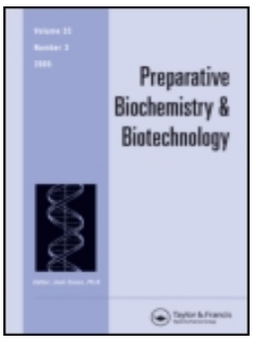

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Cryoprotective properties and preliminary characterization of exopolysaccharide (P-Arcpo 15) produced by the Arctic bacterium *Pseudoalteromonas elyakovii* Arcpo 15

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ABSTRACT

Twenty-two bacterial strains that secrete exopolysaccharides (EPS) were isolated from marine samples obtained from the Chukchi Sea in the Arctic Ocean; of these, seven strains were found to be capable of producing cryoprotective EPS. The ArcPo 15 strain was isolated based on its ability to secrete large amounts of EPS, and was identified as *Pseudoalteromonas elyakovii* based on 16S rDNA analysis. The EPS, P-ArcPo 15, was purified by protease treatment and gel filtration chromatography. The purified EPS (P-ArcPo 15) had a molecular mass of 1.7×10^7 Da, and its infrared spectrum showed absorption bands of hydroxyl and carboxyl groups. The principal sugar components of P-ArcPo 15 were determined to be mannose and galacturonic acid, in the ratio of 3.3:1.0. The cryoprotective properties of P-ArcPo 15 were characterized by an *Escherichia coli* viability test. In the presence of 0.5% (w/v) EPS, the survival percentage of *E. coli* cells was as high as $94.19 \pm 7.81\%$ over five repeated freeze–thaw cycles. These biochemical characteristics suggest that the EPS P-ArcPo 15 may be useful in the development of cryoprotectants for biotechnological purposes, and we therefore assessed the utility of this novel cryoprotective EPS.

KEYWORDS

Arctic bacterium; carbohydrate CPAs; cryoprotectant; cryoprotective properties; exopolysaccharide; *Pseudoalteromonas elyakovii*

Introduction

Freeze–thaw cycles are common in the Arctic and Antarctic regions. Cold-adapted microorganisms are accustomed to be frozen within their habitats. These types of microorganisms are also expected to have evolved adaptations to survive repeated freezing and thawing, because these processes tend to damage living cells and attenuate cell viability.[1] The production of extracellular polysaccharides (EPS), which are primarily composed of polysaccharides, has not been documented as a stress response, but is a survival mechanism for many mesophilic Antarctic marine bacteria and is also found in marine bacteria isolated in other sites.[2,16] The protective role of EPS in a wide range of bacterial species found in extreme environments, such as undersea ice or deep-sea hydrothermal vents, has been documented.[3] The physical, rheological, and chemical properties of exopolysaccharides are affected by polymer chain length, which is the principal determinant of molecular weight.[4] *Phoma herbarum*, a fungal strain isolated from Antarctic soil, generates a glucose homopolysaccharide with a molecular mass of 7.4×10^6 Da, and it has been suggested that this EPS functions as a cryoprotectant within the Antarctic environment.[5] Therefore, the production of EPS may be an effective evolutionary process for bacteria inhabiting cold environments. However, only a small number of studies exist on EPS from Arctic microorganisms, and the cryoprotective properties of these EPS are poorly understood and specified.

In an effort to improve cell preservation, a number of cryoprotectants, including glycerol and dimethyl sulfoxide (DMSO), can be used.[6] The cryoprotective properties of glycerol and DMSO, although they are the most effective cryoprotective agents (CPAs), show high toxicity,[7] and affect the differentiation of neuron-like cells.[8] Carbohydrate CPAs, such as trehalose and sucrose, cannot penetrate the cell membrane and have an exceptional ability to stabilize and preserve the membrane.[9] Although many authors have reported on the cryopreservation of stem cells or microorganisms using DMSO, glycerol, carbohydrate CPAs, or mixtures of CPAs, few studies have focused on the development of novel CPAs.

In this study, bacterial strains that secrete cryoprotective polysaccharides were isolated from Arctic marine samples. Among the isolated strains, the cryoprotective properties of the P-ArcPo 15 EPS from the ArcPo 15 strains were studied by an *Escherichia coli* viability test, and the purified EPS was partially characterized.

Materials and methods

Sample collection and isolation of bacteria

In total, 633 marine samples of various types (seawater, sediment, invertebrates, and benthic animals) were collected at different sites in the Chukchi Sea (approximate location: 73.3°N – 82.3°N, 173°E–153°W) during the Arctic Sea

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expedition cruise of the Korean icebreaker *Araon* (August 1 to September 10, 2012). Seawater samples were collected using a CTD (conductivity, temperature, depth) instrument at various depths, passed through a glass microfiber filter (pore size 0.7 µm, Whatman), and incubated on solid ZoBell medium (5 g L^{-1} peptone, 1 g L^{-1} yeast extract, 0.01 g L^{-1} FePO4, 15 g L^{-1} agar, 750 mL L^{-1} seawater, and 250 mL L^{-1} distilled water, pH 7.2) for 3 days at 15°C. For the selection of exopolysaccharide (EPS)-generating strains, primary screening was performed in solid ZoBell medium. Bacterial colonies producing large amounts of EPS were selected from these plates on the basis of their mucoid appearance. Further screening was performed by growing the selected colonies in liquid SZoBell medium (ZoBell +2% glucose). Each isolated colony was incubated in 20 mL of SZoBell for 3 days at 15°C with shaking at 120 rpm. Subsequently, the cells were separated by centrifugation at 10,000 × g for 15 min at 25°C, and 30 mL of cold ethanol was added to 15 mL of the cell-free supernatant in a test tube. Subsequently, the precipitated crude EPS were incubated for 24 hr at 4°C, and crude EPS were recovered by centrifugation at 10,000 × g for 15 min. The precipitated EPS were dried using a freeze-dryer to estimate their dry weight. EPS-generating bacterial strains were selected on the basis of the relative quantity of the resultant polymer complex that was dehydrated and then flocculated using ethanol.

Identification

Total genomic DNA was extracted from the isolates using an AccuPrep genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The 16S rRNA genes were amplified from the genomic DNA via PCR using the 27 F (5′-AGAGTTTGATC(C/A) TGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTAC-GACTT-3′) primer sets. The polymerase chain reaction (PCR) products were then sequenced, and the resulting DNA sequences were compared with the sequences of strains available in the EzTaxon database to determine taxonomic affiliation.

EPS production and purification

Bacterial cultures for EPS production were grown in liquid SZobell medium. The pH of the medium was adjusted to 7.2. The SZobell medium (500 mL), dispensed in 2-L Erlenmeyer flasks, was sterilized and then inoculated with 2% (v/v) of 12-hr-old culture in the same medium at 15°C and incubated on a rotary shaker for 3 days at 120 rpm.

EPS purification was performed according to a modified version of the procedure described by Yim et al.[10] To purify the EPS, the culture broth was diluted with an equal volume of distilled water and centrifuged at 10,000 × g for 20 min to remove cells. Two volumes of cold ethanol (4°C) were added to the supernatant, and the crude polysaccharide precipitate was dried in a dessicator overnight. Thereafter, the crude polysaccharide was dissolved in distilled water, followed by the addition of 3% (w/v) cetylpyridinium chloride (CPC) until no more insoluble CPC–polysaccharide complex was formed. The precipitate was redissolved in saline solution, washed three times with cold ethanol, and lyophilized.

Exopolysaccharide characterization

Gel permeation chromatography

The molecular weight of the EPS was determined by gel permeation chromatography (GPC) using a Waters Alliance 2695 HPLC system with a 2414 refractive index detector (Waters, Milford, MA). The sample (5 µL, 1 mg/mL buffer consisting of 0.1 *M* NaCl) was injected into the Shodex GPC columns (804 and 805 columns, connected in series) and was eluted with 0.1 *M* NaCl at a flow rate of 0.4 mL/min. The molecular weight was calculated according to a calibration curve generated using a series of pullulan standards.

Gas chromatography

The sugar composition of the EPS was determined by combined gas chromatography–mass spectrometry (GC-MS) analysis of trimethylsilyl (TMS) derivatives of the component methyl glycosides released by methanolysis. The EPS was hydrolyzed by adding methanolic HCl (1.25 *N*; Sigma, St. Louis, MO) and heating for 16 hr at 80°C. The monosaccharides were then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce, Rockford, IL) for 0.5 hr at 80°C. The samples were then dried under nitrogen gas, and the final evaporated samples were dissolved in hexane prior to analysis.

GC-MS analysis of the TMS methyl glycosides was performed on a Clarus 500 (Perkin-Elmer, Waltham, MA) GC, using an automatic injector, a flame ionization detector (FID), and an Elite-5MS low-bleed capillary column (30 m × 0.25 m; Perkin-Elmer). Sample analysis was performed following a temperature program: 120°C for 1 min, then increased by 2°C/min up to 180°C, followed by a gradient of 20°C/min until 230°C. GC was coupled with a Clarus 500 mass spectrometer (Perkin-Elmer). The peak retention time and fragmentation patterns of the TMS derivatives of each sugar component were determined from the standard samples.

Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy (FT-IR) spectra were analyzed using the KBr disc for detecting functional groups. The spectra were obtained from grinding a mixture of 2 mg EPS with S and 200 mg KBr, followed by pressing the mixture into an 8-mm-diameter mold. The FT-IR spectra were recorded on a Thermo NICOLET 6700 instrument (Thermo Scientific, Waltham, MA) from 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} and a total of 16 scans.

Cryoprotective activity assay of EPS

The cryoprotective activity assay of the EPS was performed according to the procedure described.[11] *Escherichia coli* (DH5α) was subjected to freeze–thaw cycles in the presence or absence of the purified EPS. An equal volume of the EPS was mixed with a suspension of *E. coli* in 2-mL freezing vials, after which the mixture was frozen at −80°C for 1 hr. Each tube was thawed for 20 min in a water bath at 25°C, after which it was frozen again at −80°C. For the *E. coli* cell viability test, a bacterial viability kit (LIVE/DEAD BacLight Bacterial Viability Kits; Molecular Probes, L7012, USA) was

used in conjunction with a fluorescence microplate reader (Envision 2103, PerkinElmer). The *E. coli* suspensions were adjusted to 2×10^8 cells/mL ($OD_{670} = 0.06$). The adjusted 100-µL *E. coli* sample was then mixed with an equal volume of 0.5% (w/v) EPS solution. In accordance with the manufacturer's instructions, 6 µL of staining dye A and 6 µL of staining dye B were mixed, and then 2 mL of filter-sterilized dH_2O was added and mixed thoroughly. After the freeze–thaw cycles, the mixture of 100 µL of *E. coli*, and an equal volume of exopolysaccharide was dispensed into the wells of a 96-well flat-bottomed plate. Prepared staining dye (100 µL) was then mixed with the sample. All of the mixed samples were incubated at room temperature for 15 min in darkness. The excitation wavelength was 485 nm, and the fluorescence intensity was measured at 530 nm (emission 1, green) and 630 nm (emission 2, red). The cell survival ratio was calculated as follows:

$$\text{Cell survival ratio}(\%) = (A/B) \times 100$$

in which A is the fluorescence ratio $_{G/R}$ after the freeze–thaw cycle and B is the initial fluorescence ratio$_{G/R}$:

$$\text{Fluorescence ratio}_{G/R} = \text{Emission 1 (485 nm, 530 nm)}/\text{Emission 2 (485 nm, 630)}$$

Statistical analysis

All experiments and assays were performed in triplicate, and the results were expressed as the mean ± SD (standard deviation). The significance of any differences between means was evaluated by Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

Isolation and identification of bacterial strains

In total, 22 bacterial strains that generated mucous materials were isolated from solid ZoBell medium. When cultured in liquid SZoBell medium, the production of EPS by the ArcPo 15 strain was $1.64 \pm 0.09\ g\ L^{-1}$, while other strains produced EPS in the range of $1.07 \pm 0.08\ g\ L^{-1}$ to $1.39 \pm 0.01\ g\ L^{-1}$ under the same conditions (Figure 1). The EPS produced by the ArcPo 15 strain, which was designated as P-ArcPo 15, showed the highest production (Figure 1). P-ArcPo 15 was selected for further study, including preliminary characterization and analysis of the cryoprotective properties of EPS.

The 16S rDNA sequence of the ArcPo 15 strain (1400 bp) was determined and analyzed using Advanced BLAST search software. On the basis of our 16S rDNA analysis, the ArcPo 15 strain was found to exhibit the highest similarity to *Pseudoalteromonas elyakovii* KMM 162(T), with 100% 16S rDNA similarity (Supplementary Data 1). *Gammaproteobacteria* is a highly dominant taxonomic group generally found in the Arctic and Antarctic sea ice or sea environment[12,17]; within this group, *Pseudomonas* and *Pseudoalteromonas* were the most abundant genera in the Arctic and Antarctic.[2,14]

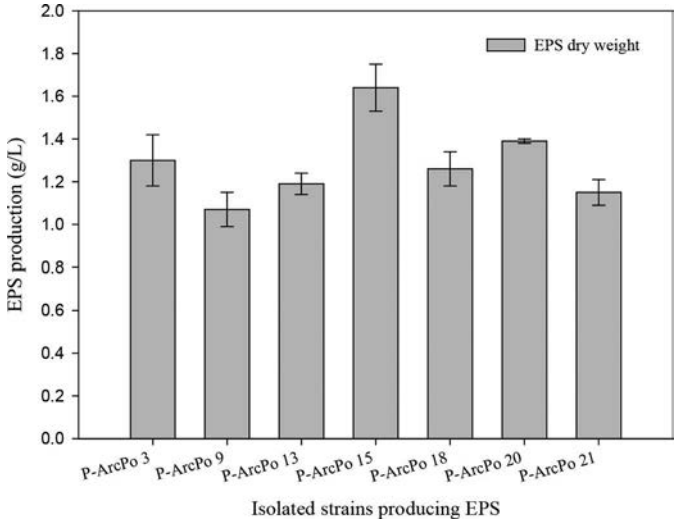

Figure 1. Exopolysaccharide (EPS) production in shake-flask cultures of bacteria. The strains were cultured for 3 days at 15°C in SZoBell medium (pH 7.0). EPS production was estimated via its dry weight. The values shown are means ± SDs from three experimental repeats.

EPS characterization

Gel permeation chromatography

The molecular mass of P-ArcPo 15 was determined by gel permeation chromatography (GPC). A single peak was obtained by GPC analysis of EPS, indicating a relatively homogeneous EPS with an average molecular mass of approximately 1.7×10^7 Da (Figure 2).

The high molecular mass of exopolysaccharide, along with its high polyhydroxyl content, would lower the freezing point of water in the low-temperature, high-salinity brine channels, especially near the cell, where exopolymer concentrations are the highest.[13] As other authors have reported,[2,3] EPS produced by Arctic Sea isolates have been shown to be between 5 and 50 times larger than the average size observed for other marine EPS.

Gas chromatography analysis

The P-ArcPo 15 was composed of mannose and galacturonic acid. Mannose was the most abundant neutral sugar; galacturonic acid was present in less abundance. The peaks showed retention times and fragmentation patterns that were identical to those of the mannose and galacturonic acid standards. The relative molar ratio of mannose and galacturonic acid in P-ArcPo 15 was 3.3:1.0 (Figure 3).

Several EPS-producing strains from Antarctic sea ice and seawater indicated that mannose represented the most abundant neutral sugar in the EPS and that uronic acids (glucuronic acid and galacturonic acid) were present as minority sugars.[3,14]

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of the EPS produced by the ArcPo 15 strain is shown in Figure 4. The FT-IR spectrum of the EPS exhibited many peaks, and the absorption at $3275\ cm^{-1}$ is attributed to the hydroxyl group (–OH) stretching band. However, the absorption at $1640\ cm^{-1}$ is due to

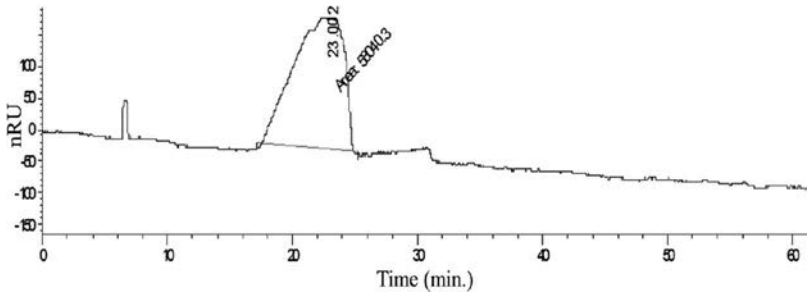

Figure 2. Gel permeation chromatography (GPC) profile of the purified polysaccharide from the ArcPo 15 strain. The purified polysaccharide appears as a symmetric peak, confirming its homogeneity.

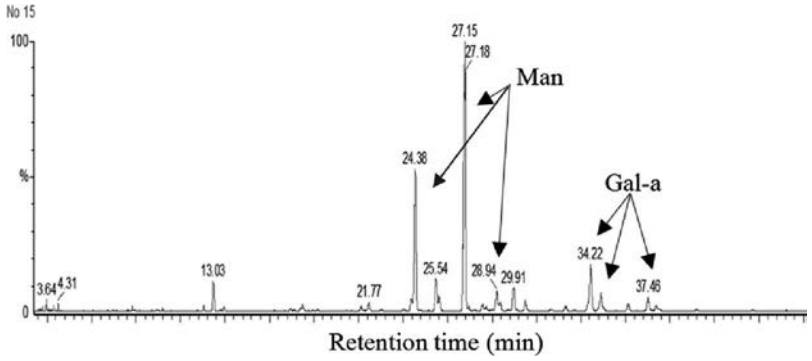

Figure 3. Gas chromatography FID profiles of the TMS methyl glycoside derivatives of P-ArcPo 15. Each peak corresponds to the monosaccharide identified by a standard sugar (Man: mannose, Gal-a: galacturonic acid).

the stretching vibration of the carboxyl group (C=O), and the intense absorptions between 1640 and 1048 cm^{-1} are characteristic of polysaccharides.[2] The sulfate content in the P-ArcPo 15 was determined by the presence of a peak at 1241 cm^{-1}. FT-IR analysis confirmed the presence of hydroxyl groups and low amounts of sulfate groups in the P-ArcPo 15. Previous reports had suggested that high concentrations of the EPS with its hydroxyl group would lower the freezing point of water at low temperatures, especially near the cell, where the EPS concentrations are highest.[2,13]

Cryoprotective effect of P-Arcpo 15 on survival of E. coli

To analyze the cryoprotective effect of purified P-ArcPo 15 on *E. coli* during freezing and thawing, *E. coli* cells were mixed with 0.5% (w/v) P-ArcPo 15 solution and subjected to freeze–thaw cycles (1–5 times). After one to five freeze–thaw cycles, the survival ratio was reduced from 96.13 ± 2.74% to 94.19 ± 7.81%. Remarkable variations in the survival ratio were not observed for *E. coli* exposed to 0.5% (w/v) P-ArcPo 15. In order to compare the cryoprotective effects of P-ArcPo 15 with those of a commonly used cryoprotectant, 20% (v/v)

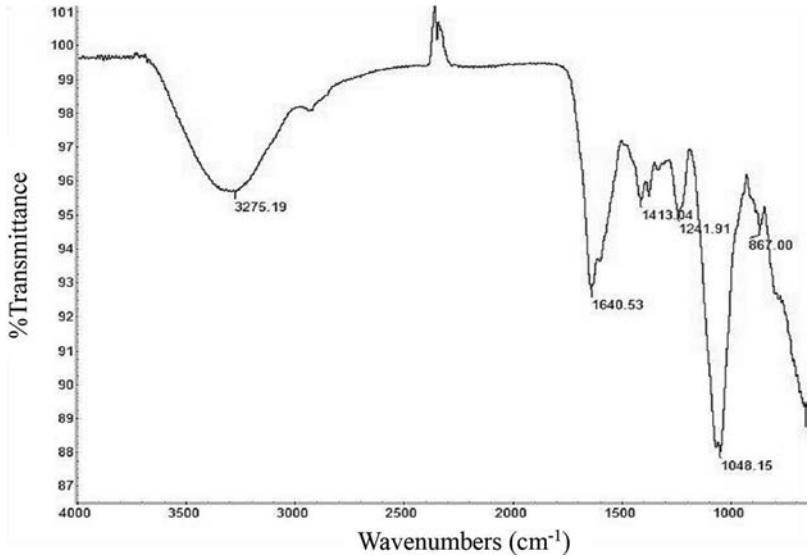

Figure 4. Fourier-transform infrared spectra of P-ArcPo 15 from the ArcPo 15 strain, Arctic marine bacteria grown in laboratory culture.

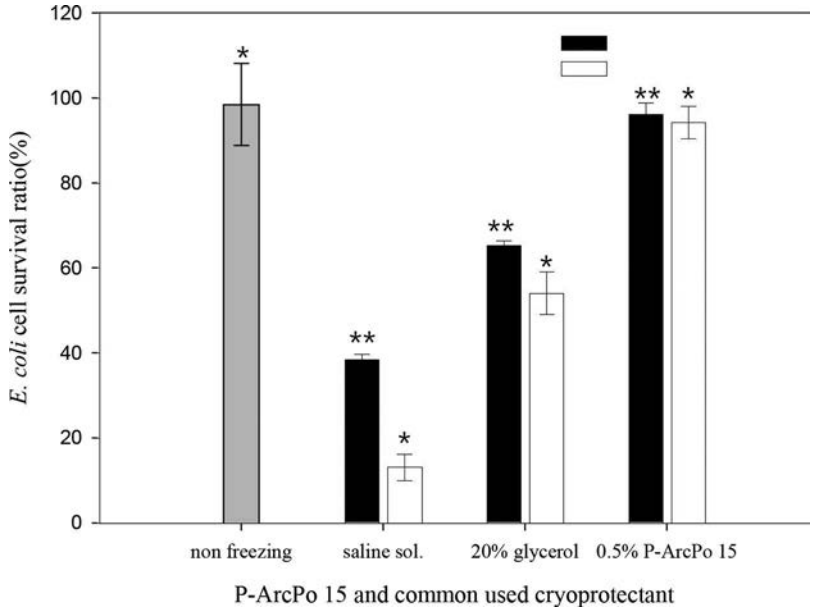

Figure 5. *Eschericha coli* cell survival ratios in the presence of 0.5% (w/v) purified P-ArcPo 15 and 20% (v/v) glycerol after various freeze–thaw cycles. An equal volume of saline solution was added to the control in lieu of EPS. The nonfreezing experiment used *E. coli* cells incubated in saline solution at room temperature (15°C) for 1 hr. The values shown are means ± SDs from three experimental repeats. Significance: $*p < 0.05$, $**p < 0.01$.

glycerol was tested. After one and five freeze–thaw cycles with 20% (v/v) glycerol, the survival ratio was reduced from $65.33 \pm 1.17\%$ to $54.11 \pm 4.97\%$, respectively (Figure 5). P-ArcPo 15 incubation resulted in a high *E. coli* cell survival ratio of more than 90% at low concentrations, compared to those of commercial cryoprotectants. These results support the finding that P-ArcPo 15 significantly improves the freeze–thaw survival ratio of *E. coli*, which is a nonpolar species. These results indicate that the cryoprotective effect of P-ArcPo 15 enabled bacteria to withstand repeated freezing and thawing under laboratory conditions. P-ArcPo 15 was shown to exhibit a highly cryoprotective effect at a low concentrations compared to that of 20% (v/v) glycerol. A fungal strain isolated from Antarctic soil, *Phoma herbarum*, produced a homopolysaccharide of glucose with a molecular mass of 7.0×10^6 Da that could improve mycelial growth after repeated freeze–thaw cycles. The authors of the study on this EPS have suggested that it plays a cryoprotective role against the rigors of the harsh Antarctic environment.[5] Nonpermeable sugars (D-allose and trehalose) and polysaccharides (hydroxyethyl starch) may provide a cryoprotection mechanism by dehydrating cells, thus reducing the amount of water present before freezing. It is also known that the viscosity of sugar solutions rapidly increases during cooling.[15] This is probably another advantage of nonpermeable sugars, because increased viscosity may reduce extracellular ice crystal growth and cryoinjury.

Therefore, P-ArcPo 15 may prove to be a useful cryoprotective agent and may offer potential commercial uses for products derived from bacterial organisms inhabiting extreme environments.

Conclusions

In summary, the mucous polysaccharide generated by the ArcPo 15 strain, designated P-ArcPo 15, had a highly cryoprotective effect as evidenced by the *E. coli* cell survival ratio. The results of 16S rDNA sequence analysis showed that the ArcPo 15 strain has a 100% sequence similarity with that of *Pseudoalteromonas elyakovii* KMM 162(T). The purified P-ArcPo 15 had a molecular mass of 1.7×10^7 Da and the infrared spectrum of P-ArcPo 15 showed absorption bands of hydroxyl and carboxyl functional groups. GC-MS analysis of EPS revealed that P-ArcPo 15 was composed of mannose and galacturonic acid. The cryoprotective effect of P-ArcPo 15 was found to enable nonpolar bacteria (*E. coli*) to withstand repeated freezing and thawing under extremely cold conditions, and P-ArcPo 15 had a highly cryoprotective effect at a low concentration. Further studies are needed to clarify how the EPS may be acting mechanically as a cryoprotectant against cryoinjury or damage and how its structural and chemical properties are related to its cryoprotective effect.

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References

[1] Mazur, P. Cryobiology: The Freezing of Biological Systems. *Science*, **1970**, *168*, 939–949.
[2] Nichols, C.M.; Garon, S.; Bowman, J.P.; Raguenes, G.; Guezennec, J. Production of Exopolysaccharides by Antarctic Marine Bacterial Isolates. *J. Appl. Microbiol.* **2004**, *96*, 1057–1066.
[3] Nichols, C.M.; Guezennec, J.; Bowman, J.P. Bacterial Exopolysaccharides From Extreme Marine Environments With Special Consideration of the Southern Ocean, Sea Ice, and Deep-Sea Hydrothermal Vents: A Review. *Mar. Biotechnol.* **2005**, *7*, 253–271.
[4] Christensen, B.E.; Kjosbakken, J.; Smidsrod, O. Partial Chemical and Physical Characterization of Two Extracellular Polysaccharides

Produced by Marine, Periphytic *Pseudomonas* sp. strain NCMB 2021. *Appl. Environ. Microbiol.* **1985**, *50*, 837–845.
[5] Selbmann, L.; Onofri, S.; Fenice, M.; Frederico, F.; Petriuccioli, M. Production and Structural Characterization of the Exopolysaccharide of the Antarctic Fungus *Phoma herbarum* CCFEE 5080. *Res. Microbiol.* **2002**, *153*, 585–592.
[6] Hubalek, Z. Protectants Used in the Cryopreservation of Microorganisms. *Cryobiology* **2003**, *46*, 205–229.
[7] Fahy, G.M. The Relevance of Cryoprotectant "Toxicity" to Cryobiology. *Cryobiology* **1986**, *23*(1), 1–13.
[8] Oh, J.E.; Karlmark, R.K.; Shin, J.H.; Pollak, A.; Hengstschlager, M.; Lubec, G. Cytoskeleton Changes Following Differentiation of N1E-115 Neuroblastoma Cell Line. *Amino Acids* **2006**, *31*(3), 289–98.
[9] Crowe, L.M.; Mouradian, R.; Crowe, J.H.; Jackson, S.A.; Wormersly, C. Effect of Carbohydrates on Membrane Stability at Low Water Activities. *Biochim. Biophys. Acta* **1984**, *769*, 141–150.
[10] Yim, J.H.; Kim, S.J.; Ahn, S.H.; Lee, C.K.; Rhie, K.T.; Lee, H.K. Antiviral Effects of Sulfated Exopolysaccharide From the Marine Microalga *Gyrodinium impudicum* Strain KG03. *Mar. Biotechnol.* **2004**, *6*, 17–25.
[11] Kim, S.J.; Yim, J.H. Cryoprotective Properties of Exopolysaccharide (P-21653) Produced by the Antarctic Bacterium, *Pseudoalteromonas arctica* KOPRI 21653. *J. Microbiol.* **2007**, *45*, 510–514.
[12] Brinkmeyer, R.; Knittel, K.; Jurgens, J.; Weyland, H.; Amann, R.; Helmke, E. Diversity and Structure of Bacterial Communities in Arctic Versus Antarctic Pack Ice. *Appl. Environ. Microbiol.* **2003**, *69*, 6610–6619.
[13] Krembs, C.; Eicken, H.; Junge, K.; Deming, J.W. High Concentrations of Exopolymeric Substances in Arctic Winter Sea Ice: Implications for the Polar Ocean Carbon Cycle and Cryoprotection of Diatoms. *Deep-Sea Res.* **2002**, *49*, 2163–2181.
[14] Nichols, C.M.; Garon, S.; Bowman, J.P.; Nichols, P.D.; Gibson, J.A. E.; Guezennec, J. Chemical Characterization of Exopolysaccharides From Antarctic Marine Bacteria. *Microb. Ecol.* **2005**, *49*, 578–589.
[15] Sui, L.; Nomura, R.; Youyi, D.; Yamaguchi, F.; Izumori, K.; Tokuda, M. Cryoprotective Effects of d-Allose on Mammalian Cells. *Cryobiology* **2007**, *55*, 87–92.
[16] Poli, A.; Anzelmo, G.; Nicolaus, B. Bacterial Exopolysaccharides From Extreme Habitat: Production, Characterization and Biological Activities. *Mar Drugs* **2010**, *8*, 1779–1802.
[17] Pesciaroli, C.; Cupini, F.; Selbmann, L.; Barghini, P.; Fenice, M. Temperature Preferences of Bacteria Isolated From Seawater Collected in Kandalaksha Bay, White Sea, Russia. *Polar Biol.* **2011**, *35*, 435–445.