Acta Oceanol. Sin., 2023, Vol. 42, No. 6, P. 70–77
https://doi.org/10.1007/s13131-022-2095-0
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Diversity of culturable alginate lyase-excreting bacteria associated with *Sargassum*

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Received 21 April 2022; accepted 12 August 2022

Abstract

Large numbers of bacteria live on the surface of various brown algae and can produce alginate lyases to consume alginate, an important component of the cell wall of brown algae. *Sargassum* is a genus of the largest canopy-forming brown algae of more than 150 species, which are widely distributed in tropical and subtropical environments. However, our knowledge about the epiphytic bacteria and the alginate lyase-excreting bacteria from *Sargassum* is still primitive. Here, we investigated the diversity of the culturable epiphytic bacteria and alginate-degrading bacteria from *Sargassum* samples collected from the coastal seawaters of Shandong Province, China. In total, 37 strains belonging to 21 genera in 3 phyla were isolated, including 15 previously unreported genera, of which *Vibrio* (6/37) and *Pseudoalteromonas* (5/37) are the dominant genera. Eight strains, mainly *Vibrio* and *Pseudoalteromonas* species, were further identified as alginate lyase-excreting strains that can utilize alginate for growth. The extracellular alginolytic activity of the 8 strains was determined, and strains *Vibrio* sp. C42 and *Pseudoalteromonas* sp. M9 showed the highest activity. These results provide a better understanding of brown algae epiphytes and alginate-degrading bacteria, and are fundamental for further studies on the interactions between brown algae and their epiphytes.

Key words: *Sargassum*, epiphyte, alginate lyase-excreting bacteria, alginolytic activity

Citation: Sun Xiaomeng, Xue Zhao, Chen Cui, Fan Shoujin, Fu Huihui, Wang Peng. 2023. Diversity of culturable alginate lyase-excreting bacteria associated with *Sargassum*. Acta Oceanologica Sinica, 42(6): 70–77, doi: 10.1007/s13131-022-2095-0

1 Introduction

Alginate is an acidic linear polysaccharide that is present in great abundance in the cell wall of brown algae (Wong et al., 2000). Alginate is composed of β-D-mannuronate (M) and its C5 epimer, α-L-guluronate (G), which are linked by 1,4-O-glycoside bonds and arranged in block structures, such as homopolymeric G block, M block, and heteropolymeric MG (GM) block (Xu et al., 2017). Alginate has been widely applied in food, cosmetic and pharmaceutical industries, due to its unique physical properties to form gels (Wong et al., 2000). Alginate lyases degrade alginate by a β-elimination mechanism, generating a product containing 4-deoxy-L-*erythro*-hex-4-enopyranosyluronic acid as the non-reducing terminal moiety (Dong et al., 2012). Bacterial alginate lyases play an important role in the decomposition and recycling of alginate in the ocean. In addition, alginate lyases are important tools for the production of alginate oligosaccharides with special bioactivities (Cheng et al., 2020). They also have potential for the production of biofuels by deconstructing the alginate-rich algal cell walls into monosaccharides (Enquist-Newman et al., 2014; Wargacki et al., 2012) and for the treatment of chronic lung infection caused by *Pseudomonas aeruginosa* (Islan et al., 2013).

Brown algae contain 20 classes, and the class of Phaeophyceae alone accounts for over 1 800 species and 66% of the total algae consumption (Holdt and Kraan, 2011), acting as important primary producers to offer nutrients for their epiphytic bacteria (Egan et al., 2008). A large number of bacteria live on the surface of brown algae, and many bacterial species have been isolated from brown algae (Lee et al., 2006; Martin et al., 2014). The composition of alga-associated microbial communities varies according to the algal species, the age of the thalli and the sampling season and site. As algae-associated bacteria constantly metabolize algal components, they produce numerous specific enzymes and secondary metabolites (Martin et al., 2014). Because brown algae possess abundant alginate in the cell walls, they are considered as a good source for isolating alginate lyase-excreting bacteria. So far, many alginate lyase-excreting bacterial strains have been isolated from a variety of brown algae (Li et al., 2011; Sawabe et al., 2000).

Sargassum, containing more than 150 species, is the largest canopy-forming brown algae, which is widely distributed in tropical and subtropical environments and has a high amount of biomass (Gouvêa et al., 2020). There are 131 species in China, 64 of which are unique, mainly distributed in the South China Sea, the East China Sea and the Yellow Sea. Only 23 species are distributed in the East China Sea and the Yellow Sea, including *S. qingdaoense*, *S. serratifolium*, *S. fusiforme*, *S. confusum*, *S. horneri*, *S. Museum*, *S. patens* and others (Huang et al., 2013). Some of them have edible (Xia et al., 2019), medicinal (Huang et al., 2006; Mao et al., 2004; Park et al., 2005) and industrial (Demirbas and Demirbas, 2011) values. They also have ecological values in repair-

Foundation item: The National Natural Science Foundation of China under contract Nos 32170127 and 42176156; the Fundamental Research Funds for the Central Universities under contract No. 202141006.
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ing the marine environment by absorbing nitrogen, phosphorus and other elements in seawater and purifying water quality (Vijayaraghavan et al., 2009). *Sargassum* species have high content of alginate, accounting for more than 50% of their dry weight (Youssouf et al., 2017). Thus, *Sargassum* species are good materials to isolate alginate lyase-excreting bacteria. In addition, large-scale *Sargassum* blooms have been increasingly observed in coastal zones in recent years, causing serious problems for seaweed and abalone farms as well as for fisheries, tourism and recreational industries (Bao et al., 2022). Isolation of alginate lyase-excreting bacteria from *Sargassum* is not only conducive to understanding the interaction between *Sargassum* and bacteria and exploiting alginate lyase resources from the ocean, but also beneficial for biological control of *Sargassum*. However, to our knowledge, so far there are only two studies on the diversity of *Sargassum*-associated bacteria (Mei et al., 2019; Menezes et al., 2010), and only one study on the isolation of alginate lyase-excreting bacteria from *Sargassum* (Wang et al., 2017). Many *Sargassum* epiphytes and epiphytic alginate lyase-excreting bacteria remain to be identified.

In this study, we investigated the diversity of cultivable bacteria associated with the *Sargassum* samples collected from coastal seawaters from five locations in Shandong Province, China. The strains with the alginate lyase-excreting ability were further isolated and identified. Moreover, the extracellular alginolytic activity of these alginate lyase-excreting strains was measured. The results provide new insight into *Sargassum*-associated bacteria and may inform biological control of *Sargassum* blooms.

2 Materials and methods

2.1 *Collection of the algal samples*

The algal samples with the same appearance forms were collected from coastal seawaters at five locations in Shandong Province, China (Table 1). The collected samples were transferred to sterile collection bags filled with sterile artificial seawater (ASW) prepared with 3% (w/v, the mass (3 g) of sea salt dissoved in the volume (100 mL) of liquid) sea salts (Sigma, America) to make temporary specimens. After collection, bags containing the samples were stored in ice and the samples were immediately processed upon returning to the laboratory. At the time of collection, the water temperature was 5.8–12.8℃, the salinity was approximately 3.2% (w/v), and the pH was approximately 7.9.

2.2 *Isolation of the bacteria associated with the Sargassum samples*

Each *Sargassum* sample was cut into small pieces using sterile scissors, and approximately 10 g of the small algal pieces were suspended in 25 mL ASW with shaking at 180 r/min, 15℃ for 10 min. The obtained suspension was serially ten-fold diluted to 10^{-6} dilution with sterile ASW. Aliquots of 200 μL diluted samples (10^{-2}–10^{-6} dilution) were spread on the plates containing tryptone, yeast extract, and artifical seawater (TYS) solid medium (pH 7.5–8.0) composed of 0.5% (w/v) tryptone (Oxoid, UK), 0.1% (w/v) yeast extract (Oxoid, UK), 1.5% (w/v) agar (Sigma, USA) and ASW. The plates were then incubated at 15℃ for 7 d. After cultivation, morphologically different colonies were separated and purified by repeatedly streaking on TYS solid medium. The purified isolates were routinely grown on TYS plates or in TYS broth (pH 7.5–8.0) composed of 0.5% (w/v) tryptone, 0.1% (w/v) yeast extract and ASW at 25℃ for 3–5 d, and stored in 20% (v/v) glycerol at –80℃.

Table 1. The sampling time and locations

Station	Time	Latitude	Longitude
1	Nov., 2018	37°09′41.7″N	122°35′11.8″E
2	Mar., 2019	37°09′24.7″N	122°35′35.9″E
3	May, 2019	37°09′24.7″N	122°35′35.9″E
4	Mar., 2019	37°09′41.7″N	122°35′11.8″E
5	May, 2019	37°09′41.7″N	122°35′11.8″E

2.3 *Screening of alginate lyase-excreting bacteria*

The purified isolates were streaked on the plates with a minimal medium (0.05% (w/v) NH_4Cl, 3% (w/v) NaCl, 0.3% (w/v) $MgCl_2{\cdot}6H_2O$, 0.2% (w/v) K_2SO_4, 0.02% (w/v) K_2HPO_4, 0.001% (w/v) $CaCl_2$, 0.000 6% (w/v) $FeCl_3{\cdot}6H_2O$, 0.000 5% (w/v) $Na_2MoO_4{\cdot}7H_2O$, 0.000 4% (w/v) $CuCl_2{\cdot}2H_2O$, 0.6% (w/v) Tris (pH 7.5–8.0) and 1.5% (w/v) agar) containing 0.5% (w/v) sodium alginate as the sole carbon source. After incubation at 25℃ for 7 d, the plates were stained by Lugol's iodine solution to detect the appearance of a clear halo of depolymerization around a strain colony as a preliminary indicator of alginate degradation. Then, the strains with a clear halo were inoculated into the liquid minimal medium (pH 7.5–8.0) containing 0.5% (w/v) sodium alginate, and cultured at 20℃ with stirring (180 r/min) for 2 d. The strains that grew in the liquid medium were selected as alginate lyase-excreting bacteria.

2.4 *Alginate lyase assay*

Alginate lyase activity was quantitatively determined by measuring the amount of reducing sugars released from alginate by using the 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959; Tang et al., 2009). The fermentation broth of each alginate lyase-excreting strain cultured at 20℃ with stirring (180 r/min) for 1.5 d was centrifuged at 16 000×*g* for 5 min to obtain the supernatant as crude enzymes. Then, 50 μL crude enzyme was mixed with 50 μL substrate solution composed of 1% (w/v) sodium alginate dissolved in 50 mmol/L Tris–HCl (pH 8.0), and the mixture was incubated at 30℃ for 60 min. Afterwards, the reaction mixture was terminated by addition of 100 μL DNS and then boiled at 100℃ for 10 min. A blank control was set by mixing DNS solution and the substrate solution followed by addition of crude enzymes to inactivate the alginate lyase activity before incubation. The absorbance values of the mixtures were determined at 540 nm. The amount of reducing sugars released into the mixture was determined with glucose as the standard. One unit of enzyme activity is defined as the amount of enzyme required to release 1 μg reducing sugars per minute.

2.5 *Sequencing of the 16S rRNA gene and phylogenetic analysis*

Genomic DNA of each strain was extracted using a BioTeke DNA extraction kit (Beijing, China). The 16S rRNA gene of each strain was amplified via PCR using the primers 1492R and 27F (1492R: 5′-GGTTACCTTGTTACGACTT-3′ and 27F: 5′-AGAGTTTGA TCCTGGCTCAG-3′) (Dong et al., 2012). The PCR product of each strain was ligated to the pMD19-T vector (TaKaRa, Japan) and sequenced on an Applied Biosystems DNA sequencer (model 3730XL). The sequence of each 16S rRNA gene was compared with those in the GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Ezbiocloud (http://www.ezbiocloud.net) databases using BLASTN. The identity values of paired sequences were obtained by the EzBioCloud server. The Software Mega X was used to construct the phylogenetic tree by the neighbor-join-

ing method (Kumar et al., 2018) based on the 16S rRNA gene sequences, and the bootstrap values of each branch of the phylogenetic tree were tested by 1 000 repetitions.

3 Results and discussion

3.1 *Identification of the algal samples*

By comparing the morphology of the five algal specimens (Fig. 1), involving the color, size, leaf shape and other appearance forms, with those of the *Sargassum* permanent specimens (preserved in Shandong Normal University, Shandong Province, China), the collected samples were all identified as *Sargassum* sp. by the botanist professor Shoujin Fan of Shandong Normal University.

3.2 *Diversity of bacteria associated with the Sargassum samples*

After 7-d cultivation of the bacteria from the *Sargassum* samples on the TYS plates, many colonies appeared on the plates with 10^{-2}–10^{-5} diluted samples. These isolates were purified and subjected to 16S rRNA gene amplification and sequencing. Isolates with two or more base difference in their 16S rRNA gene sequences were considered as different bacterial strains. Based on an alignment of the 16S rRNA gene sequences, a total of 37 strains were finally isolated from the *Sargassum* samples, which belonged to the phyla Pseudomonadota (28/37), Bacteroidota (7/37) and Bacillota (2/37) (Fig. 2). These strains were affiliated with 21 genera, including *Algibacter* (1/37), *Altererythrobacter* (1/37), *Alteromonas* (2/37), *Bacillus* (1/37), *Bernardetia* (1/37), *Colwellia* (1/37), *Erythrobacter* (3/37), *Halobacillus* (1/37), *Marinomonas* (1/37), *Octadecabacter* (1/37), *Olleya* (2/37), *Paraglaciecola* (1/37), *Photobacterium* (1/37), *Polaribacter* (2/37), *Pseudoalteromonas* (5/37), *Psychromonas* (3/37), *Rheinheimera* (1/37), *Shewanella* (1/37), *Tenacibaculum* (1/37), *Vibrio* (6/37) and *Yoonia* (1/37) (Figs 2 and 3). Among them, *Vibrio* (6/37) and *Pseudoalteromonas* (5/37) were the dominant genera.

The strains of each sampling station varied greatly. Strains belonging to *Algibacter* and *Bernardetia* (Bacteroidota) were only isolated from Station 2, and strains belonging to *Polaribacter* and *Tenacibaculum* (Bacteroidota) were only isolated from Station 4, and strains of *Olleya* of B were isolated from Station 2 and Station 4. Only two Bacillota strains were isolated, which were one of *Halobacillus* from Station 1 and the other of *Bacillus* from Station 5. Strains of Pseudomonadota were isolated from all stations (Fig. 3). Although *Vibrio* and *Pseudoalteromonas* were the dominant genera among all the 21 genera, *Erythrobacter* strains were dominant in those from Station 1 and *Psychromonas* strains were dominant in those from Station 2 (Fig. 3). Furthermore, the strains isolated from Station 2 belong to a total of 8 genera, whose diversity was higher than those isolated from other sampling stations. Although the treatment of *Sargassum* samples and the screening method of epiphytic bacteria from all stations were consistent, the epiphytes varied from each station, indicating that different sampling locations and times may have impacts on bacterial community composition.

Fig. 1. Morphology of the *Sargassum* sample collected at Station 1.

Studies on the growth cycle of *Sargassum* showed that *Sargassum* grows slowly in winter and that the growth rate of *Sargassum* accelerates with the increase in temperature. When the temperature reaches approximately 25℃, the length and weight of algae reach the maximum, and reproductive growth occurs (Chu et al., 2011; Umezaki, 1974). The sampling time at Station 1 is in winter, and the sampling time at other stations is in spring. In winter, the slow growth of *Sargassum* and reduced amount of nutrients released to the phycosphere may lead to the fact that the bacteria isolated from Station 1 were absent from other sampling stations. In March, the cold air gradually weakens and extratropical cyclone is frequent. Therefore, wind and waves often occur offshore of the Yellow Sea under the cooperation of cold air and extratropical cyclone, which probably resulted in the high diversity of bacteria on the samples from Station 2 and Station 4, which were collected in March. Samples from Station 2 and those from Station 4 were sampled on the same day but from different locations. Although their community composition was different, they all contained *Pseudoalteromonas* and *Olleya*. Similarly, samples from Station 3 and those from Station 5 were sampled on the same day but from different locations, and the dominant genus on the samples from both stations is *Vibrio*. Therefore, although differences in community composition existed between samples from different sampling locations, there were also similarities due to the proximity of the locations.

Although many strains have been isolated from *Sargassum*, including some novel species (Choi et al., 2017; Kim et al., 2016; Lee et al., 2017; Wang et al., 2020), there are so far only two studies on the diversity of *Sargassum*-associated bacteria (Fig. 4). Mei et al. (2019) reported the potential epiphytic bacteria associated with the drifting *S. horneri*, including offshore yellow *Sargassum* and nearshore brown *Sargassum*, which were analyzed using the high-throughput sequencing data of the 16S rRNA gene by a modified co-vortex method with silica sand. The dominant bacterial genera from the brown *Sargassum* are *Bacillus* (12.2%), *Propionibacterium* (9.48%), *Kocuria* (8.01%), *Pseudomonas* (7.65%), and *Bacteroides* (5.21%); and the dominant bacterial genera from the yellow *Sargassum* are *Flavobacterium* (16.5%), *Paracoccus* (11.5%), *Bacillus* (6.97%), and *Propionibacterium* (5.27%). Menezes et al. (2010) analyzed the microbial diversity associated with *Sargassum* sp. collected from Brazil. As a result, strains from *Bacillus* (7/26), *Ruegeria* (5/26), *Micrococcus* (3/26), *Staphylococcus* (2/26), *Kocuria* (1/26), *Arthrobacter* (1/26), *Brevundimonas* (1/26), *Dokdonia* (1/26), *Knoellia* (1/26), *Nocardioides* (1/26) and *Vibrio* (1/26) were isolated (Menezes et al., 2010). The *Sargassum* epiphytes from different studies are significantly different, which may be caused by the differences in *Sargassum* species,

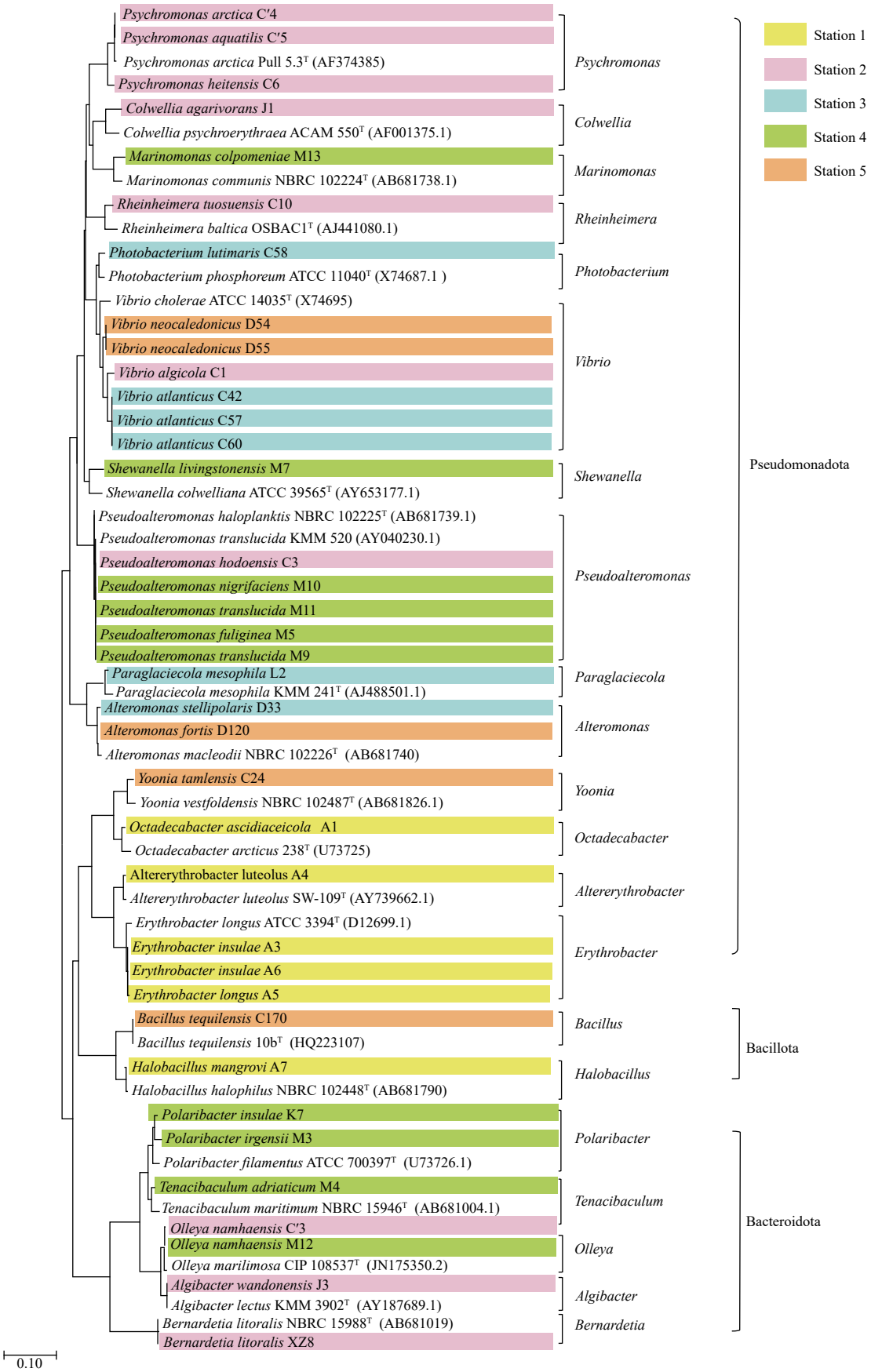

Fig. 2. A neighbor-joining phylogenetic tree of the bacteria isolated from the *Sargassum* samples based on the 16S rRNA gene sequences. The bootstrap values of each branch were tested by 1 000 repetitions. Five different colors indicate the stations which the strains were isolated from.

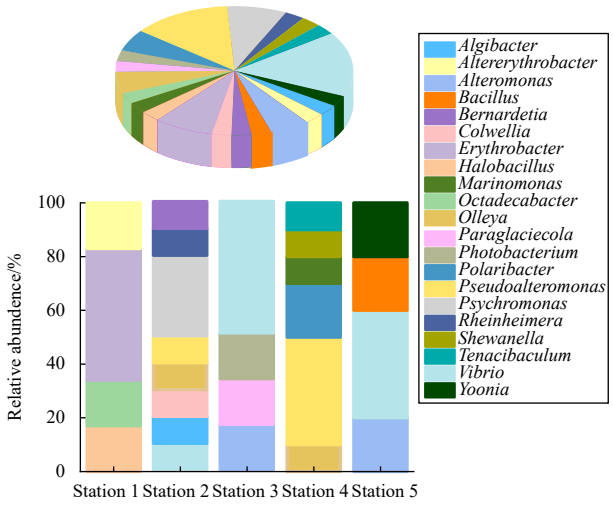

Fig. 3. The community compositions of the cultivable epiphytic bacteria from the *Sargassum* samples from all sampling stations (top) and from each station (bottom).

sampling location, sampling time, and methods for sample treatment and bacterial isolation. Our result reveals some different epiphytes from those reported, including genera *Algibacter*, *Altererythrobacter*, *Alteromonas*, *Bernardetia*, *Colwellia*, *Erythrobacter*, *Halobacillus*, *Marinomonas*, *Octadecabacter*, *Olleya*, *Paraglaciecola*, *Photobacterium*, *Polaribacter*, *Pseudoalteromonas*, *Psychromonas*, *Rheinheimera*, *Shewanella*, *Tenacibaculum* and *Yoonia*. Among them, 15 genera, including *Algibacter*, *Altererythrobacter*, *Bernardetia*, *Colwellia*, *Erythrobacter*, *Halobacillus*, *Marinomonas*, *Octadecabacter*, *Olleya*, *Photobacterium*, *Polaribacter*, *Psychromonas*, *Rheinheimera*, *Tenacibaculum* and *Yoonia*, were screened from *Sargassum* for the first time, providing a better understanding for the diversity of bacteria associated with *Sargassum*.

3.3 *Alginate lyase-excreting bacteria from the Sargassum samples*

Alginate lyase-excreting bacteria were further screened from the *Sargassum*-associated strains. Based on the alginate plate assay, we screened 15 strains that could produce a clear halo. We then inoculated these strains in the minimal medium with alginate as the sole carbon source. Finally, 8 strains grew in the medium, indicating that they utilize alginate for growth via secreting alginate lyases to degrade alginate. These strains were regarded as alginate lyase-excreting bacteria. The 8 strains are *Alteromonas* sp. D120 isolated from Station 5, *Pseudoalteromonas* strains M10, M9, M11 and M5 from Station 4, and *Vibrio* strains C42, C57 and C60 from Station 3, which all belong to Proteobacteria (Fig. 5a). Therefore, strains from *Pseudoalteromonas* (4/8) and *Vibrio* (3/8) are the main alginate lyase-excreting bacteria from the *Sargassum* samples (Fig. 5b), indicating that the predominant genera of the *Sargassum*-associated bacteria and the alginate lyase-excreting bacteria are consistent. No alginate lyase-excreting bacteria were screened out from Station 1 and Station 2, which may be caused by different sampling times. Gorham and Lewey (1984) tracked the life history of *Sargassum muticum*, and found that the amount of alginate in *Sargassum muticum* varies with the temperature, reaching the highest in May and June. In November and March, the seawater temperature at the sampling sites is low, which may lead to less alginate content in *Sargassum* than that in May.

Numerous alginate lyase-excreting strains have been isolated from brown algae. For instance, 21 alginate lyase-excreting strains were isolated from the surface of the Arctic *Laminaria* (Dong et al., 2012; Xu et al., 2020). Martin et al. (2015) identified 14 alginate lyase-excreting strains associated with the brown alga *Ascophyllum nodosum*. Targeting the diversity of alginate lyase-excreting strains from *Sargassum*, only Wang et al. (2017) reported 12 alginate lyase-excreting strains screened from *Laminaria japonica*, *S. horneri* and *S. siliquatrum* samples, which belong to 8 genera including *Paenibacillus*, *Bacillus*, *Leclercia*, *Isoptericola*, *Planomicrobium*, *Pseudomonas*, *Lysinibacillus* and *Sphingomonas*. Thus, our study reveals that some *Sargassum*-associated alginate lyase-excreting bacteria are different from those reported, including strains of *Alteromonas*, *Pseudoalteromonas* and *Vibrio*.

3.4 *Extracellular alginolytic activity of the alginate lyase-excreting strains*

We further measured the extracellular alginolytic activity of the 8 alginate lyase-excreting strains toward sodium alginate. The extracellular alginolytic activity of all the 8 strains was detectable (Fig. 6), among which *Pseudoalteromonas* sp. M9 had the highest extracellular alginolytic activity of 0.55 U/mL. Compared with the high activity of *Pseudoalteromonas* sp. M9, the extracellular alginolytic activities of the other two *Pseudoalteromonas* strains, M10 and M11, were both lower than 0.1 U/mL. The extracellular alginolytic activities of all the 3 *Vibrio* strains were higher than

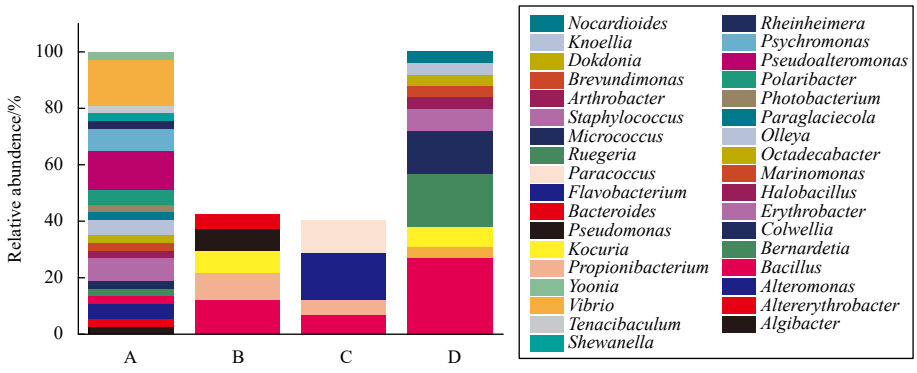

Fig. 4. Comparison of community composition of the cultivable epiphytic bacteria from *Sargassum* in this study and those from *Sargassum* samples in other reported studies. A. *S.* sp. in this study; B. the brown drifting *S. horneri* from the Yellow Sea (Mei et al., 2019); C. the yellow drifting *S. horneri* from the Yellow Sea (Mei et al., 2019); D. *Sargassum* sp. from Brazil (Menezes et al., 2010).

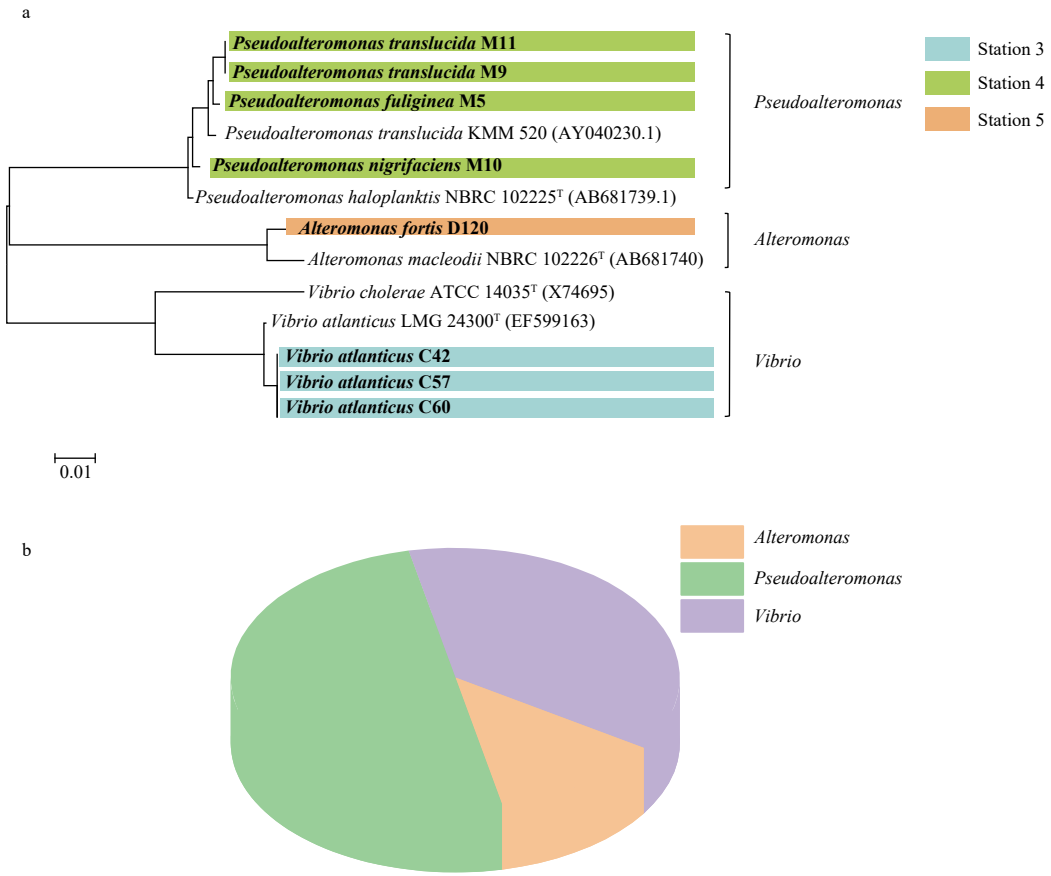

Fig. 5. The alginate lyase-excreting bacteria isolated from the *Sargassum* samples. a. A neighbor-joining phylogenetic tree of the alginate lyase-excreting bacteria isolated from the *Sargassum* samples based on the 16S rRNA gene sequences; the bootstrap values of each branch were tested by 1 000 repetitions. Three different colors indicate the stations which the strains were collected from. b. Abundances of the genera groups of the alginate lyase-excreting bacteria isolated from the *Sargassum* samples.

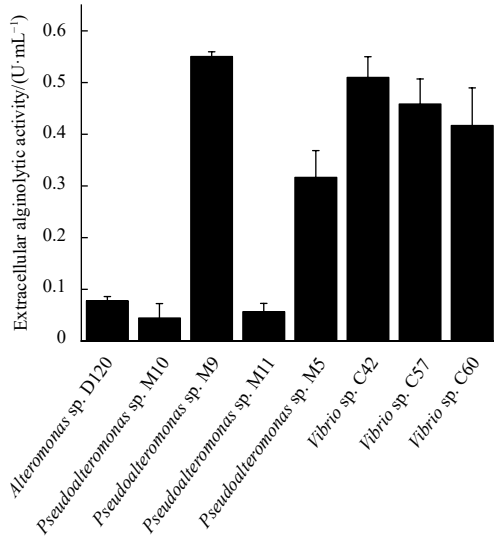

Fig. 6. Extracellular alginolytic activity of the alginate lyase-excreting bacteria. The strains were cultured in the liquid minimal medium containing 0.5% sodium alginate as the sole carbon source at 20℃ with stirring (180 r/min) for 1.5 d. The fermentation broth of each strain was centrifuged to obtain the supernatant as crude enzymes. The activity of the crude enzyme toward alginate was determined by using the 3,5-dinitrosalicylic acid method. The graph shows data from triplicate experiments (mean ± standard deviation).

0.4 U/mL, of which strain C42 was the highest, reaching 0.51 U/mL. Strain D120 belonging to *Alteromonas* showed comparatively low extracellular alginolytic activity, only 0.08 U/mL. These results indicate that these strains can secrete alginate lyases to utilize alginate as the sole carbon source for growth.

Up to now, the extracellular alginate lyase activities of many strains have been determined. For example, Dong et al. (2012) measured the extracellular alginate lyase activity of 21 alginate lyase-excreting strains at their optimal temperature using the Somogyi-Nelson method. They found that *Polaribacter* strain 2-2 had the highest extracellular alginate lyase activity, which was approximately 200 U/mL. Li et al. (2011) reported 10 alginate lyase-producing bacterial strains, of which *Pseudoalteromonas* sp. SM0524 had the highest alginate lyase activity, reaching 62.6 U/mL under the optimized conditions. So far, only Wang et al. (2017) measured the relative extracellular alginate lyase activity of 7 alginate lyase-excreting strains screened from *Sargassum*, of which *Bacillus halosaccharovorans* LJ-3 showed the highest relative enzyme activity. In contrast, the results in this study showed that, among the 8 alginate lyase-excreting strains isolated from *Sargassum*, *Pseudoalteromonas* strain M9 and *Vibrio* strain C42 had the highest extracellular alginolytic activity. Their extracellular activities were lower than those of the reported strains above (Dong et al., 2012; Li et al., 2011), maybe because their activities were not measured under the optimal enzyme production conditions and enzymatic reaction conditions. Nevertheless, these strains may be used to search for novel alginate lyases.

4 Conclusions

In this study, the diversity of epiphytic bacteria from the *Sargassum* samples collected from the coastal seawaters in Shandong Province, China was investigated. As a result, 37 *Sargassum* epiphytes belonging to 21 genera in 3 phyla were isolated, among which strains of *Vibrio* (6/37) and *Pseudoalteromonas* (5/37) were the predominant groups. Of these, 15 genera were screened from *Sargassum* for the first time. Eight strains belonging to 3 genera of Pseudomonadota were further identified as alginate lyase-excreting strains, and they were different from the previously reported strains isolated from *Sargassum*. Moreover, the extracellular alginolytic activity of the 8 strains was detected. This study characterized a few new *Sargassum* epiphytes including alginate lyase-excreting bacteria, which is instrumental for understanding the interactions between *Sargassum* and its epiphytes, and will promote the discovery of new alginate lyases and biological control of *Sargassum*.

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