Extraction, Infrared Spectral Analysis and the Antimicrobial Activity on Polysaccharide within *Nostoc Commune* Vauch.

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Abstract: In this article, the antimicrobial activity of polysaccharide from *Nostoc Commune* Vauch. was studied to provide the theoretical basis for application of polysaccharide. Contents of polysaccharide were determined by Phenol-Sulfuric acid reaction system. The chemical compositions and configuration of polysaccharide were studied by FT-IR spectroscopy. And the antimicrobial functions of polysaccharide against *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger* were investigated by inhibitory zone with filter paper. As a result, the yield of polysaccharide could achieve 15.74% in *N. commune*, polysaccharide had acylamino and hydroxy, and pyranose was the major component of the polysaccharide. Inhibiton ratio of polysaccharide on *B. subtilis* and *E. coli* was more than that on *A. niger*. And inhibition ratio of polysaccharide on *B. subtilis* was most in three kinds of bacteria. With the elongation of incubation time, the antimicrobial function of polysaccharide was decreasing. On the whole, inhibitory action increased when concentration of polysaccharide from *N. Commune* increased.

Keywords: *Nostoc commune* Vauch., Polysaccharide, FT-IR spectroscopy, Antimicrobial activity.

1. Introduction

*Nostoc* is a group of cyanobacteria, and is also called blue-green algae in China. Thallus of *Nostoc Commune* Vauch. is packed by capsule outside which is filled by winding algae silk, and is also called “Di muer” in China [1],[2]. As a terrestrial cyanobacteria, *N. commune* is widely distributed over China, and has edible nutrition and medical value, therefore, it was used as food and medicine for several thousand years.

The diverse polysaccharide in *N. commune* can obviously strengthen immunity ability of an organism, and has the anti-tumor, anti-virus, antibacterial, anti-inflammatory effects, and can be used to promote crops’ growth [3]-[5]. Extracellular polysaccharides in cyanobacteria can protect cells from various stresses in severe habitats [6], [7].

However, few studies has been done on using polysaccharide of *N. commune* to inhibit food microorganism. In this paper, extraction, structural analysis and antimicrobial effects of polysaccharide from *N. commune* were studied in order to study anticorrosion effect of polysaccharide. In this study, the phenol-sulfuric acid method was used to detect content of polysaccharides, IR spectroscopy was used to determinate the structure of polysaccharides, and antimicrobial effects of polysaccharide from *N. commune* against *Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger* which were isolated from food were investigated by inhibitory zone with filter paper.

2. Materials and Methods
2.1. Extracting polysaccharide from N. Commune samples

Fresh N. Commune samples were collected from NanBu county in SiChuan province. And the collected samples were washed and dried.

The sediment in dry N. Commune samples were rinsed with distilled water and drained. Further, N. Commune samples were soaked in distilled water for 48 hours and swelled. Lastly, the swelled samples were soaked in absolute ethyl alcohol for 15 minutes and filtered, and the step was repeated at least three times. [8], [9]

Filtered samples were dried in 60°C under 48 hours, then dried in 80°C under 6 hours. And samples were ground to a fine powder. Then, the sample powder was degreased in order that the fatty acids of samples powder was extracted by Soxhlet instrument. After degreasing, the distilled water was added in samples, and the mixture was soaked in 90°C Water bath for 6 hours, and was centrifuged in refrigerated centrifuge. And the step was repeated at least four times.

Supernatant was concentrated in 80°C Water bath for 6 hours in order to remove the protein by Sevage method.

The crude polysaccharide was precipitated by 95% ethanol in method of ethanol precipitation from concentrated solution. After vacuum filtration and freeze drying, crude polysaccharide was weighed.

2.2. Determinating Content of Polysaccharide

Standard curve of glucose was produced, and Phenol-sulfuric acid method was used to determine content of polysaccharide in N. commune.

Polysaccharide concentration=Polysaccharide Content / dry weight of N. commune ×100%

2.3. Determining structure of polysaccharide

Infrared spectrum was used to analyse structure of polysaccharide [10], structure of polysaccharide was determined by FT-IR Spectrometre (Thermo Nicolet, 470FT-IR, Thermo Electron Corporation, America)

2.4. Antimicrobial sensitivity assay

Bacillus subtilis, Escherichia coli and Aspergillus niger were separated and purificated from food. The antimicrobial effect of polysaccharide against B. subtilis, E. coli and A. niger were tested by inhibitory zone with filter paper. And the inhibition rate was assayed by the inhibition zone diameters.

The inhibition rate (%)=(the inhibition zone diameters - filter diameter)/ the inhibition zone diameters ×100%

Finally, toxicity regression equations and EC50 were got in order to determine antibacterial property of polysaccharide.

3. Results and the Analysis

3.1. Detecting the content of polysaccharide

Phenol-sulfuric acid method was used to determine the content of polysaccharide. And the glucose density (ug/ml) was denoted as abscissa, absorbance was denoted as ordinate in order to obtain the glucose density - absorbance equation: y=0.0947x+0.0159 (r²=0.9994).

According to standard curve of glucose, absorbance value of polysaccharide samples was 0.73, so the polysaccharide consistency of was figured out to be 7.54ug/ml. In the sample of polysaccharide extracted from 3g sample from N. commune, the content of polysaccharide was 93.58%. In sample, the crude polysaccharide was 0.5046g, therefore, content of polysaccharide was 15.74%.

3.2. The structure of polysaccharide determinated by infrared spectrum

As shown in figure 1, within IR region of 400-4000cm⁻¹,3423.26 cm⁻¹ absorption band peak was the characteristic absorption of hydroxy stretching vibrational absorption, 2928.01 cm⁻¹ absorption band peak was asymmetrically stretching vibration of carbon hydrogen bonds,1616.70 cm⁻¹ absorption band peak was the stretching vibrational absorption of acylamino,1408.28 cm⁻¹ absorption band peak was caused by flexural vibrations of carbon hydrogen bonds,1066.38 cm⁻¹ absorption band peak was the characteristic absorption of
hydroxy vibrate and, 875.65 cm$^{-1}$ absorption band peak was the characteristic absorption of vibration of hydrocarbon key of pyranose.

Fig. 1: Infrared spectrum analysis of polysaccharide within Nostoc Commune Vauch.

3.3. Antimicrobial activity of polysaccharide

- Antibacterial assay of polysaccharide against *B. Subtilis*

As shown in figure 2, the inhibition rate of polysaccharide against *B. subtilis* increased while contents of polysaccharide increased. When polysaccharide concentration increased from 0.001mg/ml to 1mg/ml, the inhibition rate increased slowly. When the content of polysaccharides increased from 1mg/ml to 100mg/ml, the inhibition rate increased rapidly. And the results indicated that inhibition rate of polysaccharide against *B. subtilis* was higher in the 24-hour culture than in the 48-hour culture.

Fig. 2: Inhibiting activity of polysaccharide against *Bacillus subtilis*.

- Antibacterial assay of polysaccharide against E. Coli

Figure 3 showed that the inhibitory rate of polysaccharide against *E. coli* increased when polysaccharide concentration increased. When polysaccharide concentration increased from 0.001mg/ml to 1mg/ml, the
inhibitory rate increased slowly. When the content of polysaccharides increased from 1mg/ml to 100mg/ml, the inhibitory rate increased rapidly.

And inhibitory rate of polysaccharide against E. coli was higher in the 24-hour culture than in the 48-hour culture.

- **Antifungal assay of polysaccharide against A.niger**

  Figure 4 showed polysaccharide was of highly resistance against A. niger. The inhibition rate of polysaccharide against A. niger increased when polysaccharide concentration increased. And inhibition rate of polysaccharide against A. niger was higher in the 24-hour culture than in the 48-hour culture.

![Figure 4: Inhibiting activity of polysaccharide against Aspergillus niger.](image)

- **Regression analysis of antimicrobial activity from polysaccharide**

  Antimicrobial regression equations and EC<sub>50</sub> were got when B. subtilis, E. coli and A. niger were inhibited by polysaccharide within N. Commune Vauch. Table 1 showed that polysaccharide had an inhibitory action on B. subtilis, E. coli and A. niger, and inhibitory tests showed that the EC<sub>50</sub> varied in different culturing time. EC<sub>50</sub> of polysaccharide in 24-hour culture were lower than in 48-hour culture. EC<sub>50</sub> values of polysaccharide inhibiting B. subtilis were least in all EC<sub>50</sub> in 24-hour culture and 48-hour culture.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Antibacterial time</th>
<th>Regression equation</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (mg/ml)</th>
<th>T-test</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>24h</td>
<td>y=4.7165+0.1764x</td>
<td>40.4669</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.9167)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>48h</td>
<td>y=4.5744+0.1768x</td>
<td>255.3877</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.9376)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>24h</td>
<td>y=4.6198+0.2267x</td>
<td>47.5445</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>y=4.419+0.2294x</td>
<td>340.96</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.91)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>24h</td>
<td>y=4.5914+0.2006x</td>
<td>108.8679</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.9417)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>y=4.5443+0.1599x</td>
<td>707.78</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(r&lt;sup&gt;2&lt;/sup&gt;=0.988)</td>
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</table>

EC<sub>50</sub> values of polysaccharide inhibiting B. subtilis and E. coli were less than EC<sub>50</sub> of polysaccharide inhibiting A. niger in 24-hour culture and in 48-hour culture, and there was significant difference. However, the difference between EC<sub>50</sub> values of polysaccharide inhibiting B. subtilis and EC<sub>50</sub> values of polysaccharide inhibiting E. coli was not significant.

In a word, through testing the value of EC<sub>50</sub>, the ability of the inhibition descend orderly: B. subtilis > E. coli > A. niger.

4. **Discussion**
In crude polysaccharides extracted from *N. commune*, the content of polysaccharides was 93.58%, and content of polysaccharides in *N. commune* was 15.74%. Polysaccharides had acylamino and hydroxyl, and pyranose was the major component of the polysaccharide.

Polysaccharides in *N. commune* can inhibit *B. subtilis*, *E. coli*, and *A. niger*, and inhibitory effect increases gradually with the increase of concentration. Antibacterial effect of polysaccharides to *B. subtilis* and *E. coli* was more stronger than antifungal effect to *A. niger*.

Therefore, inhibition increased gradually with increase of polysaccharide concentration. Inhibiting effect decreases gradually while the culturing time was prolonged. Such findings were similar to Chang Xiang Dong’s report [11].

The results of the present study suggested polysaccharides of *N. commune* Vauch has certain restrictive effect on bacteria and fungi, and it has certain treating function and preservative effect [12], [13]. But the mechanism for the antimicrobial activity of polysaccharide needs to be studied in the future.

5. Acknowledgements

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6. References


