PHYTOTHERAPY RESEARCH
Phytother. Res. **22**, 1269–1271 (2008)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2445

SHORT COMMUNICATION

Antibacterial Potential of Antarctic Lichens against Human Pathogenic Gram-positive Bacteria

Babita Paudel[1,2], Hari Datta Bhattarai[1], Jin Sung Lee[1], Soon Gyu Hong[1], Hyun Woung Shin[2] and Joung Han Yim[1]*

[1]Polar BioCenter, Korea Polar Research Institute, KORDI, Songdo Technopark, Songdo-dong 7-50, Yeonsu-gu, Incheon 406-840, South Korea
[2]Department of Marine Biotechnology, Soonchunhyang University, Shinchang myun, Eupnae-ri 646, Asan City, Chunchannam-do, 336-745, South Korea

Extracts from five Antarctic lichens (L3, *Stereocaulon alpinum*; L5, *Ramalina terebrata*; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis*) were tested for antimicrobial activities against several clinically important microbes by disk diffusion. The minimum inhibitory concentration (MIC) of each extract was determined by a broth dilution method. Extracts from L3, L5, L6 and L8 were active against two Gram(+) strains. *B. subtilis* was more sensitive to lichen extracts (except L5) than *S. aureus*. The MIC of lichen extracts against *B. subtilis* and *S. aureus* was observed from 36.7 ± 0.3 to 953.8 ± 85.8 μg/mL and 68.5 ± 0.6 to >1000 μg/mL, respectively. Comparisons of MIC values of Antarctic lichen crude extracts to previously published MIC values of some reported lichen metabolites against Gram(+) bacteria indicated that Antarctic lichens might be an enriched source of effective antibacterial agents against clinically relevant Gram(+) species. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: Antarctic lichens; antibacterial activity; Gram(+) bacteria; minimum inhibitory concentration (MIC).

INTRODUCTION

Pathogenic microbes, particularly Gram(+) bacteria, pose serious threats to human health and are increasing in prevalence in institutional health care settings. New alternatives for combating the spread of infection by antibiotic resistant microbes in future are necessary tools for keeping pace with the evolution of 'super' pathogens. The most successful antibiotics that have been applied to combat disease are small molecule, secondary metabolites, including penicillin derivatives that were originally isolated from fungi.

Lichens represent a symbiotic association of a fungus with an algal partner, and are important constituents of many ecosystems. Lichens produce secondary metabolites that fall into various chemical classes, which are, as a group, distinct from those produced by higher plants. These include: diterpene, triterpene, dibenbenzofuran, dibenzopyranone, depside, depsidones, anthraquinone, xanthones, usnic acids and pulvinic acids (Dayan and Romagni, 2001). Lichen secondary metabolites exhibit numerous biological activities including: antimycobacterial (Ingolfsdottir *et al.*, 1998), antiviral (Neamati *et al.*, 1997), antioxidant (Hidalgo *et al.*, 1994), analgesic (Okuyama *et al.*, 1995), cytotoxic, antimicrobial, fungicidal, herbicidal, antifeedant, photosystem inhibitory (Dayan and Romagni, 2001).

Despite several reports on bioactive compounds from lichen species of tropical and temperate regions, there are virtually no reports on bioactive compounds from Antarctic lichens. This is due to the difficulty of obtaining lichen samples from such regions. This paper presents an investigation into the antimicrobial activities of five lichen species collected from King George Island, a polar region, against various microbes as an initiative aimed at the isolation and identification of new antimicrobial therapeutics.

MATERIALS AND METHODS

Lichen specimens and collection site. Five lichen species namely L3 (*Stereocaulon alpinum*), L5 (*Ramalina terebrata*), L6 (*Caloplaca* sp.), L8 (*Lecanora* sp.) and L17 (*Caloplaca regalis*), were collected from the Korean Antarctic Research Station site on King George Island (60°13′S, 58°47′W), Antarctica. All the species were identified by comparing morphological characteristics (Vstedal and Lewis Smith, 2001). L6 and L8 were tentatively identified at generic level on the basis of morphological characteristics. The identification of L3, L5 and L17 were further confirmed by comparing ITS1 sequence analysis. The gene bank accession number of ITS1 gene for L3, L5 and L17 were EU161238, EU161239 and EU161240, respectively.

* Correspondence to: Joung Han Yim, Polar BioCenter, Korea Polar Research Institute, KOPRI, Songdo Technopark, Songdo-dong 7-50, Yeonsu-gu, Incheon 406-840, South Korea.
E-mail: jhyim@kopri.re.kr
Contract/grant sponsor: Korea Polar Research Institute, KOPRI; contract/grant number: PE07050.

Received 26 September 2007
Revised 23 October 2007
Accepted 2 November 2007

Lichen extraction. Various dry weights of specimens (Table 1) were separately extracted with 10% distilled water in methanol. Extractions were performed at room temperature. The solvent was evaporated in vacuum; samples were lyophilized and stored at –20 °C until further use.

Target microorganisms and culture conditions. Five clinical microorganisms, including two Gram(+) positive (*Bacillus subtilis* KCTC1022 and *Staphylococcus aureus* KCTC3881) and two Gram(–) negative (*Escherichia coli* KCTC1039 and *Pseudomonas aeruginosa* KCTC1636) bacteria and a fungus, *Candida albicans* KCTC 7965, were purchased from Korean Collection of Type Culture (KCTC). Bacterial strains were grown on nutrient agar (NA) at 30–37 °C and *C. albicans* was grown on yeast mannitol (YM) agar at 25 °C.

Antimicrobial test. Antimicrobial testing was carried out using a previously described paper disk assay (Bhattarai *et al.*, 2006). Sterile paper disks (Adventic, Japan) of 8 mm size were loaded with lichen extract at a concentration of 1 mg/disk in triplicate and allowed to dry at room temperature under sterile conditions. The disks were kept on the surface of NA or YM agar, which had been freshly swabbed with the overnight grown broth culture of the microbial target strain. The plates were incubated at optimum growth temperature for each strain for 24 h. The zones of inhibition around the lichen extract loaded paper disks were reflective of the antimicrobial effectiveness of the extract. Paper disks loaded with methanol, a solvent used to dissolve crude extract, were used as negative controls, while paper disks loaded with ampicillin were used as positive controls.

Determination of minimum inhibitory concentration (MIC). MIC was determined by a broth dilution method (Swenson *et al.*, 1982). Serial dilutions of lichen extract ranging from 0 to 1000 µg/mL in 5 mL of nutrient broth in triplicate were prepared in 15 mL sterile falcon tubes. The range of test concentration for the extracts was determined in an initial range finding experiment. An overnight grown broth culture target strain seed was used to inoculate fresh nutrient broth culture containing a range of test extract concentrations, to a final cell density of 10^5 CFU/mL. The inoculated tubes were incubated at optimum temperature for each target strain (Table 1) and shaken at 250 rpm for 24 h. Microbe growth was measured spectrophotometrically by absorbance at 510 nm. The MICs were analysed after regression analysis of the data.

RESULTS AND DISCUSSION

Four lichen species (L3, L5, L6 and L8) provided material with considerable antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*. The lichen extracts tested here produced inhibition zones for *B. subtilis* and *S. aureus*, but did not show antimicrobial activity against *C. albicans*, *P. aeruginosa* or *E. coli* up to 1 mg/disk concentration (lack of activity not presented). The antibacterial activity strength, as indicated by the zone of inhibition size, varied across the lichen extracts. *B. subtilis* was more susceptible than *S. aureus* to lichen extract (Fig. 1). L17 did not exhibit an inhibition zone up to 1 mg/disk of concentration indicating lack of target species specific antimicrobial metabolites. The zone of inhibition size for the other four lichen extracts (L3, L5, L6 and L8) was in the range 14 ± 1.2 to 30 ± 0 mm against *B. subtilis* and 9 ± 0 to 22 ± 0.8 mm

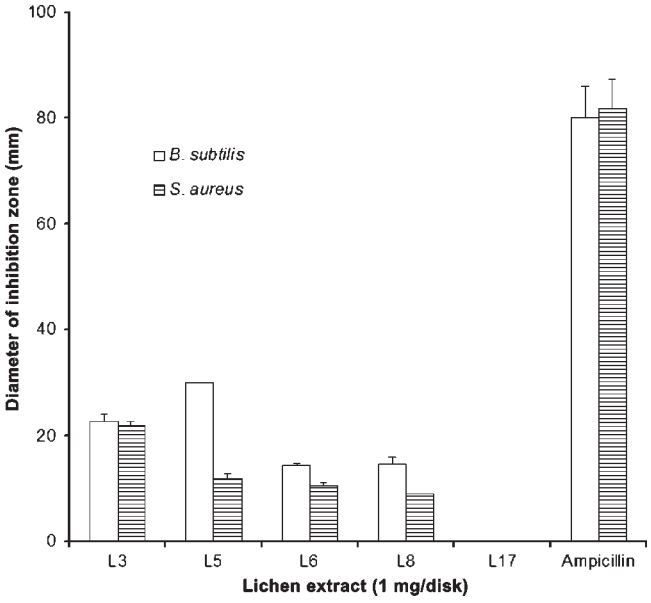

Figure 1. Antibacterial activity of methanol extracts from five Antarctic lichen species: L3, *Stereocaulon alpinum*; L5, *Ramalina terebrata*; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis*.

Table 1. Crude extract yield from five Antarctic lichens and their MICs against *Bacillus subtilis* and *Staphylococcus aureus*

	Test sample		IC_{50} (µg/mL)		MIC (µg/mL)	
Name	Lichen dry weight (g)	Crude extract yield (g)	*B. subtilis*	*S. aureus*	*B. subtilis*	*S. aureus*
L3	10.3	2.02	18.4 ± 0.2	34.3 ± 0.35	36.7 ± 0.3	68.5 ± 0.6
L5	120.3	13.6	16.9 ± 0.1	42.9 ± 3.4	33.8 ± 0.15	85.7 ± 6.7
L6	14.2	2.56	40.9 ± 0.6	168.2 ± 4.4	81.9 ± 1.2	336.3 ± 8.8
L8	22.1	3.21	476.9 ± 42.9	524.9 ± 8.2	953.8 ± 85.8	>1000
L17	127.4	20.65	>1000	>1000	>1000	>1000
Ampicillin			0.2 ± 0.005	0.17 ± 0.003	0.4 ± 0.01	0.35 ± 0.01

MIC, minimum inhibitory concentration; IC_{50}, 50% inhibition of bacterial growth; MIC, 100% inhibition of bacterial growth; L3, *Stereocaulon alpinum*; L5, *Ramalina terebrata*; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis*, ND, not determined.

DOI: 10.1002/ptr

against *S. aureus*. L5 was the most effective against *B. subtilis*, but was weaker than L3 against *S. aureus*. L3 showed almost equivalent activity against *B. subtilis* and *S. aureus*. Methanol loaded disks (negative controls) did not produce any appreciable zone of inhibition against any of the target strains.

The IC_{50} (50% of growth inhibition) and MIC (100% of growth inhibition) of antimicrobial activity showing four lichen (L3, L5, L6 and L8) extracts, against two target strains varied (Table 1). As indicated by the MIC data, the activity of lichen extracts was in the following trends: L5 > L3 > L6 > L8 against *B. subtilis* and L3 > L5 > L6 > L8 against *S. aureus*. Overall, the IC_{50} of the lichen extracts against *B. subtilis* was between 16.9 ± 0.1 and 476.9 ± 42.9 μg/mL, and against *S. aureus* was between 34.3 ± 0.35 and 524.9 ± 8.2 μg/mL. Similarly, the MIC against *B. subtilis* and *S. aureus* was between 33.8 ± 0.15 to 953.8 ± 85.8 μg/mL and 68.5 ± 0.6 to >1000 μg/mL, respectively. Ampicillin showed MICs against *B. subtilis* and *S. aureus* in the range 0.2–0.4 μg/mL. Since none of our lichen extracts were active against *E. coli*, *P. aeruginosa* (Gram-negative target strains) or *C. albicans* in the concentration ranges tested, MIC values were not determined for these strains.

This study showed that methanol extracts from Antarctic lichen species exhibit antibacterial activity that is target specific in nature. For example, L3 showed comparatively stronger antibacterial activity against *B. subtilis* than *S. aureus* but L5 showed stronger activity against *S. aureus* than *B. subtilis*. Similarly, a greater difference (in terms of MIC) in antibacterial activity of L6 was observed against the *B. subtilis* and *S. aureus* indicating the target specific nature of antibacterial activity. Lichen metabolites have been reported to have shown strong antibacterial activity, primarily against Gram(+) bacteria (Lauterwein *et al.*, 1995). Methyl β-orsellinate, and a mixture of methyl and ethyl orsellinates from *S. alpinum* (an Icelandic species), showed comparable activity against Gram(+) bacteria (MIC against *B. subtilis*, 160 μg/mL and against *S. aureus*, 60–160 μg/mL) and various levels of antimicrobial activity against Gram(−) bacteria and fungi (Ingolesdottir *et al.*, 1985). However, a methanol extract from *S. alpinum* from the Antarctic region was much more effective (Table 1) against Gram(+) bacteria, but showed no activity against other microbes, suggesting that the observed activity was due to yet uncharacterized compounds that are methanol soluble. Similarly, the other Antarctic lichens tested in this study also showed relatively strong antibacterial activity against Gram(+) strains compared with previously described lichen metabolites (Ingolesdottir *et al.*, 1985; Lauterwein *et al.*, 1995). Based on the comparison of the results from this experiment with the data from previously published reports discussed above, it is concluded that Antarctic lichen species, possibly, produce novel target specific antibacterial agents, which may be significant antibiotics molecules against Gram(+) pathogenic bacterial strains. The isolation, purification and structural characterization of metabolites from the Antarctic lichens are in progress.

Acknowledgement

This work was supported by a grant to the Korea Polar Research Institute, KOPRI, under project PE07050.

REFERENCES

Bhattarai HD, Lee YK, Cho KH, Lee HK, Shin HW. 2006. The study of antagonistic interactions among pelagic bacteria: a promising way to coin environmentally friendly antifouling compounds. *Hydrobiologia* **568**: 417–423.

Dayan FE, Romagni JG. 2001. Lichens as a potential source of pesticides. *Pestic Outlook* **12**: 229–232.

Hidalgo ME, Fernandez E, Quilhot W *et al.* 1994. Antioxidant activity of depsides and depsidones. *Phytochemistry* **37**: 1585–1587.

Ingolesdottir K, Bloomfield SF, Hylands PJ. 1985. *In vitro* evaluation of the antimicrobial activity of lichen metabolites as potential preservatives. *Antimicrob Agents Chemothe*r **28**: 289–292.

Ingolfsdottir K, Chung GAC, Skulason VG, Gissurarson SR, Vilhelmsdottir M. 1998. Antimycobacterial activity of lichens metabolites *in vitro*. *Eur J Pharm Sci* **6**: 141–144.

Lauterwein M, Oethinger M, Belsner K *et al.* 1995. *In vitro* activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrob Agents Chemother* **39**: 2541–2543.

Neamati N, Hong H, Mazumder A *et al.* 1997. Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. *J Med Chem* **40**: 942–951.

Okuyama E, Umeyama K, Yamazaki M *et al.* 1995. Usnic acid and diffractic acid as analgesic and antipyretic components of *Usnea diffracta*. *Planta Med* **61**: 113–115.

Swenson JM, Thornsberry C, Silcox VA. 1982. Rapidly growing mycobacteria: testing of susceptibility to 34 antimicrobial agents by microdilution. *Antimirob Agent Chemother* **22**: 186–192.

Vstedal DO, Lewis Smith RI. 2001. *Lichens of Antarctica and South Georgia*. Cambridge University Press: Cambridge; 77–366.