Preliminary Studies on the Isolation, Purification and Physicochemical Properties of Extracellular Acidic Polysaccharides from *Microcystis aeruginosa* var. *major*

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**Abstract** The occurrence of harmful microcystis blooms in eutrophic water bodies is a world wide problem. *Microcystis* usually forms mucilaginous sheath surrounding colony which floats on the surface of water body. The mucilaginous sheath was mainly composed of polysaccharides. To provide theoretical evidences in multipurpose use of Microcystis bloom the aim of this study was to isolate and purify acidic polysaccharides from *Microcystis aeruginosa* var. *major* cultures. Extracellular acidic polysaccharides were extracted by hot water at 80°C and pH 8.0 from *M. aeruginosa* var. *major* in the study. After being deproteinized and decolored the neutral and acidic polysaccharides were pooled from the elutes of DEAE – 52 gel column. Then the acidic polysaccharides were further loaded on Sephadex G – 150 column two fractions fraction I and EAPS I and fraction II and EAPS II of different molecular weights were obtained. Their mass ratio is 1.92:1. Detected by HPLC, the results showed that the average molecular weight of EAPS I was 7.01 × 10^4 and the mean molecular weight of EAPS II was 4.21 × 10^4. EAPS I was consisted of 10.74% uronic acid and the content of SO₄²⁻ was 44.44 µg/mg EAPS II was consisted 7.08% uronic acid and the content of SO₄²⁻ was 9.08 µg/mg. The monosaccharide constitutions of EAPS I were 9.6549% D-glucose, 19.2522% D-fructose, 71.0930% D-glucose. The monosaccharide constitutions of EAPS II were 6.4065% D-glucose, 18.0016% D-fructose, 75.5919% D-glucose.

**Keywords** *Microcystis aeruginosa* var. *major*, extra cellular acidic polysaccharide, isolation, purification, physicochemical properties
梅秋红等

铜绿微囊藻胞外酸性多糖的分离、纯化及其理化特性

用药物抑制其繁衍生长或使其毒素降解来防止水质的恶化等方面对爆发的水华的利用也还是简单地局限于将其处理成土壤肥料等较粗放的技术。

现有资料表明蓝藻门中有许多微藻富含多糖,如富含酸性基团的螺旋藻多糖具有提高免疫力和抗肿瘤的生物学效应,引起了医药界的极大关注。

前人的研究资料和我们的研究工作都表明铜绿微囊藻在胞外能产生大量的黏液层多糖,但是对于这种铜绿微囊藻胞外多糖,特别是酸性多糖的研究还未见报道,我们的工作旨在更有效的提取这种胞外酸性多糖,并力求避免藻体内的小分子肽的污染,并研究其是否具有其他蓝藻酸性多糖类似的生物学活性,为充分利用水华资源,变害为宝提供有价值的理论依据。

材料和方法

材料

铜绿微囊藻来自南京大学生科院藻类生物学研究室曾昭琪教授友情提供,藻细胞由南京师范大学生科院植物生物工程研究室培养。

纤维素、和及各种标准分子量多糖均为产品,其余试剂均为国产分析纯。

铜绿微囊藻胞外多糖的提取、分离和纯化

多糖的提取:

离心藻液,取上清液真空浓缩,收集的藻细胞加倍体积水将调至,离心,取上清液浓缩,合并浓缩液,乙醇沉淀,丙酮洗涤,乙醚干燥,得到褐色的铜绿微囊藻胞外多糖粗提物。

脱蛋白、脱色:将铜绿微囊藻胞外多糖粗提物溶于水,加胰蛋白酶反应后,蒸馏水透析,再采用反复处理去掉蛋白质和小分子肽,直至多糖的紫外光谱分析显示无蛋白吸收峰,再按比例加入活性炭粉末,水浴搅拌,冷却后过滤,滤液冷冻干燥,得到粗多糖。

多糖的分离、纯化:将适量多糖溶解于溶液中,上层析柱,先用的洗脱,然后换用浓度梯度溶液进行梯度洗脱,流速控制为收集过程中用硫酸蒽酮法跟踪检测多糖峰的出现。

收集多糖峰部位的洗脱液,浓缩,透析脱盐,加无水乙醇沉淀,乙醚干燥,再将收集的多糖上层析柱,蒸馏水洗脱,硫酸蒽酮跟踪检测,收集合并多糖峰的洗脱部分,浓缩,冷冻干燥得精品多糖。

多糖分子量的测定

参考魏远安等的方法,采用高效液相色谱法测定所得多糖的分子量,色谱采用色谱仪,粒径,凝胶分析柱,流动相为,流速示差检测器。

多糖中的糖醛酸含量的测定

采用硫酸咔唑法,样品中的己糖醛酸与浓硫酸作用形成生色原,后者定量与咔唑成色以葡萄糖醛酸作参比绘制标准曲线。

多糖中的硫酸根含量的测定

采用硫酸比浊法检测,通过酸水解从糖复合物中释放硫酸基团,即可与氯化钡起沉淀反应,形成沉淀其明胶溶液的光吸收值同样品中的硫酸根的浓度成线性关系,由标准硫酸盐作参比绘制标准曲线,根据标准曲线换算样品中硫酸根的含量。

胞外多糖各组分单糖结构组成分析

参考夏尔宁的方法,取精品多糖分别加%硫酸于沸水浴中水解,抽滤,滤液定容至,留作高效液相色谱分析用,色谱采用碳水化合物分析柱。

结果与讨论

铜绿微囊藻胞外多糖的提取

提取铜绿微囊藻胞外多糖时,藻液控制为,提取温度控制在,时间控制在,其目的是尽量提取出藻细胞外的酸性多糖,其热水抽提物脱水干燥后为黄褐色粉末,得率约为。
2.2 粗提物中含蛋白类物质较多
经反复脱色、脱蛋白至紫外检测无核酸和蛋白吸收峰(图1)为止,所得粗多糖为粗提物的1/3～1/2左右。

2.3 铜绿微囊藻胞外酸性多糖的分离与纯化
粗多糖经DE-52离子交换柱层析分离,在水洗阶段得到一个不能被DE-52吸附的数量较少的中性杂多糖峰,再经梯度洗脱,得到一个被DE-52吸附的多糖峰,洗脱曲线如图2所示,为单一对称峰(简称GHI),该洗脱峰在浓度时出现,提示GHI含有酸性基团,在偏碱性的环境中可能被DE-52所吸附,其后没有其他洗脱的糖峰出现。

2.4 GHI经透析脱盐后上图3所示的洗脱曲线,分别在管和管附近出现两个洗脱的多糖峰,这两个多糖峰均呈对称状态,且已经完全分开,分别收集得到两个组分:简称GHI和GHI(质量比为)。

多糖中糖醛酸、IO含量的测定
为进一步确定GHI和GHI中含有的酸性基团的种类和数量,对这两个样品中的糖醛酸和IO含量分别进行了测定,结果为:

GHI糖醛酸含量为,IO含量为;GHI糖醛酸含量为,IO含量为。表明这两者均含有糖醛酸和IO,特别是糖醛酸的含量分别达到,属于酸性多糖,这与其能被DE-52吸附是吻合的。

2.5 多糖中单糖组成测定
GHI和GHI经酸水解后分别作HPLC测定,根据标准单糖样品及GHI和GHI的水解样品出峰时间(图4),可以推算出GHI和GHI的单糖组成及相对含量。GHI中主要含有:的木糖,的果糖,的葡萄糖;GHI中主要含有:的木糖,的果糖,的葡萄糖。表明GHI和GHI都是杂多糖。
3

Discussion

Cyanobacteria cell wall has a mucilage layer, and the cell also contains a rich polysaccharide component. Currently, the extraction of cyanobacterial polysaccharides is usually performed with hot water extraction. However, this extraction method cannot strictly separate the extracellular and intracellular polysaccharides of Microcystis aeruginosa, so we will control the extraction temperature at 80°C and the extraction time at 2 h, and then use weak alkaline extraction to control the extraction of the extracellular polysaccharide to contain as many acidic components as possible. The experimental results show that this extraction method is effective, with a yield of up to 32%. The extracellular crude polysaccharide contains a small amount of neutral polysaccharide, but acid polysaccharide is the main component. Neutral polysaccharide cannot be adsorbed by DE-52, while the acidic component shows a single symmetric peak during the gradient elution, indicating that ion exchange chromatography can successfully separate the neutral polysaccharides and acid polysaccharides in the extracellular polysaccharides of Microcystis aeruginosa. Acid polysaccharides are further separated into two different molecular weight acid polysaccharides by a gel with molecular sieving effect, with molecular weights, monosaccharide compositions, and acid group compositions significantly different from those of the intracellular acid polysaccharides. This result not only shows that the extraction of the extracellular polysaccharides of Microcystis aeruginosa has been basically achieved, but also shows that the extracellular mucilaginous polysaccharides are more abundant, with a crude polysaccharide extraction rate of up to 27%, providing a solid foundation for the future development and application of Microcystis aeruginosa.

For the analysis of the monosaccharide composition of polysaccharides, the traditional method is to derivatize the polysaccharides and perform gas chromatography analysis, or hydrolyze the polysaccharides and perform thin-layer chromatography. However, the derivatization process of polysaccharides is very complex and difficult to control, and thin-layer chromatography often fails to show clearly. Therefore, we use hydrochloric acid to hydrolyze the polysaccharides and then use an amino column to perform chromatography on the hydrolyzed products combined with standard monosaccharides; we also adopt the high-performance liquid chromatography method to determine the molecular weight of polysaccharides. The results of the molecular weight determination show that the average molecular weight of EAPS Ⅰ is significantly larger than that of EAPS Ⅱ, with a higher proportion of xylose and a lower proportion of fructose and glucose. In particular, the uronic acid content and sulfate content of EAPS Ⅰ are significantly higher than that of EAPS Ⅱ, indicating that the acid strength should also be significantly different. This may be due to the difference in molecular size between the two polysaccharides, which results in differences in charge density.

Fig. 4 The HPLC spectrum of EAPS Ⅰ

Fig. 5 The HPLC spectrum of EAPS Ⅱ

Fig. 6 The HPLC spectrum of EAPS Ⅰ after hydrolysis

Fig. 7 The HPLC spectrum of EAPS Ⅱ after hydrolysis
对于这两种酸性多糖的结构上的特点分析和比较，我们正在用红外、紫外光谱、核磁共振的谱和一些化学方法作深入的研究，将进一步报道由于这两种酸性多糖在分子大小、糖醛酸等酸性基团数量上不同和结构上存在的差异，可能导致他们的生物学效应不同，两者的生物学活性比较的实验我们也正在进行之中。

参考文献