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Antioxidant Properties of Various Microorganisms Isolated from Arctic Lichen *Stereocaulon* spp.

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Lichens are symbiotic organisms composed of fungi, algae, or cyanobacteria which are able to survive in extreme environmental conditions ranging from deserts to polar areas. Some lichen symbionts produce a wide range of secondary metabolites that have many biological activities such as antibacterial, antifungal, antiviral, antitumor, antioxidant and anti-inflammatory *etc*. Among the symbionts of lichens, of the bacterial communities of lichen symbionts little is known. In this study, we isolated 4 microbial species from the Arctic lichen *Stereocaulon* spp. and evaluated their antioxidant properties using 1,1-diphenyl-2-picryl-hydrazyl assay as well as 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) assay. Total phenolic contents and total flavonoid contents were also measured. A potent radical scavenging activity was detected in a number of the lichen extracts. Among the 4 species tested in this study, the ethyl acetate extract of *Bosea vestrisii* 36546(T) exhibited the strongest free radical scavenging activity, with an inhibition rate of 86.8% in DPPH and 75.2% in ABTS assays. Overall, these results suggest that lichen-bacteria could be a potential source of natural antioxidants.

Keywords: ABTS, antioxidant property, Arctic lichen, DPPH, *Stereocaulon* spp, TPC

Introduction

Lichens are typical land plant of Arctica and they can survive in extreme environmental conditions from desert to polar area through their symbiotic relationship between a fungus, algae, or cyanobacteria [35]. Lichens and their natural products have been used as food, dyes, perfumes and decoration [36]. Also, they have used as natural medicines because they show antiviral, anti-proliferative, anti-inflammatory, anti-tumor, anti-mycobacterial and analgesic activities [22, 26, 27, 32, 34] but most of these are come from lichen or fungal symbionts of lichen [29, 48, 49]. Recent report about bacterial symbioses in lichen suggested that some bacterial symbionts have antibacterial and antioxidant activity [17, 38, 46].

Stereocaulon is genus of lichen and commonly found on the rocks or on the ground in humid regions or warmer regions [11]. Although *Stereocaulon* species have a number of subspecies, they have not been extensively investigated except for their habitat and simple characteristics [11, 21]. There are few studies that metabolite of *Stereocaulon* species show antioxidant, antibacterial, antimitotic and protein tyrosine phosphatase 1B inhibitory activities [8, 9, 33, 45]. In addition, diversity of bacterial community and their biological activity is not known in *Stereocaulon*.

Antioxidants are important in the prevention of human disease [19]. Living organisms have a natural defense mechanism of antioxidant, but sometime low level of antioxidant molecules or inhibition of these antioxidant enzymes causes oxidative stress and may cause damage or kill cells [15]. Several strong synthetic antioxidants have already been reported such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) which have proven to be highly carcinogenic compound [18, 50]. For this reason, finding new antioxidants from natural sources is highly desirable.

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In our previous study, 4 bacterial species were isolated from the Arctic lichens including *Stereocaulon* spp. There are very few reports about microorganism isolated from lichens in Arctic region and their biological properties have not been fully explored yet. We expected that Arctic region have potential to provide valuable and variety of natural resources. On this background, the aim of this study is to evaluate antioxidant activities of cell extracts from four novel bacterial species isolated from the Arctic lichens *Stereocaulon* spp. for explore the properties as natural antioxidants.

Materials and Methods

Lichen species

Lichen *Stereocaulon* spp. was collected by Korean Polar Research Institute (KOPRI, Incheon, Korea) in 2010. Stereocaulon was located around the Dasan, Korean Arctic Station at Ny-Alesund, Svalbard, Norway (S78°, E11°). All microorganisms were identified by KOPRI (Table 1) and the numbers were also assigned by KOPRI.

Screening of polar microorganisms associated with lichens

For isolation of bacteria from Arctic lichen *Stereocaulon* spp., we carried out screening experiment according to Yamamoto method with some modifications [51]. A segment of lichen thallus was separated by scissors and then added the 1 ml of sterilized 0.85% saline solution. Vortex in ten minute and then discard the solution and repeat above steps about two more times. Break the tissue with mortar added 1 ml of sterilized 0.85% saline solution and then spread on selective media. Cultures were incubated at 28°C for 15-21 days. Selective media is Humic acid vitamin agar (Humic acid 10.0 g, Na_2HPO_4 0.5 g, KCl 1.71 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $CaCO_3$ 0.02 g, vitamin 1.0 ml, distilled water 1.0 L, agar 16.0 g), Bennett's vitamin agar (D-glucose 10.0 g, yeast extract 1.0 g, peptone 2.0 g, beef extract 1.0 g, vitamin 1.0 ml, distilled water 1.0 L, agar 16.0 g), ISP4 (Difco soluble starch 10.0 g, K_2HPO_4 anhydrous 1.0 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, NaCl 1.0 g, $(NH_4)_2SO_4$ 2.0 g, $CaCO_3$ 2.0 g, ISP trace salt solution 1.0 ml, distilled water 1.0 L, agar 16.0 g) and water agar (distilled water 1.0 L, agar 16.0 g). Agar was purchased from Difco (USA) and all other reagents were purchased from Daejung (Korea). Obtained single colony was taken from the Bennett's vitamin agar media and ISP4 agar media. They have kept on fresh media that mentioned above, respectively. To identify these isolates, we carried out colony PCR. Each fresh single colony were picked in PCR tube with 20 ul of distilled water and used as PCR template. 16S rRNA gene sequences were amplified by PCR using universal primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). The sequences were submitted to the Basic Local Alignment Search Tool (BLAST) search program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the closely related organisms.

Preparation of the lichen associated with bacterial cell culture extract

We used solvent extraction system which is the commonly used technique to recover bioactive compound from bacteria cell culture according to Coleman's method (2011) with some modifications [14]. For the extraction, 50 ml of total 4 microorganisms were cultured on Bennett's vitamin liquid media and ISP4 liquid at 15°C for 10-15 days. Culture broth centrifuged and the supernatant were taken and added to double volume of various solvents including aqueous, acetone, methanol, ethyl acetate, chloroform, ethanol, diethyl ether and petroleum ether (Daejung, Korea) at room temperature. Subsequently solvent was evaporated in vacuum at 40°C. The residuals were then dissolved with solvent and stored at –20°C until further study.

Table 1. Bacterial species isolated from the Arctic lichen.

KOPRI No.	Lichen Source	Bacterial species (Closest strain)	Similarity (%)[a]
26639	*Stereocaulon* spp.	*Pseudomonas graminis* DSM11363(T)	99.754
26640	*Stereocaulon* spp.	*Mucilaginibacterrigui* WPCB133(T)	96.283
26641	*Stereocaulon* spp.	*Pseudomonas graminis* DSM11363(T)	99.574
26642	*Stereocaulon* spp.	*Boseavestrisii* 34635(T)	98.242

[a]The value are expresses as sequence similarity with the closely related organisms and generated by Basic Local Alignment Search Tool (BLAST) search program (http://blast.ncbi.nlm.nig.gov/Blast).

Evaluation of total phenolic contents (TPC) and total flavonoid contents (TFC)

Total quantity of total phenolic content of the lichen-bacterial cell extract was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton with some modifications [47]. All of chemical reagents were purchased from Sigma-Aldrich (USA). 30 μl of test extract was thoroughly mixed with 30 μl of 1 N Folin-Ciocalteu reagent and incubated for 3 min at room temperature. Subsequently, 600 μl of 2% Na_2CO_3 was added to the reaction mixture and the mixture was incubated for 30 min at room temperature. Finally the absorbance was measured at 760 nm. Gallic acid was used as a positive control, whereas reaction mixture without the extraction sample was used as negative control. The concentration of TPC was expressed in micrograms of gallic acid equivalent per milligram of lichen-bacterial cell extract. A single extract were measured three times. Total flavonoid content was determined by colorimetric method described previously [53]. Dried solvent extracts (0.2 g) were dissolved in 20 ml of 80% methanol, extracted for 2 hr at room temperature and centrifuged at 3,000 rpm for 15 min. The volume of the extract was made up to 100 mL with 80% methanol. A portion of 0.5 ml was taken and 0.5 ml of 2% $AlCl_3$ ethanol solution was added to it. After 1 hr at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as catechin from a calibration curve.

Evaluation of free radical scavenging activity using DPPH and ABTS

The free radical scavenging activity of the lichen-bacterial cell extract was determined by DPPH and ABTS methods. The DPPH free-radical scavenging activity of the 4 lichen-bacterial cell extract was determined by the method of Blois with some modifications [10]. All of chemical reagents were purchased from Sigma-Aldrich (USA). 0.1 mM of 1, 1-diphenyl-2-picryl-hydazil (DPPH) was prepared in methanol. Then, 950 μl of DPPH solution was mixed with 50 μl of lichens extraction samples with various solvents. The mixture was incubated for 30 min at room temperature and the absorbance was measured at 517 nm using a UV-Visible spectrophotometer (Biochrome, USA). For ABTS assay, the procedure followed the method of Arnao with some modifications [3]. 7.4 mM of 2, 2'-azino-bis (3-ethyl benzthiazoline-6-sulfolic acid, ABTS) was prepared in methanol. ABTS was kept in the dark for 12 h to generate free radicals from the ABTS salt and then 950 μl of ABTS solution was mixed with 50 μl of lichens extraction samples with various solvents. The mixture was incubated for 30 min at room temperature and the absorbance was measured at 734 nm using a UV-Visible spectrophotometer (Biochrome, USA). 1 mM of ascorbic acid was used as positive control and a reaction mixture without the test sample was taken as a negative control in both assays. Free radical scavenging activity described as the inhibitory percentage of DPPH and ABTS was calculated according to the following equation. A single extract were measured three times.

$$\text{Scavenging activity (\%)} = [\,1 - (\text{Abs sample}/\text{Abs control})] \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was done according to the modified Benzie and Strain method with some modifications [6]. All of chemical reagents were purchased from Sigma-Aldrich (USA). Briefly, 900 μl of FRAP reagent, freshly prepared and warmed at 37°C, were mixed with 90 μl distilled water and either 30 μl of samples in different concentrations or standard or appropriate blank reagent. The FRAP reagent contained 2.5 ml of a 10 mM 2,4,6-tripyridyl-striazine (TPTZ) solution in 40 mM HCl, plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 mM acetate buffer pH 3.6. Readings at the absorption maximum (593 nm) were taken every 15 sec, using a spectrophotometer (Epoch, Biotech Instruments). Temperature was maintained at 37°C. The readings at 30 min were selected for calculation of FRAP values.

Statistical analysis

Data were expresses as Mean ± SD. Statistical analysis was done using Microsoft Office Excel 2007 and a one-way analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$.

Results

Screening and identification of polar microorganisms from the Arctic lichen *Stereocaulon* spp

A total of 4 microorganisms were isolated from the Arctic lichen *Stereocaulon* spp. They grew well on the Bennett's vitamin agar media at 28°C and obtained colonies have pigments and their shapes were either circlar or irregular (data now shown). Their 16S rRNA sequence analysis

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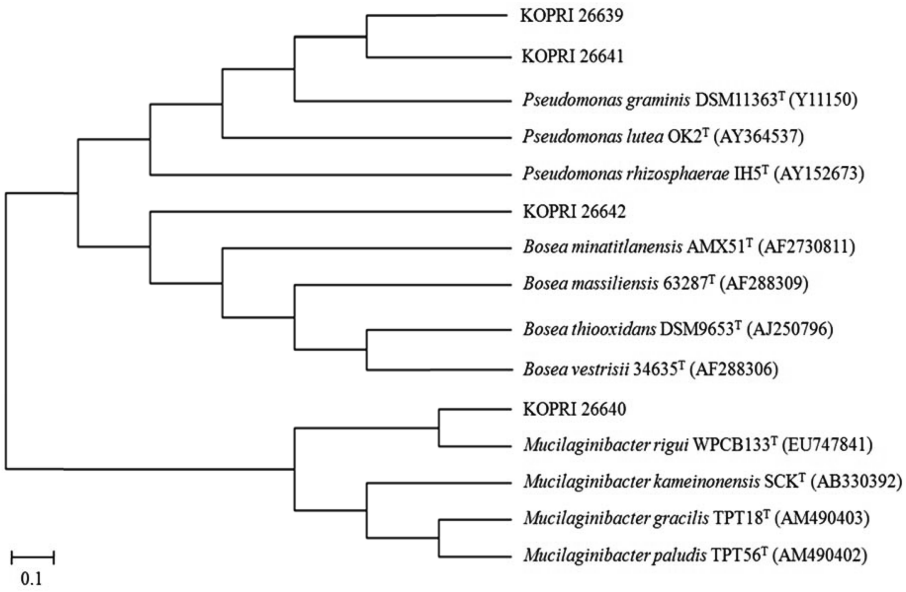

Fig. 1. Phylogenetic dendrogram of polar microorganisms isolated from the Arctic lichen *Stereocaulon* spp.
The tree was constructed by the neighbor-joining method. Bar 0.02 changes per nucleotide. KOPRI26639, *Pseudomonas graminis* DSM11363T; 26640, *Mucilaginibacter rigui* WPCB133T; 26641, *Pseudomonas graminis* DSM11363T; 26642, *Bosea vestrisii* 34635T.

showed the identity of the each microorganism (Table 1). Based on these results, we constructed phylogenetic tree (Fig. 1).

Total phenolic and flavonoid contents of lichen-associated with bacterial cell extracts

Generally, antioxidant activities are dependent on their phenolic constituents [16]. Thus, we evaluated the total TPC of lichen-bacterial cell culture extracts using various solvent such as aqueous, acetone, methanol, ethyl acetate, chloroform, ethanol, diethyl ether and petroleum ether. As shown in Table 2, phenolic compound of some lichen-bacterial species showed high-level of TPC. Especially, extract of *Bosea vestrisii* 34635(T) had the highest TPC in comparison to other bacterial species. In case of solvents, ethyl acetate and chloroform extracts showed higher TPC than others. Finally, these high levels of TPC indicate that lichen-bacteria have properties as useful natural antioxidant. Fla-

Table 2. Total phenolic/flavonoid contents of lichen-bacterial cell culture extracts.

Sample No.		Solvents							
		Aqueous	Acetone	Chloroform	Diethyl ether	Ethanol	Ethyl acetate	Methanol	Petroleum ether
TPC[a]	26639	5.22 ± 0.91	12.01 ± 0.21	13.11 ± 1.20	15.14 ± 2.32	19.12 ± 0.37	65.37 ± 1.90	33.12 ± 0.82	14.2 ± 2.33
	26640	9.92 ± 2.02	27.33 ± 1.30	28.12 ± 0.32	50.32 ± 0.47	44.39 ± 0.22	88.32 ± 2.16	44.00 ± 0.12	24.18 ± 0.36
	26641	5.84 ± 1.42	8.20 ± 1.32	32.02 ± 0.30	44.33 ± 0.58	39.99 ± 0.61	83.05 ± 0.33	30.21 ± 1.02	10.20 ± 1.38
	26642	17.01 ± 3.62	36.42 ± 3.41	81.03 ± 0.52	76.42 ± 0.42	53.21 ± 0.32	99.84 ± 0.22	55.02 ± 0.23	34.15 ± 0.32
TFC[b]	26639	0.31 ± 0.31	5.34 ± 1.02	8.02 ± 0.41	7.98 ± 0.98	8.84 ± 1.15	19.84 ± 1.98	13.59 ± 0.97	6.31 ± 0.21
	26640	1.32 ± 1.02	10.39 ± 2.03	11.07 ± 1.01	11.74 ± 0.99	15.34 ± 1.34	25.32 ± 0.88	16.76 ± 0.78	8.07 ± 0.31
	26641	1.22 ± 0.11	3.07 ± 0.59	19.09 ± 1.12	13.65 ± 0.78	17.33 ± 1.98	30.26 ± 1.51	11.09 ± 0.52	3.14 ± 0.09
	26642	3.31 ± 0.54	4.31 ± 0.51	32.31 ± 1.07	28.47 ± 0.57	20.29 ± 2.01	35.64 ± 1.05	18.01 ± 0.37	10.98 ± 0.04

[a]Total phenolic contents are expresses as gallic acid equivalents (mg GAE/g extract).
[b]Total flavonoids contents are expresses catechin equivalents (mg CE/g extract).
Each value is expresses as mean ± SD (n = 3). Data with different superscript letters are significantly different ($p \leq 0.05$).

vonoids are one of the most powerful antioxidants found in plants [20, 39]. Typically, they possess one or more of the following structural elements that are considered important to their antioxidant activities [42-44]. Thus, total flavonoid content was measured with TFC assay. As shown in the bottom of Table 2, total flavonoid content in all samples was lower than quantity of total phenolic contents. Pattern of quantity is similar with TPC though in order of 26642 > 26641 > 26640 > 26639. Both TPC and TFC level indicated that these strains have antioxidant activities.

DPPH and ABTS free radical scavenging activities of lichen-bacterial cell extracts

The 4 lichen-bacterial species were extracted to evaluate the antioxidant activities. Free radical scavenging activity was described as the percentage of DPPH and ABTS as summarized in Table 3. The DPPH free radical scavenging activity of each lichen-bacterial cell extract was compared with that of the natural antioxidant, ascorbic acid (vitamin C). All the tested extracts and the control (ascorbic acid) exhibited DPPH and ABTS free radical scavenging activity was depend on their concentration. Also, the rate of scavenging activity was variable for each extract and each solvent. Among 4 bacterial species, *Bosea vestrisii* 34635(T) showed a stronger activity than ascorbic acid about 1.7 fold and 2.9 fold in DPPH and ABTS assay, respectively. In almost all cases, ethyl acetate extracts of bacterial species that have high level amount of TPC showed high antioxidant activity.

Ferric reducing antioxidant power of microorganisms

The FRAP assay measures the reduction of ferric iron

Table 3. Evaluation of antioxidant properties using various solvents.

Sample No.		Solvents							
		Aqueous	Acetone	Chloroform	Diethyl ether	Ethanol	Ethyl acetate	Methanol	Petroleum ether
DPPH[a]	26639	5.9 ± 1.21	23.6 ± 1.52	39.4 ± 0.98	13.2 ± 2.10	9.1 ± 1.22	70.1 ± 1.03	13.9 ± 1.12	10.1 ± 0.23
	26640	8.4 ± 0.71	26.3 ± 3.20	20.4 ± 1.17	34.9 ± 1.23	40.9 ± 0.93	82.1 ± 0.91	7.2 ± 0.82	29.1 ± 0.87
	26641	8.9 ± 0.91	22.3 ± 0.92	31.2 ± 2.14	32.3 ± 0.82	39.2 ± 0.44	71.2 ± 0.82	12.6 ± 0.71	31.4 ± 1.40
	26642	11.3 ± 1.31	44.1 ± 0.52	60.8 ± 1.51	49.1 ± 0.77	41.9 ± 1.12	86.8 ± 1.02	41.7 ± 2.13	46.9 ± 2.33
Control		Ascorbic acid (1 mM) : 51.3 ± 0.32							
ABTS[b]	26639	3.3 ± 0.42	13.7 ± 1.11	23.1 ± 0.30	13.2 ± 1.10	9.8 ± 0.91	44.1 ± 1.10	14.5 ± 1.33	10.4 ± 0.73
	26640	5.3 ± 0.79	22.1 ± 0.32	28.0 ± 0.29	13.2 ± 3.23	24.3 ± 0.30	62.0 ± 0.39	22.5 ± 2.39	9.3 ± 0.62
	26641	5.7 ± 0.92	8.1 ± 0.36	19.2 ± 1.19	12.3 ± 0.52	19.4 ± 0.33	48.2 ± 2.22	13.9 ± 1.14	12.9 ± 1.66
	26642	10.2 ± 1.26	24.8 ± 0.45	28.3 ± 0.51	24.9 ± 0.67	8.6 ± 1.54	75.2 ± 0.94	19.7 ± 0.82	23.8 ± 1.39
Control		Ascorbic acid (1 mM) : 25.8 ± 0.41							

[a]DPPH : 1, 1-diphenyl-2-picryl-hydazil.
[b]ABTS : 2'-azino-bis (3-ethyl benzthiazoline-6-sulfolic acid).
Each value is expressed as mean ± SD (n = 3). Data with different superscript letters are significantly different ($p \leq 0.05$).

Table 4. Ferric reducing antioxidant power activity of extracts.
FRAP activities are expressed as mM Fe(II)/mg extract and ascorbic acid was used as control.

Sample No.	Solvents							
	Aqueous	Acetone	Chloroform	Diethyl ether	Ethanol	Ethyl acetate	Methanol	Petroleum ether
26639	0.11 ± 0.11	0.13 ± 0.02	0.20 ± 0.16	0.19 ± 0.01	0.17 ± 0.07	0.18 ± 0.01	0.16 ± 0.04	0.16 ± 0.01
26640	0.12 ± 0.13	0.10 ± 0.01	0.33 ± 0.21	0.21 ± 0.02	0.17 ± 0.11	0.42 ± 0.02	0.19 ± 0.01	0.15 ± 0.02
26641	0.17 ± 0.09	0.15 ± 0.01	0.21 ± 0.06	0.19 ± 0.03	0.16 ± 0.05	0.34 ± 0.02	0.25 ± 0.02	0.23 ± 0.04
26642	0.15 ± 0.14	0.21 ± 0.03	0.59 ± 0.04	0.30 ± 0.01	0.15 ± 0.06	0.92 ± 0.03	0.17 ± 0.01	0.34 ± 0.12
Ascorbic acid	6.81 ± 0.44							

Each value is expressed as mean ± SD (n = 3). Data with different superscript letters are significantly different ($p \leq 0.05$).

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(Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants which are reducer with half-reaction reduction potentials above Fe^{3+}/Fe^{2+} [6, 7]. This assay is also commonly used for the routine analysis of single antioxidants and total antioxidant activity [40]. The FRAP values in various solvent extracts of polar microorganisms are summarized in Table 4. To evaluate the reducing (antioxidant) potential of tomato fractions, the reduction of Fe^{3+}-TPTZ complex to Fe^{2+} in the presence of antioxidants were calculated. The assay is based on the total amount of antioxidant to the reducing capacity of the sample. The results showed the ferric reducing antioxidant power were in the order of 26642 > 26640 > 26641 > 26639, which is similar with DPPH and ABTS results.

Discussion

Although many lichen investigations have been reported [23, 30, 31, 37, 41, 49], most of these are based on lichens or their fungal symbionts. Even though bacterial symbionts of lichen are previously demonstrated structurally and ecologically [5, 12, 13], detail investigation has not been conducted yet. Based on this background, we isolated the total 4 microorganisms from the Arctic lichen *Stereocaulon* spp. and evaluated their antioxidant activities. In their 16S rRNA sequence analysis of 4 microorganisms, KOPRI 26639 and KOPRI 26641 looks like same strain (Table 1). Nevertheless, they have not only different color of colony and shape (data not shown), but variation of antioxidant activities (Table 2-4). In the further studies, analysis of morphological traits (e.g., pigmentation, cell wall characteristics) and metabolic characteristics are needs to identify their major differences. Little is known about these microorganisms such as their source, general morphological and biochemical characteristics *etc* [4, 25], except *Pseudomonas graminis* that is known as control the foodborne pathogen on fresh-cut fruit [1, 2]. Actually, finding microorganisms from the lichen have been investigating continuously [12, 13] but important thing is that these microorganisms have some biological activity like our results. As we mentioned above, most of active compound come from fungal symbionts in lichen [29, 47, 48] but our results showed the microorganisms associated with lichen have antioxidant activities. We can guess these microorganisms have specific and unique bio-mechanisms because they were taken from lichen which lives in polar area. Therefore, these kinds of works are very important to understand their unique mechanisms.

Based on our results, among the various solvents used for extraction such as aqueous, acetone, methanol, ethyl acetate, chloroform, ethanol, diethyl ether and petroleum ether, ethyl acetate extracts showed highest activity in all antioxidant activity tests. In particular, ethyl acetate extracts of *Bosea vestrisii* 34635(T) exhibits highest antioxidant activities in all antioxidant assays including total TPC/TFC assays. In TPC assay, interestingly, chloroform extract of *Pseudomonas graminis*(T) (26639) showed high antioxidant activities than *Mucilaginibacter rigui*(T) (26640) albeit total phenol contents and total flavonoid contents of 26639 is lower than ones in 26640. This means that all phenolics do not have same antioxidant activity (i.e. even if amounts of phenolic are high, its antioxidant activity can low). It may be due to the synergistic of phenolic compounds or antagonistic interactions with other phenolics, or differing types of components such as carbohydrates and proteins [30, 42]. In bacterial communities, some lactic acid bacteria (LAB) such as *Lactobacillus acidophilus*, *Bifidobacterium longum* and *Lactobacillus ferementum* possess antioxidant activity and they can decrease the risk of accumulation of ROS [24, 28]. Recently, Zhang *et al.* (2011) reported the antioxidative activity of lactic acid bacteria in yogurt [52]. Although experiment condition from each study have some difference such as culture condition, type of solvents, and evaluation methods *etc*, our results showed 4 microorganisms isolated from *Stereocaulon* spp. exhibited higher antioxidant activity than lactic acid bacteria in DPPH assay. These results open that microorganisms isolated from lichen possess antioxidant activity and are able to use as natural antioxidant.

This study is the first time to investigate the polar microorganisms isolated from the Arctic lichen *Stereocaulon* spp. As mentioned above, several widely known strong synthetic antioxidants are often carcinogenic compound [18, 50]. Therefore, trials to find potential antioxidants are important and meaningful investigation, especially in undeveloped environment like polar area. In the future study, we will optimize the growth of our polar microorganisms and then investigate other biological activities of these strains and will carry out screening experiment using nature source of polar area.

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국문초록

북극 지의류 *Stereocaulon* spp로부터 분리한 여러 미생물의 항산화 성질. 김미경[1], 박현[2], 오태진[1]*. [1]*선문대학교 제약공학과,* [2]*극지연구소*

지의류는 사막에서 북극지방까지 이르는 극한 환경에서도 생존 가능한 곰팡이, 조류 또는 시아노박테리아 등으로 구성된 공생체이다. 몇몇 지의류 공생체들은 항균, 항곰팡이, 항바이러스, 항암, 항산화 및 항염증 등과 같은 많은 생물학적 활성을 지닌 넓은 범위의 이차대사물질을 생산한다. 지의류와 공생 관계인 박테리아에 관하여는 아주 일부 알려져 있다. 최근 본 연구팀은 북극 지의류 *Stereocaulon* spp로부터 4종류의 미생물을 분리하였으며, DPPH와 ABTS 측정법을 이용하여 그들의 항산화능을 조사하였다. 또한 총 폴리페놀 함량과 총 플라보노이드 함량 분석 등도 측정되었다. 강력한 라디컬 소거능은 지의류 추출물을 이용하여 수행하였다. 본 연구에서 조사된 4종류 중, *Bosea vestrisii* 36546(T)의 에틸아세테이트 추출액은 DPPH 분석에서 86.8% 그리고 ABTS 분석에서 75.2%에 달하는 억제력과 함께 가장 강력한 자유 라디컬 소거능을 보여주었다. 따라서 이러한 결과들로부터 지의류 유래 박테리아 종들이 천연 항산화제로서 잠재적인 소재가 될 수 있다는 것을 제안한다.