The changes of proteins and polysaccharides in extracellular polymeric substance for Spirogyra fluviatilis under different salinity

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Abstract. Spirogyra is a genus of widely distributed, large green fresh water algae. This study discovered that changes in salinity can induce Spirogyra fluviatilis to produce amounts of extracellular polymeric substance (EPS) when controlling other environmental conditions. If culturing S. fluviatilis with salinity greater than a 3.0‰ medium for 4 hours, the secretion EPS will be changed. And the level of polysaccharides and proteins, the primary components of EPS, is slightly increased in accordance with the increase in the salinity. But the proteins to polysaccharides ratio changes are not significantly

1. Introduction

As recent research has shown, the EPS of the algae is highly sensitive to salinity. Once the salinity of the growing environment is increased, the species of *S. fluviatilis* starts to change the composition of polysaccharides and protein in EPS. In recent years, interest in the exploitation of valuable EPSs has been increasing for various industrial applications and renewable natural resources representing an important class of biotechnological importance [1, 2]. Therefore, making the in-depth study of EPS is also important in term of development of value-added EPS applications [3, 4, 5]. Algal is the photosynthetic organism can grow in the different environment and EPS production which makes algae a perfect candidate for biotechnological exploration. [6]

This study investigated EPS production under different salinity by exploring the features of *S. fluviatilis*. This study also investigated the changes of polysaccharides and proteins, the primary components in EPS, and hopefully the study results can be used as a reference for valuable EPS.

2. Methods

2.1. Algae

Spirogyra (Chloropyta, Zygnematale, Zygnemaceae) is a filamentous macro algal algae. It is one of the commonest fresh water algae which is worldwide in distribution. It occurs in extensive masses, free floating on the surface of water, forming familiar pond scum, water silk or pond silk. The bright green slippery shining tangled masses of filaments are found in the stagnant water of ponds, puddles, ditches, tanks, and also slow flowing rivers. The alga appears in great abundance during and after rains [7].

2.2. Influence of salinity on S. fluviatile EPS

The *S. fluviatile* was harvested from the campus of Kun-Shan University (N 23º25´, E 120º21´) in Taiwan. The pond water was also brought back to the laboratory to prepare a culture medium with different concentrations of salinity, including 8 different salinity levels such as unadjusted (assuming it was 0.0 ‰), 0.5‰, 1.0‰, 3.0‰, 5.0‰, 10.0‰, 20.0‰, and 30.0‰, so as to culture *S. fluviatile*. Individual aquariums contained 10 liters of river water with different salinity, and the water was circulated using a small motor to create an adequate environment in which to culture *S. fluviatile* Samples were then taken at 4h, 8h, 12h, 24h, 48h and 72h to measure the changes of polysaccharides and proteins in EPS.

2.3. EPS extraction using ultra sonication

This study used ultra sonication to extract EPS. 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. 1).

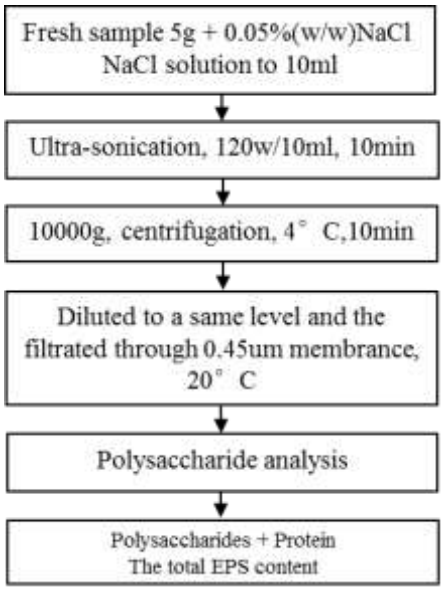

Figure 1. Procedure for the extraction process of *S. fluviatilis*.

2.4. Analysis method

All chemicals used in this work were of analytical grade. Polysaccharide content was determined by the phenol-sulfuric acid [8] using glucose as a standard. Protein content was determined according to Bradford (1976) [9] with bovine serum albumin BSA, Sigma A2153 100mg/ml) [10]. The total EPS content was measured as the sum of these two substances.

3. Results and discussion

3.1. The influence of different salinity on the level of polysaccharides in EPS

The changes in polysaccharides levels in EPS under different salinity and culture time are shown in Fig. 2. From the results, salinity impacts of *S. fluviatilis* EPS production. The polysaccharides is slightly increased in accordance with the increase (about 15% increase) in salinity.

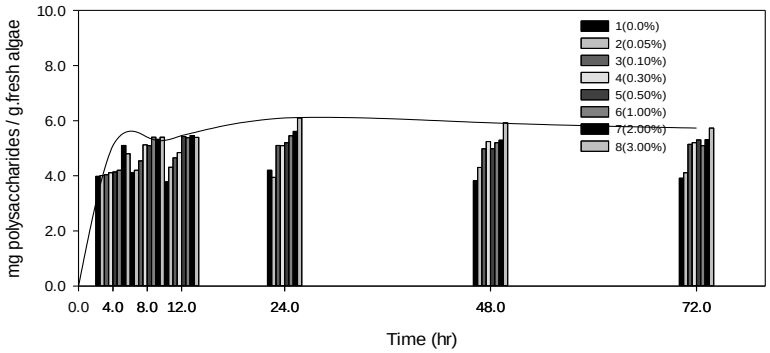

Figure 2. Change of polysaccharide level under different salinity and culture time.

3.2. The influence of different salinity on the level of proteins in EPS

In this study, we isolated EPS from *S. fluviatilis* cultured under different salinity. The extracellular salt stress affects the protein content in EPS, and significant advancement about increast 9% in the protein production was observed at higher salinity (Fig 3), is contrarily slightly impact by culture time.

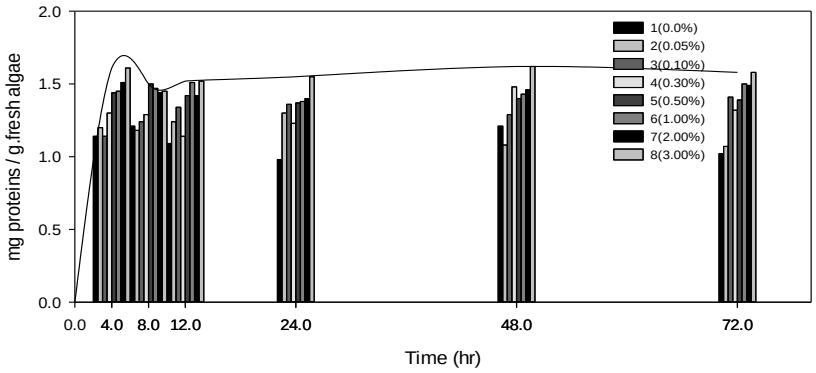

Figure 3. Change of proteins level under different salinity and culture time.

3.3. The influence of salinity on the S. fluviatilis EPS

Table 1 and Fig. 4. Demonstrate the changes of *S. fluviatilis* EPS amounts under culture conditions with different salinity. The results indicated that although EPS secretion was not obvious change if the *S. fluviatilis* was cultured lower 3.0‰ condition, once the culture salinity increased ($\geqq$ 3.0‰), EPS secretion also greatly increased accordingly. However, the increment of EPS secretion was not in a portion to the increase in culture time. In this case, *S. fluviatilis*, start to change EPS 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 is 3.0‰.

Table 1. Amounts of EPS and polysaccharide proteins ratio in different salinity.

Salinity	Culture time					
	4hr	8hr	12hr	24hr	48hr	72hr
1(0‰)	5.12/0.29*	5.32/0.29	4.87/0.29	5.18/0.23	5.03/0.32	4.93/0.26
2(0.5‰)	5.21/0.30	5.38/0.28	5.550.29	5.24/0.33	5.38/0.25	5.18/0.26
3(1.0‰)	5.18/0.28	5.78/0.27	5.99/0.29	6.46/0.27	6.27/0.26	6.55/0.27
4(3.0‰)	5.41/0.32	6.41/0.25	5.98/0.24	6.32/0.24	6.72/0.28	6.52/0.25
5(5.0‰)	5.58/0.35	6.68/0.31	6.84/0.26	6.57/0.26	6.38/0.28	6.69/0.26
6(10.0‰)	5.65/0.35	6.87/0.27	6.89/0.28	6.83/0.25	6.63/0.28	6.59/0.29
7(20.0‰)	6.61/0.30	6.76/0.27	6.88/0.26	7.01/0.25	6.75/0.28	6.8/0.28
8(30.0‰)	6.41/0.34	6.85/0.27	6.91/0.28	7.65/0.25	7.54/0.27	7.32/0.28

*EPS/ratio：EPS= polysaccharide + proteins, ratio= proteins/ polysaccharide

In this study, we discovered that ratio of proteins to polysaccharides between 0.23-0.35(mean=0.29), the changes are not significant, an increase in salinity can accelerate the secretion of EPS, which thickens the extracellular mucilage layer.

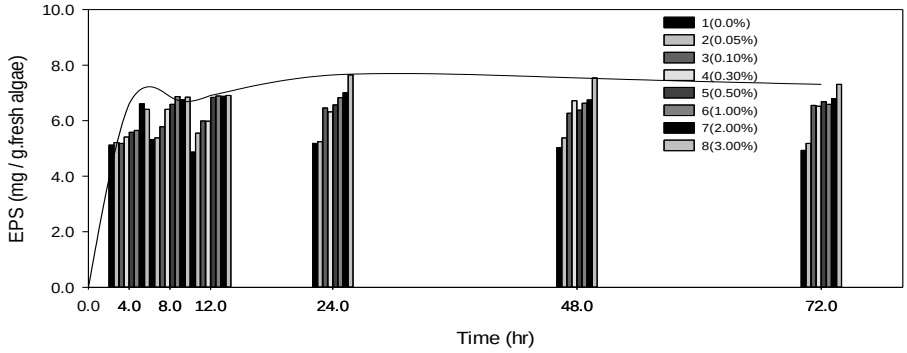

Figure 4. Changes of the EPS level under different salinity and culture time.

4. Conclusion

Spirogyra is a genus widely spread all over the world and is also a dominant species, large green algae in fresh water. The species of *S. fluviatilis* used in this study originally grew in freshwater areas, EPS could be secreted due to the changes in salinity in the habitat. Consequently the mucilage layer outside of the cell walls of the algae becomes thicker. This study revealed that EPS secretion was increased when the *S. fluviatilis* was cultured in 3.0‰ of salinity for 4 hours, but the amount of EPS merely slightly increased in accordance with culture time.

The total amount of EPS is slightly increased in accordance with the increase in salinity; however, the effects for ratio of proteins to polysaccharides not significant. This study may allow further exploration of *S. fluviatilis*. Studies on the biotechnological importance and ecological significance of the extracellular polymeric substances of *S. fluviatilis* deserve further attention.

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