Kelp microbiology: linking bacteria and viruses to carbon cycling within kelp forests

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ABSTRACT

Kelps are large marine algae that use photosynthesis to convert carbon dioxide into organic carbon which they store as biomass. This organic carbon represents a major energy source for many of the other organisms in the ecosystem. Due to this conversion and their large biomass, kelp forests are among the most productive ecosystems on the planet yet very little of the kelp carbon enters the food web by direct grazing on the kelp itself. In fact, bacteria liberate a majority (70-80%) of the carbon in kelp making it available for consumption by other organisms via a pathway called the microbial loop. However, little is understood about the movement and cycling of kelp carbon through this loop. As part of a larger project designed to determine the importance of viruses in cycling kelp carbon, we isolated several (7) marine bacteria that use kelp carbon for growth. Using standard microbial techniques, we grew these bacterial cultures, classified them using colony and cell morphology and determined aspects of their metabolic capabilities by testing for the presence of enzymes that catalyze specific metabolic reactions. Furthermore, in a following experiment we examined the application of a Most-Probable-Number assay to determine the ability of viruses to infect our isolated bacteria. We observed infections in one of the two tested bacterial cultures (2B5). This result clearly indicates the potential of using this assay in future experiments to quantify the number of viruses present in a seawater sample that can infect bacteria growing on kelp carbon and thus will allow for a deeper understanding of how microbes are ultimately driving the high productivity found in kelp forests.

INTRODUCTION

In marine ecosystems, the transfer of carbon to higher trophic levels and the movement of it throughout the environment is critical to the survival of marine organisms. Kelp, as large plant-like algae, are an important source of carbon in many of the marine environments around British Columbia. Identifying how the carbon in kelp cycles through the ecosystem is important for understanding why kelp forests are some of the most productive ecosystems on the planet. Only 10% of kelp is grazed upon, so very little of the carbon stored in kelp is transferred to higher trophic levels directly. Most kelp carbon passes through the microbial loop before it enters the grazer food web. The microbial loop is mediated by bacteria (Azam *et al*., 1983) . In this case, bacteria use the carbon in kelp to grow, they are then eaten by higher trophic organisms, such as protozoans, which are eaten by zooplankton and ultimately fish. In this way, kelp carbon indirectly enters the food web through the bacteria in the microbial loop.

Viruses are also known to play a role in the cycling of carbon via the lysis of marine bacteria. The carbon from lysed bacteria enters the dissolved organic carbon pool in the ocean and acts as a carbon source for other marine bacteria, encouraging bacterial proliferation and growth at the expense of organisms at higher trophic levels. In order to determine the importance of viruses in cycling kelp carbon, we want to quantify the number of viruses infecting marine bacteria that use alginate as their primary carbon source. Alginate is a major component (35-45%) of kelp cell walls (Preiss & Ashwell, 1962) and as such is a large source of carbon. To investigate the relationship between the bacteria and the viruses present in kelp forests and their role in cycling kelp carbon this study had two goals: (1) to isolate and characterize bacteria that grow using the alginate in kelp as a carbon source and (2) to test the use of a Most-Probable-Number assay to assess viral infections of our isolated bacteria.

METHODS AND MATERIALS

Seawater near the kelp forests off Stanley Park (Figure 1) were collected in October 2017. Following the process used by Clasen and Shurin (2015) to isolate bacteria that use kelp carbon for growth, dilution of the seawater were plated onto agar plates containing artificial (fake) seawater supplemented with alginate (FSW+alginate). Bacterial colonies (Figure 2a) were classified using size, shape, elevation, texture, margin and pigmentation. Visually different colonies were grown in liquid FSW+alginate in tissue culture plates which were incubated at room temperature. These cultures were propagated at various dilutions in order to ensure only one bacterial species was present in each culture. The most diluted cultures with the most prominent growth were then grown in large liquid cultures with FSW+alginate (Figure 2b).

Liquid cultures and their corresponding colonies were used in a number of experimental tests designed to identify two unique bacterial cultures for use in further studies. Specifically, spread plate colonies were gram stained (Figure 3) and viewed with a compound light microscope to determine the structure of their cell walls. Cultures were also subjected to 7 biochemical tests that evaluated the bacteria's ability to tolerate salt and bile, to ferment mannitol, lactose and ribose, to hydrolysis esculin and starch, and to produce catalase (Bergey *et al.* 1974).

Once two different and pure bacterial cultures were identified, large liquid cultures of these bacteria were made for use in Most-Probable-Number viral assays to see if we could visualize viral infections of the bacteria. Based on Clasen *et al*. (2013), one pure bacterial culture was added to each well of tissue culture plates along with varying dilutions of seawater collected from Stanley park. After 30 minutes of incubation, FSW+alginate was added to all the wells and then the plates were monitored for 2 weeks for evidence of viral infections. If viruses infected the bacteria and caused them to lyse, the culture should look clear compared to the control wells, confirming the presence of viruses in the seawater sample.

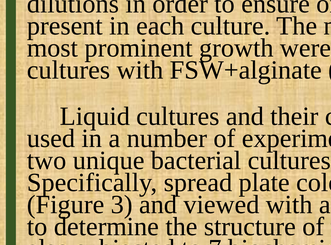

Figure 2a. Bacterial colonies isolated from seawater growing on agar plates supplement with alginate as a carbon source.

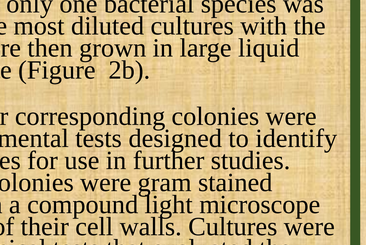

Figure2b. Liquid bacterial cultures grown from colonies isolated on agar plates. Notice the difference in the colour of the cultures. 2B5 and 2C5 were used for further biochemical tests.

Figure 1. Sampling location just off Stanley Park, Vancouver, BC. Seawater samples were collected near *Nereocystis* (commonly called Bullwhip kelp) kelp forest.

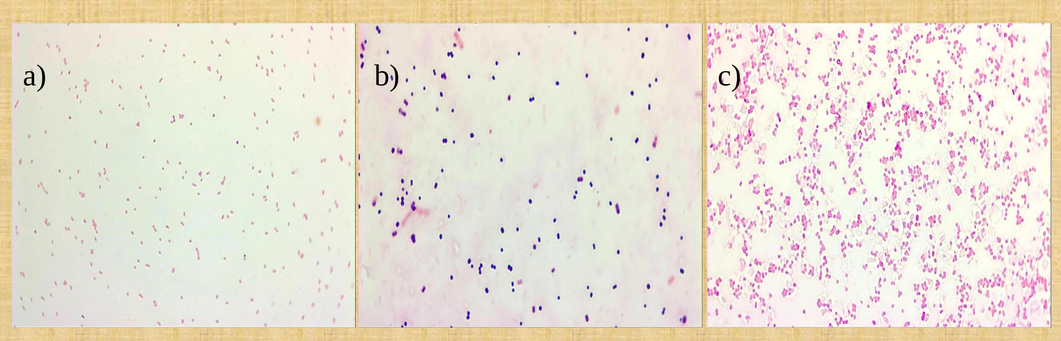

Figure 3. Gram stain reactions for three bacterial cultures. A gram reaction is a differential stain that indicates the structure of the bacterial cell wall. All images were obtained using a compound light microscope at 100x magnification. a) 2B5: gram negative. b) 2C5: gram positive. c) 1A4: gram negative.

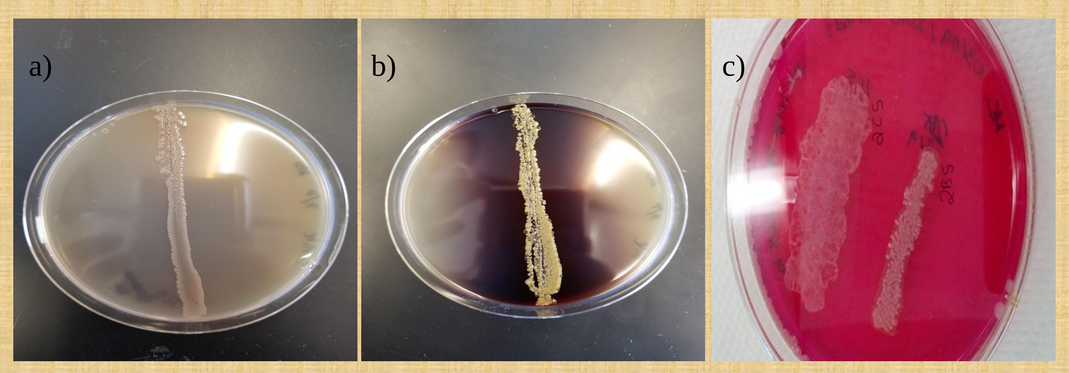

Figure 4. Biochemical test results from two different pure cultures. a) 2C5: positive bile test. b) 2B5: positive for bile tolerance and positive for hydrolysis of esculin test. c) 2C5 and 2B5 are both positive for salt tolerance but negative for the fermentation of mannitol.

EXPERIMENTAL TEST	Bacterial culture 2B5	Bacterial culture 2C5
Gram reaction	Gram Negative	Gram Positive
Salt tolerance	Positive	Positive
Ferment mannitol	Negative	Negative
Bile tolerance	Positive	Positive
Hydrolysis esculin	Positive	Negative
Hydrolysis starch	Negative	Negative
Possess catalase	Negative	Positive
Ferment lactose	Negative	Negative
Ferment ribose	Negative	Negative

Table 1. Reactions of culture 2B5 and culture 2C5 to the biochemical tests performed. The columns in green show the differing results.

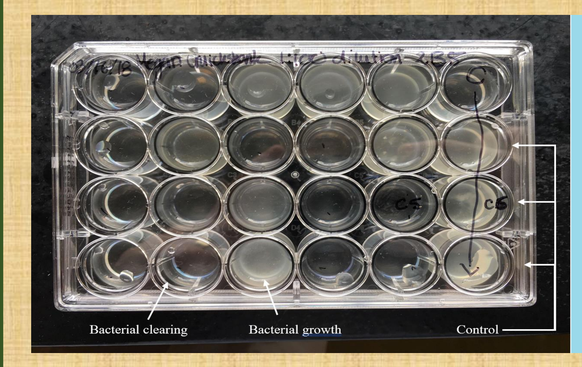

Figure 5. Most-Probable-Number (MPN) assay to determine the ability to use this assay to visualize viral infection of our isolated bacteria. Clearing of a well (as compared to the control wells in the column furthest to the right) indicates that the bacteria were infected and lysed by viruses.

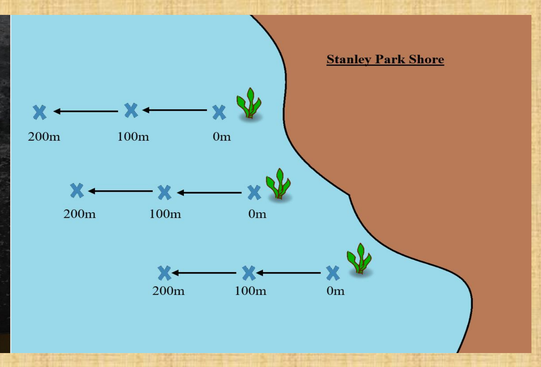

Figure 6. Schematic representation of the sampling that will be conducted in late Spring to quantify the number of viruses infecting our isolated bacteria that grow using kelp carbon.

RESULTS

ISOLATION

Seven marine cultures were isolated from FSW+alginate plates. Of the cultures that grew, 3 cultures were considered impure as their gram stain slides showed the presence of both gram negative and gram positive bacteria. Culture 2C5 also contained two different species of bacteria. The colony morphology of the first 2C5 species was 3mm in size, circular with an entire margin, dull and smooth, raised elevation, and white in colour. The second species of 2C5 was 1mm in size, circular with an entire margin, shiny and smooth, convex elevation, and also white in colour. Species 2B5 was 2mm in size, circular with an entire margin, smooth and shiny, slightly convex, and yellow in colour. The smaller species of 2C5 was identified as gram positive while 2B5 was identified as gram negative (Figure 3a and 3b). Based on the combination of differing colony morphologies and the gram staining reactions, the small colony 2C5 species was deemed different than 2B5 and both were used in additional tests to further confirm that 2B5 and 2C5 were different species of bacteria.

BIOCHEMICAL TESTS

Biochemical tests were performed on the two pure species of bacteria to further identify the differences between the cultures (Table 1). The species were grown on mannitol & salt plates, bile esculin plates and starch plates (Figure 4). The results showed that 2B5 and 2C5 were positive for growth on salt but did not ferment mannitol. Furthermore, on the bile esculin plate, both 2B5 and 2C5 grew. However, 2B5 showed a positive result for the hydrolysis of esculin. The starch plates showed a negative result for both 2B5 and 2C5. The liquid cultures of 2C5 and 2B5 were subjected to fermentation tests in tubes to test for fermentation of lactose and ribose, both cultures were negative for fermentation of these sugars. Catalase tests were also performed on the cultures. Culture 2B5 showed a negative result and 2C5 showed a positive result.

MOST- PROBABLE -NUMBER VIRAL ASSAY

MPN results were mixed when the two isolated cultures (2B5 and 2C5) were used to determine the ability of this type of assay to visualize viral infections. The 2B5 tissue culture plates showed evidence of viral clearing (Figure 5) whereas the 2C5 tissue culture plates did not. This is the first time this type of assay has been applied to this system and it suggests that a MPN assay can be used in the future to quantify the amount of viruses found in seawater that can infect bacteria growing on kelp carbon.

FUTURE RESEARCH

We have two further avenues of research to explore. First, to realistically quantify the viruses, more pure cultures of bacteria will need to be isolated from the kelp forests. We plan on continuing to isolate and/or characterize more bacteria that grow using kelp carbon (for example culture 1A4 on Figure 3c). Secondly, in late Spring, we will collect seawater along three transects off the kelp forest in Stanley park (Figure 6) and use our proven MPN assay to quantify the number of viruses capable of infecting our bacterial cultures. Results from this experiment will allow the role of viruses in cycling kelp carbon to be expressed for the very first time.

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