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Antioxidant and moisture-retention activities of the polysaccharide from *Nostoc commune*

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ABSTRACT

The main supporting matrix of the edible *Nostoc commune* colony is polysaccharide, which plays important roles in protecting the cyanobacterium itself from extreme desiccation and high radiation. To explore biomedical potential from the inherent nature of the polysaccharide, we investigated antioxidant and moisture-retention capacities of the polymer. We report here that the polysaccharide is capable of scavenging both superoxide anion and hydroxyl radicals *in vitro*. Using the model animal *Caenorhabditis elegans*, we further show that the polysaccharide can increase antioxidant enzyme activity, decrease lipid peroxidation level, and reduce paraquat-induced oxidative damage. We also reveal that the polysaccharide has strong *in vitro* moisture-absorption and -retention capacities as compared to chitosan and urea, and is able to improve water retention in mouse stratum corneum under dry conditions. Together, these data demonstrate the potent *in vitro* as well as *in vivo* antioxidant activities and strong moisture-retention capacities of the polysaccharide from *N. commune*.

1. Introduction

Nostoc is a genus of widespread nitrogen-fixing cyanobacteria capable of forming macroscopic or microscopic colonies. The cosmopolitan *Nostoc commune*, for instance, is able to form macroscopic colonies in natural habitats with filaments of moniliform cells embedded in its mucilaginous matrix. Traditionally, some species of *Nostoc* have been used in China as a food source or as medicine to treat illness. For example, *N. commune* was among the 1892 medicaments outlined in *Compendium of Materia Medica* (Bencao Gangmu) compiled by Li Shizhen (1518–1593) in Ming Dynasty. *Nostoc sphaeroides* (or *N. commune* var. *sphaeroides*) was also on the imperial meal list of Qing Dynasty as disclosed in the autobiography of the last Chinese emperor. According to ethnobiological studies, *N. commune* was spread on the surface of rocks and the colonies formed were collected as a tidbit by Scandinavian tribes (Brüll et al., 2000). A recent study has suggested the potential of *N. commune* as a natural food to reduce the risk of coronary heart disease (Rasmussen et al., 2008).

It is well-known that *N. commune* can resist extreme desiccation and readily restore metabolic activity upon rehydration (Potts, 1994). This ability is closely associated with its abundant sheath or capsular jelly, which consists largely of polysaccharide (Helm et al., 2000; Tamaru, Takani, Yoshida, & Sakamoto, 2005). On the other hand, the field *Nostoc* colonies are often exposed to high intensity of solar irradiation, where excessive UV radiation induces reactive oxygen species (ROS) and impairs cellular function. However, the effective ROS clearance by *Nostoc* cells suggests that *Nostoc* has a set of unique antioxidant components to tackle the stress (Wang, Chen, et al., 2008).

Since the main supporting matrix of *N. commune* colonies is polysaccharide, which plays important roles in protecting the organism itself from a range of physical as well as biological stresses, we are particularly interested in the potential biomedical applications of the polysaccharide. We have previously shown that the polysaccharide from *N. commune* has a high kinematic viscosity in solution (Huang, Liu, Paulsen, & Klaveness, 1998) and a strong effect on the complement system (Brüll et al., 2000). Using *in vitro* assays the polysaccharide from *N. sphaeroides* has also been shown to be effective in scavenging ROS (Tang, Hu, & Chen, 2007), suggesting the biomedical potential of polysaccharides from *Nostoc* species. However, *in vitro* antioxidant capacity may not directly correlate with *in vivo* antioxidant activity (Halliwell, 2008; Kelly

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et al., 2008), and thus *in vivo* investigations are needed to further assess the effect of the polysaccharides.

In this study, we first investigated the *in vitro* scavenging effect of *N. commune* polysaccharide on superoxide anion and hydroxyl radical, and then the *in vivo* effect of the polysaccharide on antioxidant enzyme activities, lipid peroxidation level and superoxide-mediated paraquat toxicity using the model animal *Caenorhabditis elegans*. We also determined the moisture-absorption and -retention properties of the polysaccharide using moisture weight gain-and-loss assay, and further evaluated its moisture-retention function in mouse stratum corneum using attenuated total-reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy.

2. Materials and methods

2.1. Preparation of N. commune polysaccharide

N. commune colonies were collected from field sites in Xuan'en County (30°04′N and 109°47′E; Hubei Province, China). The colonies were washed, air dried and stored at room temperature until use. The polysaccharide was extracted and isolated as described previously (Huang et al., 1998). Briefly, after extraction with 80% ethanol (v/v) at 50 °C and removal of the solvent, the materials were immersed overnight in distilled water at room temperature and then extracted at 95 °C. The extract was centrifuged, and the supernatant was collected and concentrated under reduced pressure at 40 °C. The solution was precipitated with 4 volumes of ethanol, and the precipitate was collected by filtration and redissolved in distilled water. After removal of proteins by Sevag method, the polysaccharide solution was dialyzed (molecular weight cutoff 3500) and freeze dried. Prior to use, the polysaccharide was dissolved in water and diluted into a series of concentrations as indicated. The carbohydrate content of the solution was determined by phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.2. In vitro antioxidant assays

The superoxide anion scavenging activity of the polysaccharide was assessed according to Marklund and Marklund (1974). Briefly, 1 ml of polysaccharide solution, 2 ml of 50 mM Tris–HCl buffer (pH 8.2) and 0.85 ml of water were mixed, and then 0.15 ml of 3 mM pyrogallol solution was added to initiate the reaction. The absorbance change of the reaction at 325 nm within 5 min was recorded, and the superoxide anion scavenging activity was calculated as described (Marklund & Marklund, 1974). The hydroxyl radical scavenging activity was determined according to Smirnoff and Cumbes (1989). The reaction mixture contained 0.5 ml of 10 mM ferrous sulfate solution, 0.5 ml of 10 mM salicylic acid solution and 0.5 ml of the polysaccharide solution, and the reaction was initiated by addition of 2 ml of 300 μM hydrogen peroxide. After incubation at 37 °C for 30 min and cooling to room temperature, the absorbance of the reaction was measured at 532 nm and the hydroxyl radical scavenging activity was calculated as described (Smirnoff & Cumbes, 1989).

2.3. In vivo antioxidant assays

The *C. elegans* wild-type strain (N2) was obtained from the *Caenorhabditis* Genetics Center (University of Minnesota), and maintained at 20 °C on NGM plates with *Escherichia coli* OP50 (Brenner, 1974). Synchronization of the worms was performed as described (Emmons, Klass, & Hirsh, 1979). Food clearance assay was used to determine the suitable range of the polysaccharide concentration (Voisine et al., 2007).

Age-synchronized worms were grown to young adult in S medium and treated with 5-fluoro-2′-deoxyuridine for 2 days at 25 °C to block reproduction. After growing for another 18 days at 25 °C, the worms were treated with *Nostoc* polysaccharide at indicated concentrations or resveratrol (positive control; 50 μg/ml) for 2 days. The worms were harvested, cleaned from bacteria and debris by sucrose floatation, and washed with S medium. The cleaned worms were suspended in 2 ml of phosphate buffer (50 mM, pH 7.8) and homogenized in glass homogenizer. The homogenate was centrifuged at 5000 × *g* and 4 °C for 10 min, and the supernatant was collected for determination of superoxide dismutase (SOD) activity (Giannopolitis & Ries, 1977), catalase (CAT) activity (Aebi, 1984), glutathione peroxidase (GPX) activity (Paglia & Valentine, 1967) and malondialdehyde (MDA) content (Mihara & Uchiyama, 1978). Protein content was determined by Bradford method (Bradford, 1976).

To evaluate the *in vivo* antioxidant effect of the polysaccharide against paraquat-induced oxidative stress, the paraquat assay was performed according to Pun et al. (2010). The synchronized young adult worms were treated with the polysaccharide in S medium for 2 days at 25 °C. The worms were then transferred to 96-well plates (~10 worms/well; >100 worms for each treatment) and exposed to 0.1 mol/l of paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) in S medium containing heat-killed *E. coli*. The numbers of live and dead worms were scored microscopically every 3 h on the basis of their movement; before counting, the plate was gently vibrated to stimulate movement of worms.

2.4. Evaluation of moisture absorption and retention

Nostoc polysaccharide, chitosan and urea were ground into fine powder and oven-dried at 100 °C for 4 h. For determination of moisture absorption, the samples (100 mg each) were placed in a sealed humidity chamber maintained by saturated K_2CO_3 (43% relative humidity; RH) or saturated $(NH_4)_2SO_4$ (81% RH) at 25 °C for indicated times. The moisture absorption rate (R_a) of a sample was evaluated by the gain of weight: $R_a\ (\%) = 100 \times (W_t - W_0)/W_0$, where W_0 was the weight of an oven-dried sample and W_t was the weight of the sample after moisture absorption for a specific time in the humidity chamber.

For evaluation of moisture retention, the samples (100 mg each) were first placed in a humidification chamber containing distilled water to humidify at 25 °C for 24 h and then transferred to a humidity chamber containing saturated K_2CO_3 (43% RH) to dehydrate at 25 °C for 12 h. The samples were then transferred to a desiccation chamber containing dried silica gel for further desiccation at 25 °C for the indicated times. The moisture retention rate (R_r) was evaluated by weight loss of the sample: $R_r\ (\%) = 100 \times (W_m - W_t)/(W_m - W_0)$, where W_0 was the weight of an oven-dried sample, W_m was the weight of the sample after moisturization in the water chamber, and W_t was the weight of the moisturized sample after dehydration for a specific time in K_2CO_3 or silica gel chamber.

2.5. FTIR spectral analysis of water retention on stratum corneum

Mouse stratum corneum was isolated from male BALB/c mice (8 weeks old). The mice were obtained from Wuhan University Laboratory Animal Center and handled according to the guidelines of the Animal Care and Use Committee of Wuhan University. The stratum corneum sheets were washed with cold hexane to remove superficial lipids, air-dried, and cut into small pieces (1 cm × 1 cm). After treating with *Nostoc* polysaccharide (20 mg/ml), urea (20 mg/ml) or water for 1 h, the stratum corneum sheets were transferred into a sealed chamber containing silica gel. The ATR-FTIR spectra of stratum corneum were recorded using Nicolet 5700 FTIR spectrometer

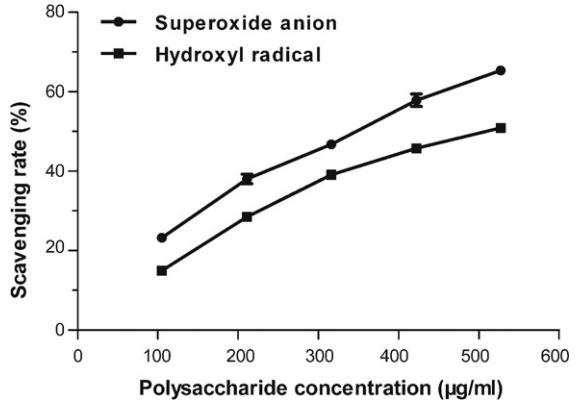

Fig. 1. Scavenging effect of the polysaccharide from *Nostoc commune* on superoxide anion and hydroxyl radical.

(Nicolet, Madison, WI, USA) at indicated times after desiccation. All spectra were collected in the 4000–800 cm^{-1} spectral range at 4 cm^{-1} interval with an average of 64 scans. Omnic FTIR software was used to calculate the peak areas associated with amide I and amide II bands.

2.6. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with a Student's *t*-test, and differences were considered to be statistically significant if $p < 0.05$, except for the analysis of *C. elegans* survival, where the data were analyzed by the Kaplan–Meier method and the statistical significance of mean survival time was determined by Peto's log-rank test. Data are presented as mean ± SD of three repeats.

3. Results

3.1. In vitro ROS scavenging effect of Nostoc polysaccharide

Since the overproduction of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radical, is directly associated with oxidative stress, we tested the ability of the polysaccharide from *N. commune* to scavenge ROS. As shown in Fig. 1, the *Nostoc* polysaccharide is capable of scavenging both superoxide anion and hydroxyl radical *in vitro* in a dose-dependent manner; the highest scavenging rates to superoxide anion and hydroxyl radical were 65% and 51%, respectively, demonstrating the *in vitro* antioxidant activity of the polysaccharide.

Table 2
Effect of the polysaccharide from *Nostoc commune* on the mean survival time of paraquat-treated *Caenorhabditis elegans*.

Polysaccharide (μg/ml)	Mean survival time (h)	p[a]	n[b]
0	14.5 ± 0.48		115
26	15.0 ± 0.53	0.414	106
53	15.8 ± 0.52	0.023	117
105	16.6 ± 0.57	3.9E-4	112

[a] Determined by log-rank test.
[b] Number of worms observed

3.2. Effect of Nostoc polysaccharide on antioxidant enzyme activity and lipid peroxidation level in C. elegans

Oxidative stress develops when an imbalance between ROS production and elimination occurs. Therefore, we examined the effect of *Nostoc* polysaccharide on the antioxidant defense system of *C. elegans*, a favorable animal model in oxidative stress and aging studies with a short lifespan (Melov et al., 2000; Sampayo, Olsen, & Lithgow, 2003). As shown in Table 1, the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), was significantly increased when the worms were treated with *Nostoc* polysaccharide for 2 days at concentration >50 μg/ml. When the polysaccharide was at 105 μg/ml, the increase level of the antioxidant enzyme activity was at a comparable scale with the treatment of 50 μg/ml resveratrol, a known polyphenolic antioxidant (Baur & Sinclair, 2006). On the other hand, the level of lipid oxidation product MDA was decreased about 13.5% when the worms were treated with 105 μg/ml of *Nostoc* polysaccharide (Table 1). Together, these data indicate the *in vivo* antioxidant effect of the polysaccharide from *N. commune*.

3.3. Effect of Nostoc polysaccharide on superoxide-mediated paraquat toxicity in C. elegans

Paraquat, a highly toxic herbicide to animals and humans, is known to increase intracellular superoxide anion level, which may result in generation of more toxic hydrogen peroxide and hydroxyl radicals (Suntres, 2002). To test the effect of the polysaccharide from *N. commune* on oxidative stress-mediated toxicity in general, we examined the survival rate of paraquat-challenged *C. elegans*. As shown in Fig. 2, treatment with the polysaccharide increased the survival rate of the worms exposed to paraquat. The mean survival time of the worms exposed to 0.1 mol/l paraquat was increased 3.4%, 9.0% and 14.5% when treated with 26, 53 and 105 μg/ml of *Nostoc* polysaccharide respectively (Table 2), suggesting the reduction of paraquat-induced oxidative stress and toxicity.

Table 1
Effect of the polysaccharide from *Nostoc commune* on the antioxidant enzyme activity and malondialdehyde content of *Caenorhabditis elegans*.

Treatment	Antioxidant enzyme activity[a]			MDA content[b]
	SOD	CAT	GPX	
Control	24.5 ± 1.76	31.9 ± 0.85	14.7 ± 0.51	2.15 ± 0.159
Nostoc polysaccharide				
26 μg/ml	25.0 ± 1.88	33.8 ± 0.76[c]	15.1 ± 0.59	2.14 ± 0.136
53 μg/ml	29.8 ± 1.26[c]	35.7 ± 0.98[d]	15.7 ± 0.32[c]	1.98 ± 0.171
105 μg/ml	32.3 ± 1.98[d]	36.2 ± 0.71[d]	16.5 ± 0.41[d]	1.86 ± 0.079[c]
Resveratrol[e]	34.7 ± 1.15[d]	34.2 ± 0.91[c]	16.8 ± 0.54[d]	1.91 ± 0.097

[a] SOD, U/mg protein; CAT, k/mg protein; GPX, U/mg protein.
[b] MDA, nmol/mg protein.
[c] $p < 0.05$.
[d] $p < 0.01$.
[e] Resveratrol, 50 μg/ml.

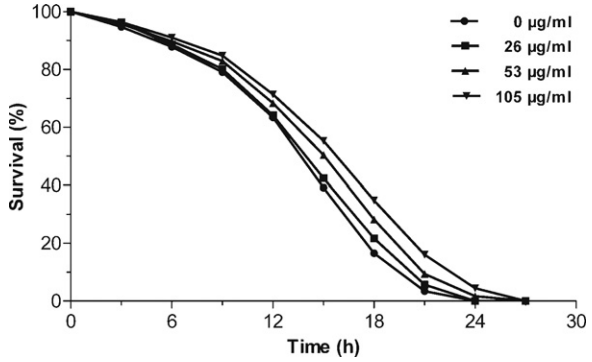

Fig. 2. Effect of the polysaccharide from *Nostoc commune* on the survival rate of paraquat-treated *Caenorhabditis elegans*. Following pretreatment with the polysaccharide at indicated concentrations for 2 days, the N2 worms were exposed to 0.1 mol/l paraquat at 25 °C and surviving worms were scored microscopically every 3 h based on their movement.

3.4. Moisture-absorption and -retention properties of Nostoc polysaccharide

The *in vitro* moisture-absorption and -retention properties of *Nostoc* polysaccharide were examined gravimetrically and compared with those of chitosan and urea, which are frequently used as hygroscopic and humectant agents (Rinaudo, 2006; Stebbins, Alexis, & Levitt, 2008). As shown in Fig. 3A, the moisture absorption rate (R_a) of all the samples at 43% RH increased in the first 12 h, and the R_a of *Nostoc* polysaccharide increased much faster than that of chitosan and urea. After exposed to 43% RH for 24 h, the R_a of *Nostoc* polysaccharide (10.1%) was much higher than that of chitosan (6.3%) and urea (5.8%) (Fig. 3A). The R_a of all the samples at 81% RH was higher than that at 43% RH, and continued to increase until 24 h; the R_a of *Nostoc* polysaccharide, chitosan and urea at 24 h was 15.0%, 10.2% and 12.7%, respectively (Fig. 3B).

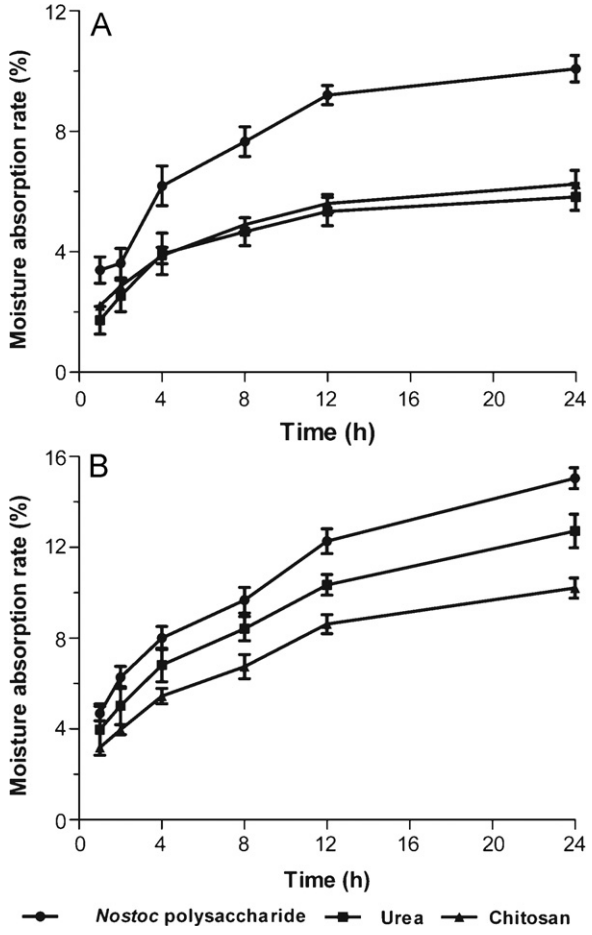

Fig. 3. Moisture-absorption rate of the polysaccharide from *Nostoc commune*, chitosan and urea. The samples were oven-dried at 100 °C for 4 h and placed in a saturated K_2CO_3 chamber (A; 43% RH) or saturated $(NH_4)_2SO_4$ chamber (B; 81% RH) at 25 °C for the indicated times. Moisture absorption was examined gravimetrically.

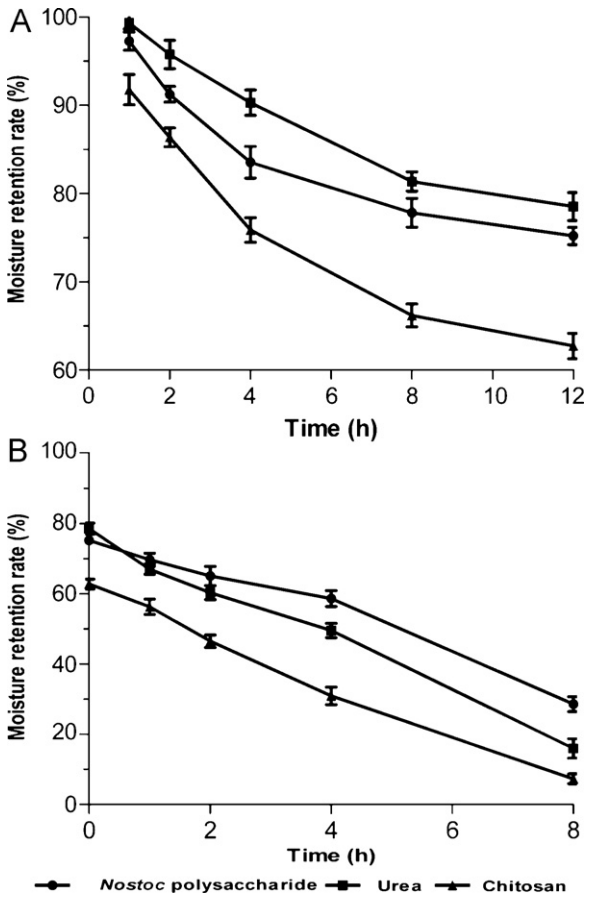

Fig. 4. Moisture-retention rate of the polysaccharide from *Nostoc commune*, chitosan and urea. Dry samples were placed in a water chamber to humidify at 25 °C for 24 h and transferred to a saturated K_2CO_3 chamber (A; 43% RH) to dehydrate at 25 °C for 12 h. The samples were then further dried in a silica gel chamber (B) at 25 °C for the indicated times. Moisture retention was assessed by weight loss of the samples.

To evaluate the moisture-retention properties, *Nostoc* polysaccharide, chitosan and urea were first placed in a water-humidified chamber to absorb moisture for 24 h and then dehydrated at 43% RH. As shown in Fig. 4A, water was lost gradually in all the samples but much slower in urea and *Nostoc* polysaccharide than in chitosan. After dehydration for 12 h at 43% RH, the moisture retention rate (R_r) of urea, *Nostoc* polysaccharide and chitosan was 78.5%, 75.2% and 62.7% respectively. To further characterize the moisture retention properties, the samples dehydrated at 43% RH for 12 h were further desiccated in a silica gel chamber. As shown in Fig. 4B, water loss of all the samples in silica gel chamber was much faster than that at 43% RH within 8 h. After desiccation in the silica gel chamber for 8 h, the R_r of *Nostoc* polysaccharide, urea and chitosan was 28%, 15.9% and 7.3% respectively. Together these results demonstrate the strong moisture-retention capacity of *Nostoc* polysaccharide as compared to chitosan and urea.

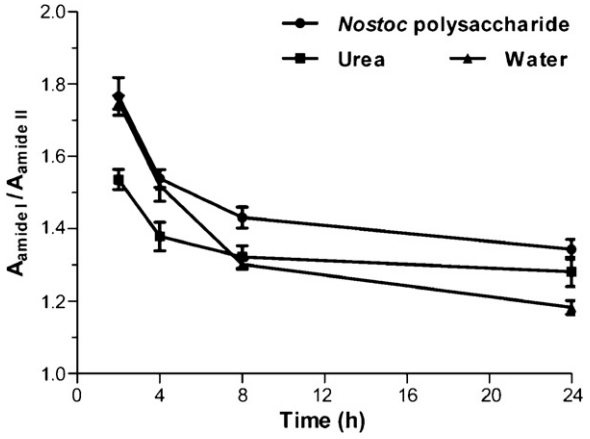

Fig. 5. Effect of the polysaccharide from *Nostoc commune* and urea on the water content of mouse stratum corneum. The stratum corneum sheets were treated with *Nostoc* polysaccharide (20 mg/ml), urea (20 mg/ml) or water for 1 h and transferred to a silica gel chamber to dehydrate for the indicated times. The ATR-FTIR spectra of stratum corneum were recorded and the absorbance ratio of amide I (1648 cm^{-1}) and amide II (1540 cm^{-1}) was calculated.

3.5. Effect of Nostoc polysaccharide on water retention of mouse stratum corneum

The ATR-FTIR spectroscopy, a surface analytical technique, has been successfully used in a variety of *in vitro* as well as *in vivo* biological systems, including measuring constituent status of skin and percutaneous absorption of chemicals (Kazarian & Chan, 2006). Among the ATR-FTIR absorption bands of animal skin, the absorbance of amide I band (carbonyl of the amide bond stretch; ~1650 cm^{-1}) is affected by keratin and water, while that of amide II band (NH deformation; ~1545 cm^{-1}) is only affected by protein (Bello et al., 2006). Therefore the ratio of amide I/amide II absorbance ($A_{amide\ I}/A_{amide\ II}$) can be used as a measure of relative moisture content in stratum corneum (Gloor, Hirsch, & Willebrandt, 1981). As shown in Fig. 5, the $A_{amide\ I}/A_{amide\ II}$ ratio of all stratum corneum samples gradually declined upon dehydration in a silica gel chamber, but the decrease level of *Nostoc* polysaccharide (20 mg/ml) and urea (20 mg/ml) treatments was lower than that of water. In the first 4 h of dehydration, the $A_{amide\ I}/A_{amide\ II}$ ratio of stratum corneum treated with *Nostoc* polysaccharide and water was higher than that with urea ($p < 0.05$). After dehydration for 8 h and 24 h, the $A_{amide\ I}/A_{amide\ II}$ ratio of *Nostoc* polysaccharide treated stratum corneum was higher than that of urea and water treatments ($p < 0.05$). These data demonstrate the water-retention efficacy of *Nostoc* polysaccharide in stratum corneum.

4. Discussion

The delicate balance between ROS production and clearance is critical to maintain normal physiology of cells. Although most ROS generated under normal conditions can be detoxified by endogenous antioxidant systems, excessive production of ROS often triggers oxidative stress, which is involved in aging and a range of diseases. Since antioxidants are capable of scavenging the deleterious ROS, it is widely accepted that appropriate supplementation of exogenous antioxidants may help reduce ROS-induced oxidative damage. However, due to concerns about the side-effects of some synthetic antioxidants, there is an increasing interest in replacing synthetic antioxidants with natural antioxidants in food, pharmaceutical and cosmetic industries. Among the natural antioxidants are polysaccharides, including those from microalgae (Chen, You, Huang, Yu, & Chen, 2010; Tannin-Spitz, Bergman, van-Moppes, Grossman, & Arad, 2005). The present study has revealed that the polysaccharide from the edible *N. commune* has both *in vitro* and *in vivo* antioxidant activities.

Superoxide anion and hydroxyl radical are the most representative forms of ROS in oxidative reactions. Although hydroxyl radical is the strongest and most harmful ROS, superoxide anion is a relatively weak oxidant species (Smirnoff and Cumbes, 1989). Nevertheless, superoxide anion can generate other, more toxic forms of free radicals including hydroxyl radical and singlet oxygen, which may trigger further lipid peroxidation and lead to more damage to cellular components (Radi, Peluffo, Alvarez, Naviliat, & Cayota, 2001). We have shown that the polysaccharide from *N. commune* is able to scavenge both superoxide anion and hydroxyl radical (Fig. 1); the higher activity of the polysaccharide in scavenging superoxide anion over hydroxyl radical may indicate its preventive effect against ROS formation and oxidative damage. The underlying *in vitro* scavenging mechanisms of *Nostoc* polysaccharide may involve direct participation in oxidation reaction as well as interaction with hydroxyl radical as discussed for other polysaccharides (Wang, Zhang, Zhang, & Li, 2008). Interestingly, the extracellular polysaccharide from *N. commune* was reported to have a strong ability to chelate metal ions (De Philippis, Paperi, & Sili, 2007), and thus chelating ferric ion, which is needed for generation of hydroxyl radical and decomposition of hydrogen peroxide in Fenton reaction (Gutteridge, 1986), may also contribute to the scavenging activity of *Nostoc* polysaccharide on hydroxyl radical.

The antioxidant status of an organism provides essential information on its capability to resist oxidative stress. Since the overall antioxidative capacity of the organism is more important than the antioxidant level of any dietary supplement *per se*, the antioxidant enzyme activities and the lipid peroxidation levels in an *in vivo* system are commonly used to assess the antioxidant properties of an antioxidant additive. The antioxidant enzymes, including SOD, CAT and GPX, jointly form the primary *in vivo* antioxidative defense system to scavenge ROS (Inal, Kanbak, & Sunal, 2001). On the other hand, the content of the lipid peroxidation product MDA reflects the *in vivo* oxidation level and is considered a marker of oxidative stress (Valenzuela, 1991). Using the model animal *C. elegans*, we have revealed that the *Nostoc* polysaccharide is capable of increasing antioxidant enzyme activities and decreasing MDA content (Table 1). The antioxidant efficacy of the polysaccharide is comparable with that of resveratrol, an antioxidant polyphenol able to suppress *in vitro* as well as *in vivo* peroxidation of lipids and other macromolecules (Baur & Sinclair, 2006). Together with the *in vitro* antioxidant results, our data suggest that the polysaccharide from *N. commune* is able to exert its potent antioxidant activity through direct scavenging of free radicals, inhibition of enzymes involved in generation of free radicals, chelation of metal ions implicated in ROS production, and stimulation of antioxidant enzyme activities.

A number of studies have reported the lifespan extension of *C. elegans* by antioxidants via resistance of oxidative stress (Kim et al., 2008; Wood et al., 2004; Wu et al., 2002). Although antioxidant capacity is not necessarily predictive of lifespan benefits (Pun et al., 2010), an agent with *in vivo* antioxidant activity does decrease oxidative damage in general. As shown in Fig. 2 and Table 2, the polysaccharide from *N. commune* increased the survival rate of *C. elegans* in paraquat resistance assay. Since *Nostoc* polysaccharide is capable of enhancing endogenous antioxidant activity (Table 1) and paraquat is a well-known *in vivo* ROS generator, these results suggest that the protective effect of the polysaccharide is through its power of ameliorating oxidative damage in paraquat-challenged worms. As several polysaccharides have been reported to exert their effects via stress signaling pathways (ERK, JNK, and p38) (Lin et al., 2006; Yu et al., 2007) and insulin pathways (Chuang, Chiou, Huang, Yang, & Wong, 2009; Liu, Wu, Mao, Wu, & Ouyang, 2010), it would be interesting to further investigate the effect of the polysac-

charide on lifespan and survival of *C. elegans* models related to oxidative pathways.

Since the polysaccharide of *N. commune* plays an important role in its tolerance to extreme desiccation, it is plausible to make use of the polysaccharide in biomedical applications in terms of moisture absorption and retention. As expected, *Nostoc* polysaccharide has shown strong moisture-absorption and -retention capacities at the humidity and desiccation conditions tested, as compared to chitosan and urea (Figs. 3 and 4). Interestingly, *Nostoc* polysaccharide is also able to help maintain water content in mouse stratum corneum when dehydrated in a silica gel chamber (Fig. 5). As the outermost layer of skin, one of the most important functions of stratum corneum is to prevent water loss from hydrated skin. Therefore, water content in the stratum corneum is an important physiological index in dermatology and cosmetics. As shown in Fig. 5, the water-retention efficacy of *Nostoc* polysaccharide in stratum corneum was higher than that of urea, a commonly used humectant in cosmetics. The moisture-retention competence of the polysaccharide may stem from its chemical structure and three-dimensional network: the presence of special peripheral groups such as nosturonic acid may help to modulate its rheological properties (Helm et al., 2000), and the stereoscopic network may be beneficial to its moisture absorption and retention (Shaw et al., 2003). Therefore, the polysaccharide from the edible *N. commune* is an ideal moisture-retention additive in biomaterials including living cells and tissues.

5. Conclusions

We have revealed that the polysaccharide from *N. commune* is capable of scavenging both superoxide anion and hydroxyl radicals in a dose-dependent fashion. We further show that treatment with the polysaccharide can increase the activities of antioxidant enzymes (SOD, CAT and GPX) and decrease the content of lipid peroxidation product MDA in *C. elegans* at a comparable scale with resveratrol treatment. We also demonstrate that the polysaccharide is able to increase the survival rate of paraquat-challenged *C. elegans*. On the other hand, we reveal that the *Nostoc* polysaccharide has strong *in vitro* moisture-absorption and -retention capacities as compared to chitosan and urea, and is capable of increasing the water-retention power of mouse stratum corneum at dry conditions. Taken together, these results demonstrate the potent *in vitro* as well as *in vivo* antioxidant activities and strong moisture-retention capacities of the polysaccharide from *N. commune*, and thus provide pivotal evidence for its potential application in food, pharmaceutical and cosmetic industries.

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