The Effects for Extracellular Polymeric Sustances of *Cladophora glomerata* under Different Culture pH and Salinities

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Abstract: The extracellular polymeric sustances (EPS) of algae have been studied as high-value natural resources for a number of application. *Cladophora glomerata* is the most abundant algae in freshwater throughout the world. This study discovered that under algal favoured pH environment, changing culture salinity can induce *C. glomerata* to secrete massive amount of EPS. Under pH 7.0 and pH 8.0, the total EPS increased from 3.10 mg g^{-1} fresh algae to 6.47 mg g^{-1} fresh algae, the protein to polysaccharide ratio (P/S) decreased from 0.31 to 0.21 with the increase in the salinity from 0 ‰ to 30.0‰. For pH 5.0, 6.0 and pH 9.0, 10.0 culture conditions, the total EPS between1.17 mg g^{-1} fresh algae to 4.32 mg g^{-1} fresh algae, the P/S ratio between 0.24 to 0.29 with the increase in the salinity from 0 ‰ to 30.0‰. Therefore, the total EPS and P/S ratio are influenced by culture salinity and pH obviously.

Key words: *Cladophora glomerata*, extracellular polymeric substances (EPS), protein, polysaccharide, salinity, pH.

1. Introduction

Algae can excrete various EPS into their immediate living environment during their life cycle. These algal EPS mainly consist of polysaccharides, proteins, nucleic acid and lipid [1]-[4]. EPS are an abundant source of structurally and compositionally diverse biopolymers.

In recent years, EPS have been studied and used as renewable natural resources for wide spectrum of application, including food, pharmaceuticals/cosmetics, nutraceuticals, and so no [5]-[7]. Therefore, making the in-depth study of EPS is also important in term of development of value-added EPS applications [8]-[11]. Algal is the photosynthetic organism can grow in the different environment and EPS production which makes algae a perfect candidate for biotechnological exploration [12]-[14].

As recent research has shown, the EPS of the algae is highly sensitive to salinity [15]. Once the salinity of the growing environment is increased, the species of *C. glomerata* starts to change the composition of polysaccharide and protein in EPS.

Bellis (1968) found that cultures of *C. glomerata* were killed at initial pH values less than 7.0 and above 10.0. At initial pH values immediately adjacent to these limits the cultures produced only akinetes, but branched filaments developed where initial pH values ranged from 7.25-9.01 [16]. However, the changes of algal secretions (EPS) with pH changes are unknown.

Cladophora glomerata (Chlorophyta, Cladophoraceae) is widely distributed around the world and receive

a significant amount of attention from biologists. Wen *et al*. (2007) [17] investigated these types of green algae in rivers in southern Taiwan and measured that the biomass of these algae reached up to 117 g/m^2. Surveys of the Great Lakes further found green algae biomass to be as great as 940 g/m^2 [18]. These macrobenthic algae up take nutrients from the water. They tential to be developed as biological materials.

This study investigated EPS production under different salinity and pH by exploring the features of *C. glomerata*. This study also investigated the changes of polysaccharides and proteins, and protein to polysaccharide ratio (P/S).

2. Materials and Methods

2.1. Algae

Cladophora is a branched and filamentous algae, the cell walls of which comprise an inner layer of cellulose, a middle layer of pectin, and an outer layer of chitin. In fresh water. The surface of the algae is rougher, hosting a number of epiphytes, primarily Bacillariophyta as well as Cladophora, Cyanophyta, and Euglenophyta. *C. glomerata* usually is the dominant species in its habitat. In this study, the species *C. glomerata* was collected from a main river in southern Taiwan, the upstream of the Zengwun River(23°04'N, 120°04'E), and then transferred to aquariums with different salinities and pH for further culturing.

2.2. Influence of Salinity and pH on *C. glomerata* EPS

The sessile *C. glomerata* grew healthily on the carriers (pebbles) and was harvested from the upstream of the Zengwun River in southern Taiwan. The river water above the tidal river reaches was also brought back to the laboratory to prepare a culture medium with different concentrations of salinity, including 5 different salinities such as unadjusted (assuming it was 0.0 ‰), 3.0‰, 10.0‰, 20.0‰, and 30.0‰. *C.glomerata* was cultured in an outdoor open system without CO_2 addition and the culture presented a pH values naturally ranging from 6.2-8.1. therefore to test the influence of pH on EPS production under different salinities. six pH values (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) were tested after adjustment using 1.0 mol/L HNO_3 or 1.0 mol/L NaOH solution. Individual aquariums contained 10 liters of river water with different salinities, and the water was circulated using a small motor to create an adequate environment in which to culture *C. glomerata*. Samples were then taken at 48h after treated.

2.3. Thickness of Extracellular Mucilage Layer

To measure the mucilage layer to determine the EPS, we employed the methods used by Tien *et al*. (2002) [9] and Chiou *et al*. (2010) [10]. Fig. 1 shows measurements of the mucilage layer of the algae. Algae samples were placed on glass slides with an appropriate quantity of India ink and then observed using an optical microscope. The green regions in the image are the cell walls of *C. glomerata*; the uniform halo on the outside was used to estimate the thickness of the mucilage layer.

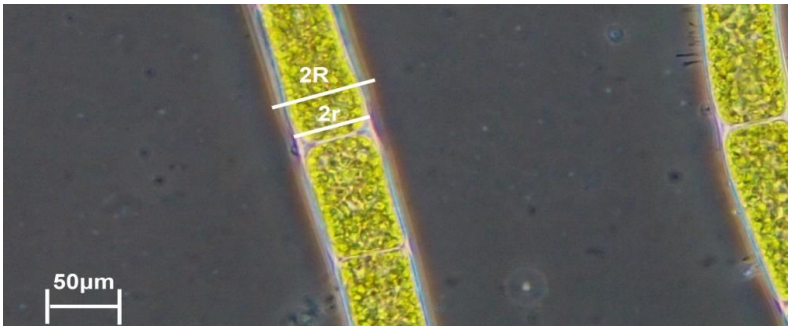

Fig. 1. Illustration of how the measurement of mucilage layer (the thickness of mucilage layer=R-r, scale bar=50μm).

2.4. EPS Extraction and Analysis

The ultrasonication method was used for the EPS extraction [12], [19]-[21]. The algae was harvested from the aquarium, washed twice with tap water, and drained in a strainer at room temperature for 2 h until it dried naturally. Afterward extraction procedures were started (as indicated in Fig. 2). All chemicals used in this work were of analytical grade. Polysaccharide content was determined by the phenol-sulfuric acid using glucose as a standard [22]. Protein content was determined according to Bradford (1976) with bovine serum albumin BSA, Sigma A2153 100mg/ml [23]. The total EPS content was measured as the sum of these two substances [24], [25]. All sample measurements were 6 repeated.

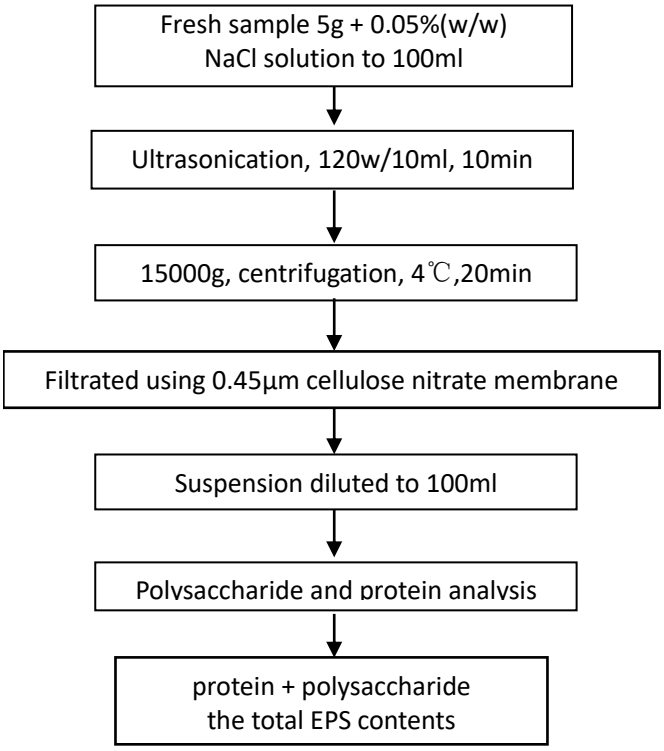

Fig. 2. Procedure for the EPS extraction process for *C. glomera.*

3. Results and Discussion

3.1. The Influence of Salinity and pH on the *C. glomerata* Mucilage Layer

To determine the influence of variations in salinity and pH on the EPS and epiphytes of *C. glomerata*, we conducted experiments using onsite river water and various concentrations of NaCl, (0.0‰, 3.0‰, 5.0‰, 10.0‰, 20.0‰ and 30.0‰ salinity) under six different pH values to cultivate well-grown *Cladophora* algae samples.

Table 1 and Fig. 3 demonstrate the changes of mucilage layer thickness under different culture conditions with different salinities and pH values. Under pH 7.0 and pH8.0, the results indicated that the culture salinity increased (≧3.0‰), EPS secretion also greatly increased accordingly. In this case, *C. glomerata,* which grows naturally and favourable pH conditions in freshwater areas and thus hardly forms a mucilage layer outside of its cell walls, may start to formulate mucilage layers in accordance with the increase in salinity. To protect itself, the algae could change its physiological conditions to adapt to the environmental changes. But the study results implied that critical salinity for creating significant changes in EPS production was 3.0‰. Under the pH value (5.0, 6.0, 9.0, 10.0), *C. glomerata* decreased the influence of algal

secretion with the changes of salinites. In the culture experiments, *C. glomerata* became colourless within 48h after being placed in a pH 5.0 and pH10.0 culture conditions.

Table 1. Changes of the Thickness of Mucilage Layer in Different pH and Salinity

salinity	pH					
	5.0	6.0	7.0	8.0	9.0	10.0
1(0.0‰)	0.75±0.02	2.20±0.04	2.39±0.04	2.46±0.04	2.51±0.04	1.00±0.04
2(3.0‰)	0.85±0.03	3.12±0.07	4.28±0.08	4.36±0.09	3.17±0.09	1.12±0.09
3(10.0‰)	0.87±0.03	3.26±0.07	4.45±0.08	4.79±0.09	3.21±0.10	1.37±0.09
4(20.0‰)	0.87±0.03	3.31±0.07	4.70±0.08	5.20±0.09	3.32±0.09	1.42±0.09
5(30.0‰)	0.91±0.05	3.45±0.10	5.22±0.11	5.51±0.12	3.40±0.13	1.49±0.12

*Unit:μm

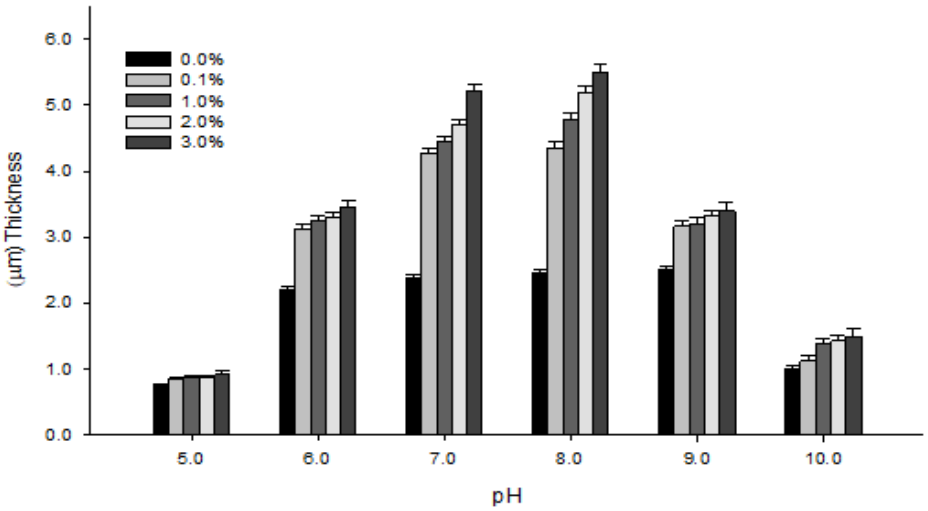

Fig. 3. Changes of the thickness of mucilage.

In this study, we discovered that on *C. glomerata*, an increase in salinity can accelerate the secretion of EPS, which thickens the extracellular mucilage layer, thereby affecting the attachment of epiphytic algae also.

3.2. The Influence of Different Salinity on the Level of Polysaccharide and Protein in EPS of *C. glomerata*

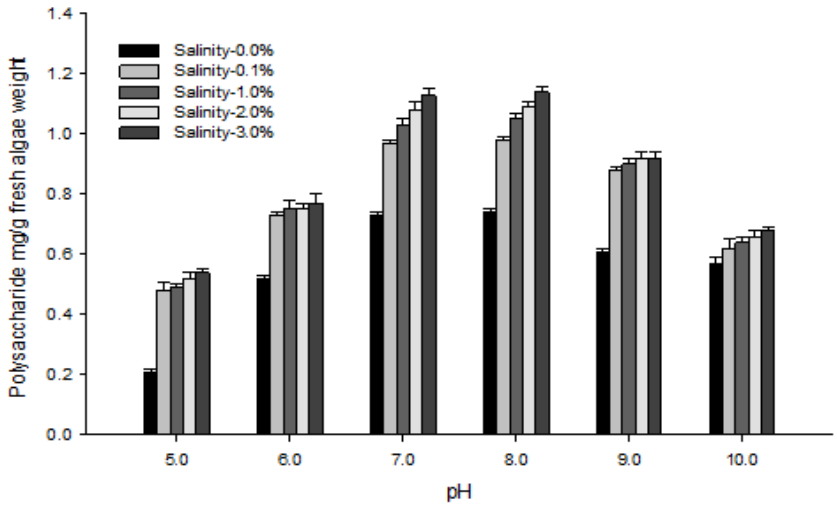

Fig. 4. Polysaccharide level under different culture pH and salinity.

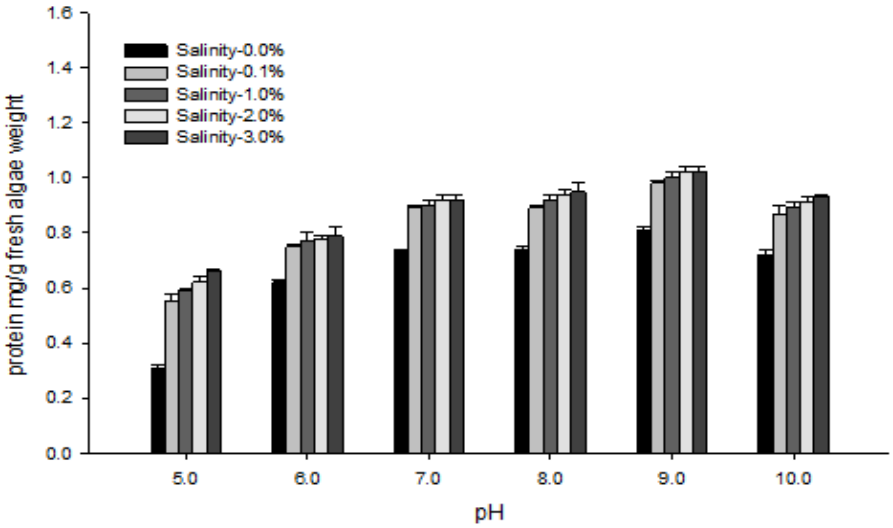

Fig. 5. Protein level under different culture pH and salinity.

The changes in polysaccharide levels in EPS under different salinities and pH values are shown in Fig. 4. The changes in protein levels in EPS under different salinities are shown in Fig. 5. From previous results, salinity impacts *C. glomerata* protein and polysaccharide.

Therefore, changes in salinity may stimulate algae originally grown in freshwater areas into producing massive amount of EPS. And the thickness of mucilage layer is increased in accordance with the increase in salinity. The salinity-response was significantly affected by pH. Both for protein and polysaccharide, the maximun level was 1.13 mg g^{-1} fresh algae of protein, 5.18 mg g^{-1} fresh algae of polysaccharide at pH 7.0, and the 5.34 mg g^{-1} fresh algae of protein, 1.14 mg g^{-1} fresh algae of polysaccharide at pH 8.0. But the salinity-response for pH 9.0, 10.0 and pH 5.0, 6.0 were not significantly different.

By exploring the euryhaline feature of *C. glomerata,* this study discovered that mucilage layer could be massively induced by regulating environmental salinity under favoured pH range.

3.3. The Influence of Salinity and pH on the *C. glomerata* EPS

To determine the influence of variations in salinity and pH on the EPS and epiphytes The total EPS content was measured as the sum of polysaccharide and protein Fig. 6 and Table 2 shows the change of the total EPS of *C. glomerata* at different salinities under six different pH values.

Table 2. EPS and P/S Ratios under Different Culture pH and Salinity

salinity	pH					
	5.0	6.0	7.0	8.0	9.0	10.0
1(0‰)	1.17±0.02/ 0.22±0.01*	2.71±0.04/ 0.24±0.01	3.10±0.05/ 0.31±0.01	3.18±0.05/ 0.30±0.01	3.09±0.08/ 0.24±0.01	2.91±0.05/ 0.25±0.01
2(3.0‰)	2.25±0.04/ 0.27±0.01	3.32±0.12/ 0.28±0.01	5.22±0.12/ 0.23±0.01	5.31±0.12/ 0.23±0.01	3.92±0.10/ 0.29±0.02	3.02±0.10/ 0.26±0.00
3(10.0‰)	2.30±0.03 /0.27±0.00	3.59±0.11/ 0.27±0.02	5.45±0.12/ 0.23±0.01	5.80±0.13/ 0.22±0.01	4.08±0.12/ 0.29±0.02	3.20±0.12/ 0.25±0.01
4(20.0‰)	2.39±0.04/ 0.28±0.01	3.71±0.08/ 0.26±0.01	5.75±0.10/ 0.23±0.01	6.24±0.11/ 0.21±0.00	4.24±0.13/ 0.28±0.02	3.27±0.13/ 0.25±0.01
5(30.0‰)	2.46±0.02/ 0.28±0.00	3.82±0.14/ 0.25±0.02	6.31±0.14/ 0.22±0.01	6.47±0.10/ 0.21±0.01	4.32±0.12 /0.27±0.02	3.35±0.12/ 0.25±0.01

*(EPS / P/S)
EPS=P+S, P/S: Protien/ Polysaccharide

Unit: mg g^{-1} fresh algae

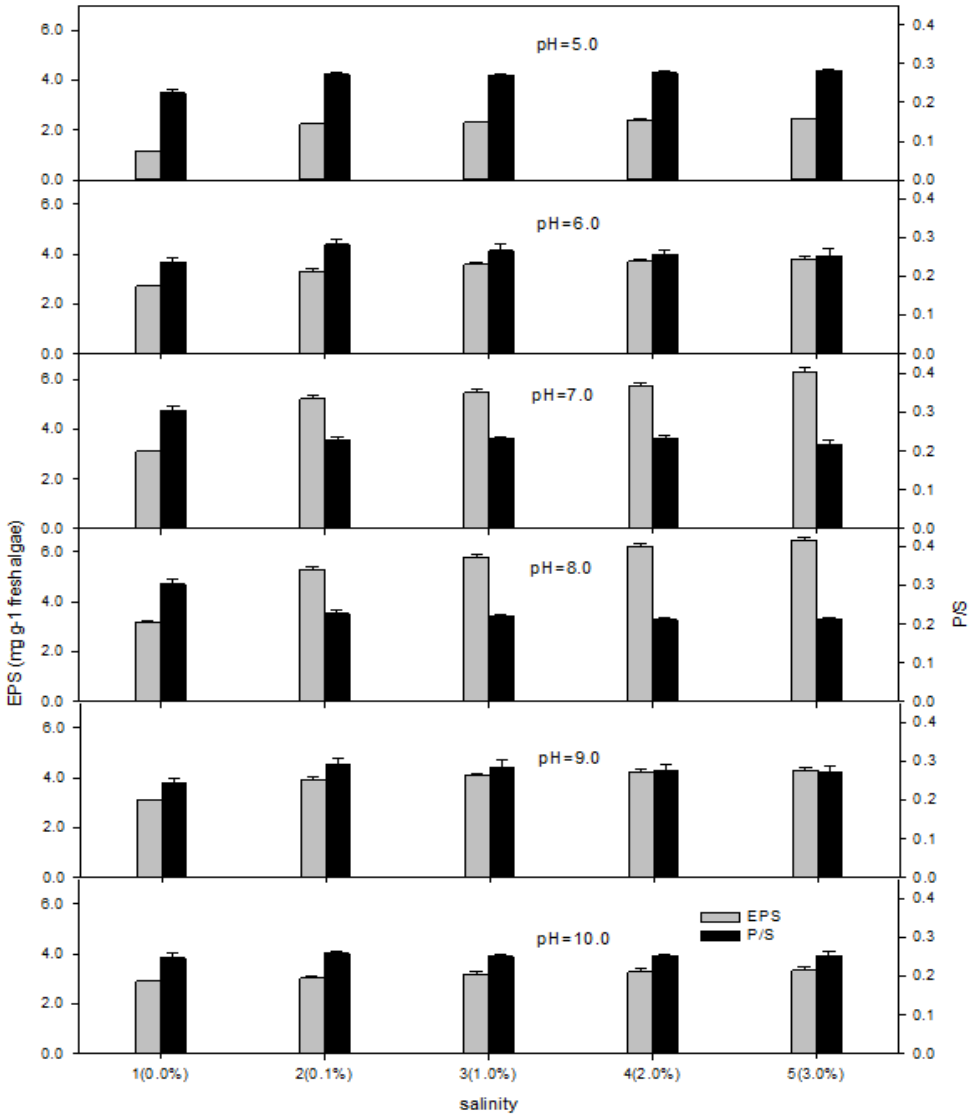

Fig. 6. Variation of EPS and P/S of *C. glomerata* under different culture salinity and pH.

The total EPS increased from 3.10 mg g^{-1} fresh algae to 6.31 mg g^{-1} fresh algae mg g^{-1} fresh algae with the increase in the salinity from 0 ‰ to 30 ‰ under pH 7.0. Similarly the total EPS also increased from 3.18 mg g^{-1} fresh algae to 6.47 mg g^{-1} fresh algae under pH 8.0 culture condition. The phenomenon of pH influences the salinity-response effect, which was strongly observed at pH 7.0 and 8.0, but not for pH 5.0, 6.0 and pH 9.0, 10.0 culture conditions. Under the pH 7.0 and 8.0 culture conditions, the protein to polysaccharide ratios in EPS decreased from 0.31 to 0.21 with the increase in the salinity from 0 ‰ to 30 ‰. For pH 5.0, 6.0 and pH 9.0, 10.0 culture conditions the P/S ratio between 0.24 to 0.29. Some studies have reported the increase in salinity could cause microoganisms to produce more exopolysaccharide due to the variation of osmotic pressure between microoganisms and the bulk solution [19], [26].

The study discovered that EPS could be massively induce by regulating salinity under favourable pH condition, and the contents of protein and polysaccharide in EPS can also be controlled using the different salinities of culture condition. And the level of polysaccharide / protein ratio in EPS can also be controlled using the different salinity of habitats when controlling other environmental factors.

4. Summary

This study compared the content and the EPS production from the *C. glomerata* under different culture

salinity and pH. The results showed that 1) *C. glomerata* has great development potential for EPS production. 2) under pH7.0 and 8.0, the culture salinity increased the mucilage layer thickness (EPS) increased accordingly. 3) The salinity-respone for EPS was significantly affected by algal favoured pH range. 4) under pH 7.0 and pH 8.0 P/S between 0.21 to 0.31, for pH 5.0, 6.0 and pH 9.0 between 0.24 to 0.29. 5) The EPS can be massively induce by regulating salinity under favourable pH condition, and the contents of protein and polysaccharide in EPS can also be controlled using the different salinities of culture condition.

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