2013 2nd International Conference on Environment, Energy and Biotechnology
IPCBEE vol.51 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V51. 12

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

Yi Diao[1,2], Hongbo Han[2], Yong Li[2], Jianping Zhou [1], Zujun Yang [1]

[1] School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, Sichuan,People's Republic of China.

[2] College of Biological and Chemical Engineering, PanZhiHua University, Panzhihua 617000, Sichuan, People's Republic of China.

Abstract: In the article,the antimicrobial activity of polysaccharide from *Nostoc Commune* Vauch. was studied to provide the theoretical basis for application of polysaccharide. Concents of polysaccharide was 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 hydroxyl,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 inhibiton 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 spectroscop, 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 [1]inhibitory zone with filter paper.

2. Materials and Methods

+ Yi Diao. Tel.: + 86 08123373938; fax: ++86 08123371021.
E-mail address: diaoy163@163.com
+ Corresponding author. Tel.: +0086+08123373938; fax: +0086+08123371021.

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℃ under 48 hours, then dried in 80℃ 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℃ 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℃ 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 producted, 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 EC_{50} 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: $y=0.0947x+0.0159$（$r^2=0.9994$）.

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 vibrateand, 875.65 cm^{-1} absorption band peak was the characteristic absorption of vibration of hydrocarbon key of pyranose.

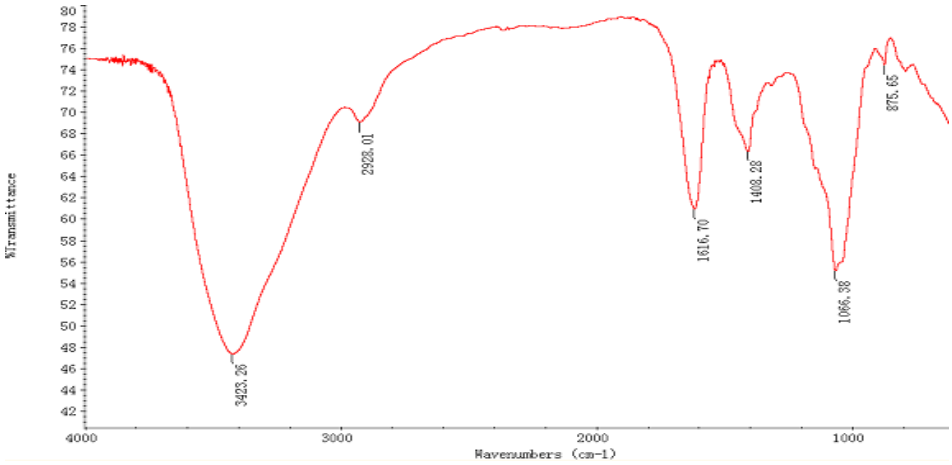

Fig. 1: Infrared spectrum analysis of polysaccharide within *Nostoc Commune* Vauch.

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.

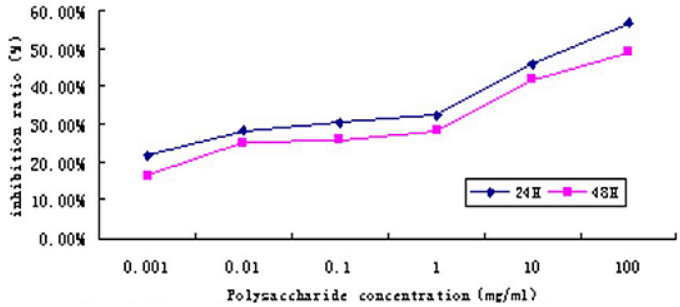

Fig. 2: Inhibiting activity of poysaccharide agianst *Bacillus subtilis*.

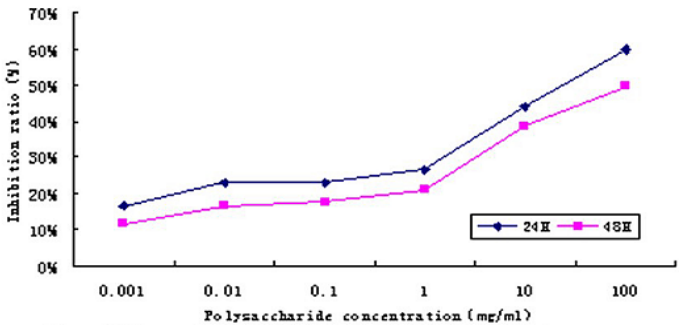

Fig. 3: Inhibiting activity of poysaccharide agianst *Escherichia coli*.

- 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.

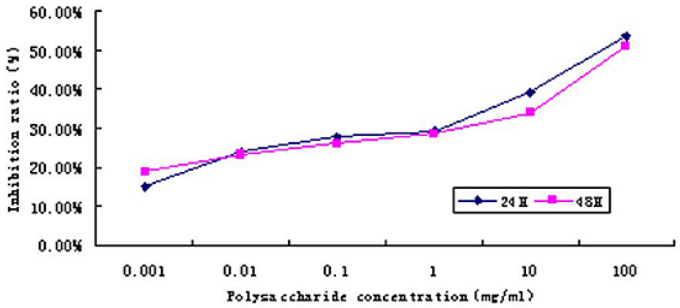

Fig. 4: Inhibiting activity of poysaccharide agianst *Aspergillus niger*.

- Regression analysis of antimicrobial activity from polysaccharide

Antimicrobial regression equations and EC_{50} 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_{50} varied in different culturing time.EC_{50} of polysaccharide in 24-hour culture were lower than in 48-hour culture.EC_{50} values of polysaccharide inhibiting *B. subtilis* were least in all EC_{50} in 24-hour culture and 48-hour culture.

Table1: the antimicrobial activity of polysaccharide within *N. Commune*

Strains	Antibacterial time	Regression equation	EC_{50} (mg/ml)	T- test		F - test
				T0.05	T0.01	
Bacillus subtilis	24h	y=4.7165+0.1764x (r^2=0.9167)	40.4669	D	D	48.15**
	48h	y=4.5744+0.1768x (r^2=0.9376)	255.3877	B	B	
Escherichia coli	24h	y=4.6198+0.2267x (r^2=0.93)	47.5445	D	D	
	48h	y=4.419+0.2294x (r^2=0.91)	340.96	B	B	
Aspergillus niger	24h	y=4.5914+0.2006x (r^2=0.9417)	108.8679	C	C	
	48h	y=4.5443+0.1599x (r^2=0.988)	707.78	A	A	

EC_{50} values of polysaccharide inhibiting *B. subtilis* and *E. coli* were less than EC_{50} of polysaccharide inhibiting *A. niger* in 24-hour culture and in 48-hour culture,and there was significant difference.However, the difference between EC_{50} values of polysaccharide inhibiting *B. subtilis* and EC_{50} values of polysaccharide inhibiting *E. coli* was not significant.

In a word, through testing the value of EC_{50}, 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 XiangDong'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

The present work was partly supported by national natural science fund project (NO.30800685) and common project of Panzhihua Sci ence and Technology Bureau (2012CY-S-22(9)). We would like to thank Jiang Chao,Liao Wei, Li Xingcai,Jiang Jian for valuable contributions toward collecting samples of *N. commune* Vauch.

6. References

[1] FanQunyan, Wu Xiangyang, Yang Liuqing, Zhu Xiaohuan, Mao Guanghua.Research Progress of *Nostoc commune* vauch [J].*Journal of Changshu Institute of Technology(Natural Sciences)*. 2007, **21**(4):55-59.

[2] LiHaiFeng, LiZhangWei, Liu YongMei, HuangZeBo. Prospect for Present Status and Application of Polysaccharides from *Nostoc* [J].*Journal of Agricultural Science and Technology*.2011, **13**(1):105-110.

[3] Roberto De Philippisa, Massimo Vincenzinia. Exocellular polysaccharides from cyanobacteria and their possible applications [J].*FEMS Microbiology Reviews*. 1998, **22**(3):151-175.

[4] Kenji Kanekiyo, Jung-Bum Lee,Kyoko Hayashi,Hiroyuki Takenaka,Yumiko Hayakawa,Shunro Endo,Toshimitsu Hayashi.Isolation of an antiviral polysaccharide, Nostoflan, from a terrestrial Cyanobacterium, Nostoc flagelliforme [J].*J.nat.prod*.2005, **68**:1037-1041.

[5] Sheng Jiarong, Zeng Linghui, Sun Haizhen, Huang Anqi.Biological Activities of Protein-polysaccharides from *Nostoc Commune* [J].*Journal of Guangxi Academy of Sciences*.2001, **17**(1):20-23.

[6] Eric Shaw, Donna R. Hill, Nicole Brittain, Debbie J. Wright, Uwe Tauber, Herve Marand, Richard F. Helm, Malcolm Potts.Unusual Water Flux in the Extracellular Polysaccharide of the Cyanobacterium *Nostoc commune* [J]. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. 2003, **69**:5679-5684.

[7] Yoshiyuki Tamaru, Yayoi Takani, Takayuki Yoshida, Toshio Sakamoto. Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial Cyanobacterium *Nostoc commune* [J].*APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. 2005, **71**:7327-7333.

[8] Mo Kaiju, Xie Bijun, Wang Xingping, Liu Huaichen. Study on Extraction，Seperation and Purification of *Nostoc Sphaeroides* Kutzing Polysaccharide[J].*Food Science*.2004, **25**(10):103-108.

[9] Zhang TangWei, Li Tiancai. Optimal Extraction Conditions on Polysaccharide of *Nostoc Commune* Vauch [J].*Chinese Journal of Spectroscopy Laboratory*. 2011, **28**(1):144:148.

[10] Zhang Saijin, Li Wenquan, Deng Yongzhi. Analysis of Marine Microalgal Polysaccharides by Gas *Chromatography and IR Spectroscopy [J]. Journal of Xiamen University (Natural Science).* 2005, **44**(supplement):212-214.

[11] Chang Xiangdong, Li Bingbing, Deng Yiling. Antibacterial Research on the Water-soluble Polysaccharide within *Nostoc Sphaeroides* Kotzing [J]. *J Med Theor & Prac*. 2009, **22**:1032-1034.

[12] Kunshan Gao, Chinese studies on the edible blue-green alga, Nostoc flagelliforme:a review[J].*Journal of Applied Phycology*,1998, **10**:37-49.

[13] Richard, F.Helm, Zebo Huang, Devin Edwards, Heidi Leeson, William Peery,Malcolm Potts.Structural Characterization of the Released Polysaccharides of Desiccation-Tolerant *Nostoc commune* DRH-1[J].*Journal of Bacteriology*, 2000, **2**:974-982.