC-LR degradation. *Bacillus* sp. was suggested as a critical role player in MC-LR degradation in the bacterial consortium collected from Isahaya-Shin-ike-Pond and Isahaya-bay due to bacterial community analysis. In addition, 97% of the added MC-LR was degraded until 29 hours by the bacterial consortium obtained from the Isahaya-bay of about 4,900 m from the reclamation dike. Thus, almost MCs might be degraded even if MCs leaked into Isahaya-bay. As the microbial communities, including MCs-degrading bacteria in the freshwater and seawater environment, differ depending on the presence or absence of salinity, microbial communities adapted to each environment contribute to the decomposition of MCs. It is clarified that the freshwater ecosystem in Isahaya-Shin-ike-Pond and the seawater ecosystem in Isahaya-bay effectively degraded MCs.

This study focused on MC-LR because it is particularly highly toxic. Generally, cyanobacteria produce MC-LR and other MCs such as MC-RR and MC-YR. Therefore, further investigation of the degradation of other MCs is necessary. However, these MCs might be degraded like a case of MC-LR as references of the present study. Furthermore, in this study, it was clarified that MCs can be efficiently decomposed in seawater. This fact provides a helpful perspective to evaluate the environmental risk of MCs in the sea, including stability and bioaccumulation. Although the consortium had many families of bacteria, an actual role player of MC-LR degradation might be a few bacterial species. Thus, a clarification of these bacteria is expected in the future. Finally, this report is expected to provide helpful knowledge for solving the problems of cyanobacterial blooms and MCs in the sea.

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References

Alamri, S. A. (2010). Biodegradation of microcystin by a new *Bacillus* sp. isolated from a Saudi freshwater lake, *African Journal of Biotechnology*, *9*(39), 6552-6559. https://doi.org/10.5897/AJB10.1096

Alamri, S. A. (2012). Biodegradation of microcystin-RR by *Bacillus flexus* isolated from a Saudi freshwater lake, *Saudi Journal of Biological Sciences*, *19*(4), 435-440. https://doi.org/10.1016/j.sjbs.2012.06.006

Arman, T., & Clarke, J. D. (2021). Microcystin toxicokinetics, molecular toxicology, and pathophysiology in preclinical rodent models and humans, *Toxins*, *13*(8), 537, 1-21. https://doi.org/10.3390/toxins13080537

Bourne, D. G., Jones, G. J., Blakeley, R. L., Jones, A., Negri, A. P., & Riddles, P. (1996). Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR, *Applied and Environmental Microbiology*, *62*(11), 4086-4094. https://doi.org/10.1128/aem.62.11.4086-4094.1996

Campos, A., & Vasconcelos, V. (2010). Molecular mechanisms of microcystin toxicity in animal cells, *International Journal of Molecular Sciences*, *11*(1), 268-287. https://doi.org/10.3390/ijms11010268

Chernova, E., Sidelev, S., Russkikh, I., Voyakina, E., & Zhakovskaya, Z. (2019). First observation of microcystin- and anatoxin-a-producing cyanobacteria in the easternmost part of the Gulf of Finland (the Baltic Sea), *Toxicon*, *157*, 18-24. https://doi.org/10.1016/j.toxicon.2018.11.005

Hagiwara, T., Yagi, O., Takamura, Y., & Sudo, R. (1984). Isolation of bacteria-free *Microcystis aeruginosa* from Lake Kasumigaura, *Japan Journal of Water Pollution Research*, *7*(7), 437-442. (in Japanese) https://doi.org/10.2965/jswe1978.7.437

Harada, K.-I., Tsuji, K., & Watanabe, M. F. (1996). Stability of microcystins from cyanobacteria - III. Effect of pH and temperature. *Phycologia*, *35*(Sup 6), 83-88. https://doi.org/10.2216/i0031-8884-35-6S-83.1

Hu, L., Zhang, F., Liu, C., & Wang, M. (2012). Biodegradation of microcystins by *Bacillus* sp. strain EMB, *Energy Procedia*, *16*(C), 2054-2059. https://doi.org/10.1016/j.egypro.2012.01.312

Kansole, M. M. R., & Lin, T.-F. (2016). Microcystin-LR biodegradation by *Bacillus* sp.: Reaction rates and possible genes involved in the degradation, *Water*, *8*(11), 1-19. https://doi.org/10.3390/w8110508

Kasuya, T., & Shitama, M. (2014). Phytoplankters and Benthoses of the Detention Pond originated from Isahaya-Bay Land Reclamation (Investigation from April 2013 to March 2014), *Annual Report of Nagasaki Prefectural Institute*

for Environmental Research and Public Health, *59*, 74-76. (In Japanese)

MAFF (Ministry of Agriculture, Forestry and Fisheries of Japan), Kyushu Regional Agricultural Administration Office (2013 a), *3. Results of the analysis in sediments collected from the Isahaya-bay and the Ariake-sea*. Retrieved June 25, 2019, from MAFF Web site: https://www.maff.go.jp/kyusyu/seibibu/info/ 20060823.html (in Japanese)

MAFF, Kyushu Regional Agricultural Administration Office (2013 b). *Document 3: Investigation plan to figure out the alteration in the environment caused by the opens of the drainage gate of the reclamation dike as a tide-barrier in the reclamation project of the Isahaya-bay*, Retrieved November 1, 2023, from the Ministry of the Environment of Japan Web site: https://www.env.go.jp/council/20ari-yatsu/y202-04b.html (in Japanese)

Maruyama, T., Kato, K., & Park, H.-D. (2004). Population dynamics of free-living bacteria related to the microcystin-degrading strain Y2 in Lake Suwa and in microcystin amended enrichments, *Microbes and Environments*, *19*(2), 137-146. https://doi.org/10.1264/jsme2.19.137

Massey, I. Y., & Yang, F. (2020). A mini review on microcystins and bacterial degradation, *Toxins*, *12*(4), 268, 1-21. https://doi.org/10.3390/toxins12040268

Mazur, H., & Pliński, M. (2001). Stability of cyanotoxins, microcystin-LR, microcystin-RR and nodularin in seawater and BG-11 medium of different salinity, *Oceanologia*, *43*(3), 329-339.

Okano, K., Maseda, H., Sugita, K., Saito T., Utsumi M., Maekawa T., Kobayashi M., & Sugiura N. (2006). Biochemical characteristics of Microcystin-LR degradation by typical protease, *Japanese Journal of Water Treatment Biology*, *42* (1), 27-35. https://doi.org/10.2521/jswtb.42.27

Park H.-D., (2014). Studies on the toxins produced by blue-green algae, *Journal of Japan Society on Water Environment*, *37A*(5), 169-174. (in Japanese)

Peacock, M, B., Gibble, C. M., Senn, D. B., Cloern, J. E., & Kudela, R. M. (2018). Blurred lines: Multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California, *Harmful Algae*, *73*, 138-147. https://doi.org/10.1016/j.hal.2018.02.005

Preece, E. P., Hardy, F. J., Moore, B. C., & Bryan, M. (2017). A review of microcystin detections in estuarine and marine waters: Environmental implications and human health risk, *Harmful Algae*, *61*, 31-45. https://doi.org/10.1016/j.hal.2016.11.006

Ruike, K., Inamori, R., & Inamori, Y. (2020). Quantitative evaluation of the microcystin produced by blue-green algae and its decomposition characteristics at inside and outside of the dike in Isahaya pond, *Journal of Water and Waste*, *62*(10), 722-729. (in Japanese)

Takahashi, T., Umehara, A., & Tsutsumi, H. (2014). Diffusion of microcystins (cyanobacteria hepatotoxins) from the reservoir of Isahaya Bay, Japan, into the marine and surrounding ecosystems as a result of large-scale drainage, *Marine Pollution Bulletin*, *89*(1-2), 250-258. https://doi.org/10.1016/j.marpolbul.2014.09.052

Takahashi, T. (2019). The drainage from Isahaya Bay reservoir has further adverse effects on the damaged ecosystem of Ariake Sound, *Japanese Journal of Benthology*, *73*(2), 123-128. (in Japanese) https://doi.org/10.5179/benthos.73.123

Takahashi, T. (2020). Advections of microcystine from Isahaya bay reservoir to the sea area, *Journal of Japan Society on Water Environment*, *43A* (10), 350-353. (in Japanese)

Umehara, A., Komorita, T., Tai, A., Takahashi, T., Orita, R., & Tsutsumi, H. (2015). Short-term dynamics of cyanobacterial toxins (microcystins) following a discharge from a coastal reservoir in Isahaya Bay, Japan, *Marine Pollution Bulletin*, *92*(1-2), 73-79. https://doi.org/10.1016/j.marpolbul.2014.12.053

Umehara, A., Takahashi, T., Komorita, T., Orita, R., Choi, J.-W., Takenaka, R., Mabuchi, R., Park, H.-D., & Tsutsumi, H. (2017). Widespread dispersal and bio-accumulation of toxic microcystins in benthic marine ecosystems, *Chemospehre*, *167*, 492-500. https://doi.org/10.1016/j.chemosphere.2016.10.029

Umehara, A., Tsutsumi, H., & Takahashi, T. (2012). Blooming of *Microcystis aeruginosa* in the reservoir of the reclaimed land and discharge of microcystins to Isahaya Bay (Japan), *Environmental Science and Pollution Research*, *19*(8), 3257-3267. https://doi.org/10.1007/s11356-012-0835-y

Xie, L., & Park, H.-D. (2007). Determination of microcystins in fish tissues using HPLC with a rapid and efficient solid phase extraction, *Aquaculture*, *271*(1-4), 530-536. https://doi.org/10.1016/j.aquaculture. 2007.07.004

Yagi, O., Okada, M., & Sudo, R. (1979). Cultivation of *Microcystis* and red-tide-organisms. *Research Report from the National Institute for Environmental Studies*, *6*, 223-229. (in Japanese).

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Authors contributions

Dr. Y. Inamori and Dr. R. Inamori were responsible for study design, drafted and revised the manuscript. Dr. K. Ruike was responsible for mainly data collection of the degradation experiment. Prof. K. Murakami was responsible for data collection of the molecular biological analysis. Dr. R. Suzuki was responsible for the LC analysis. All authors read and approved the final manuscript.

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Data sharing statement

No additional data are available.