Extremophiles (2020) 24:63–70
https://doi.org/10.1007/s00792-019-01113-8

SPECIAL FEATURE: ORIGINAL PAPER

12th International Congress on Extremophiles

Cell-wall associated polysaccharide from the psychrotolerant bacterium *Psychrobacter arcticus* 273-4: isolation, purification and structural elucidation

Angela Casillo[1] · Annarita Ricciardelli[1] · Ermenegilda Parrilli[1] · Maria Luisa Tutino[1] · Maria Michela Corsaro[1]

Received: 24 May 2019 / Accepted: 1 July 2019 / Published online: 15 July 2019

Abstract
In this paper, the structure of the capsular polysaccharide isolated from the psychrotolerant bacterium *Psychrobacter arcticus* 273-4 is reported. The polymer was purified by gel filtration chromatography and the structure was elucidated by means of one- and two-dimensional NMR spectroscopy, in combination with chemical analyses. The polysaccharide consists of a trisaccharidic repeating unit containing two residues of glucose and a residue of a *N,N*-diacetyl-pseudaminic acid.

Keywords Capsular polysaccharide · Pseudaminic acid · Extracellular polysaccharide (EPS) · Psychrotolerant

Introduction

Extracellular polysaccharides (EPSs) are produced by a variety of microorganisms including fungi, algae, and bacteria. They can be associated with the cell surface, as a capsule (capsular polysaccharides, CPSs), or completely released in the surrounding medium (medium-released polysaccharides, MRPs) (Sutherland 1972; Casillo et al. 2018a). The presence of these polymers in a so different range of microorganisms suggests that their production represents an advantage in several situations. They can play an important role in the protection of the cell and communication with the external environment, in adhesion to a surface, and in resistance to the host's adaptive immune response (Corbett and Roberts 2008). In the last few years, the interest in the research of bacterial polysaccharides is increased due to their several industrial applications, like emulsifiers (Bejar et al. 1996; Lee et al. 2001; Yim et al. 2004; Martínez-Checa et al. 2007), biosorption agents of heavy metal (Zhang et al. 2010, 2015; Caruso et al. 2018, 2019), antioxidants (Sun et al. 2015; Roca et al. 2016), and many others. These compounds are a substantial component of the extracellular matrix in microbial cells that populate extreme environments, such as Antarctic ecosystems, saline lakes, or geothermal springs. Recently, the study of the role of EPS as cryoprotectants and their involvement in cold adaptation mechanism has been reported (Carillo et al. 2015; Casillo et al. 2017a). In the sea ice, several diatoms species and many bacteria produce copious amount of EPS, which accumulate as thick gels around the cells (Ewert and Deming 2013; Carillo et al. 2015; Casillo et al. 2017a, b). In particular, abundant EPS production from cold-adapted microorganisms can alter the microstructure and the desalination process of growing ice (Krembs et al. 2011). These polymers are able to interact with ice boundaries and inhibit their growth, to avoid cell lysis.

To better correlate these polymers to the bacterial survival in extreme habitats, it is necessary to characterize new polysaccharides. In addition, since the technological potential of these polymers is high in food industry,

Communicated by M. Moracci.

This manuscript is part of a special issue of Extremophiles journal for the 12th International Congress of Extremophiles (Extremophiles 2018) that was held on 16–20 September 2018 in Ischia, Naples, Italy.

✉ Angela Casillo
angela.casillo@unina.it

✉ Maria Michela Corsaro
corsaro@unina.it

[1] Department of Chemical Sciences, Università degli Studi di Napoli Federico II, Complesso Universitario Monte S. Angelo, Via Cintia, 80126 Naples, Italy

cryopreservation, and agriculture, the discovering of relationships among the structure and the properties is mandatory.

Psychrobacter arcticus strain 273-4 was isolated from permafrost sediment cores in the Kolyma lowland (Siberia, Russia) (Bakermans and Nealson 2004); it is considered a psychrotolerant bacterium and represents a model for cold-adaptation mechanisms in permafrost (Ayala-del-Rio et al. 2010). In previous papers, the core and the lipid A from *P. arcticus* have been in-depth analyzed (Casillo et al. 2015, 2018b). Transmission electron microscopy analysis of *P. arcticus* revealed the presence of a capsule layer around the cells grown in high salinity conditions (Ponder et al. 2008). In this paper, we report the isolation, purification and structural characterization of the capsular polysaccharide produced by *P. arcticus*. Furthermore, its thermal hysteresis (TH) and ice recrystallization inhibition (IRI) activity are reported.

Materials and methods

Bacterial growth and polysaccharide extraction

Psychrobacter arcticus strain 273-4 was grown in Luria–Bertani (LB) medium supplemented with 5% w/v NaCl at 4 °C in aerobic condition. When the liquid cultures reached late exponential phase, bacterial cells were separated from the supernatant through centrifugation (9000 rpm, 4 °C for 20 min). Cells were extracted with phenol/chloroform/petroleum ether (PCP) (2:5:8) (Galanos et al. 1969), and then through the phenol/water method (Westphal and Jann 1965). The PCP extract was analysed elsewhere (Casillo et al. 2015, 2018b). The aqueous phase was extensively dialyzed against water (cut-off 3500 Da) and freeze-dried (240 mg).

TEM

The samples were prepared for Transmission Electron Microscopy (TEM) observations as detailed in Escalera et al. 2014. Briefly, specimens were fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated in a graded ethanol series further substituted by propylene oxide and embedded in Epon 812 (TAAB, TAAB Laboratories Equipment Ltd, Berkshire, UK). Ultrathin sections (60 nm thick) were collected on nickel grids and stained with uranyl acetate and lead citrate and TEM images were acquired with a Zeiss LEO 912AB TEM (Zeiss, Oberkochen, Germany) operating at an accelerating voltage of 80 kV.

DOC-PAGE analysis

Polyacrylamide gel electrophoresis (PAGE) analysis was performed using the system of Laemmli (1970) with sodium deoxycholate (DOC) as detergent (Ricciardelli et al. 2019). The extract was visualized after silver staining and Alcian Blue as previously described (Tsai and Frasch 1982).

Polysaccharide purification

The freeze-dried material (240 mg) was resuspended in 30 mL of digestion buffer containing TRIS/EDTA (10 mM/1 mM) and 10 mM $MgCl_2$. After addition of 4 mg of RNAse and DNAse (Sigma) the sample was incubated for 16 h at 37 °C, followed by 60 °C for 2 h after addition of 2 mg of protease (Protease from *Streptomyces griseus*, Sigma). After dialysis against water (cut-off 3500 Da) it was freeze-dried. The material (68 mg) was resuspended in 7 mL of 5% aqueous CH_3COOH and hydrolyzed at 100 °C for 3 h, then centrifuged at (11.5*g*-force, at 4 °C for 30 min. The pellet was washed three times with water, and the supernatants obtained following each centrifugation were combined and lyophilized.

The material (48 mg) was resuspended in water and fractionated on a Biogel P-10 column (Biorad, 1.5 × 110 cm, flow rate 14.4 mL/h, fraction volume 2.5 mL), eluted with pyridinium acetate buffer (pH 5.0, 0.05 M pyridine, and 0.05 M acetic acid). The first fraction, which eluted with the void volume, contained polysaccharidic material (5 mg), while later fractions contained oligosaccharide core material (data not shown). The polysaccharidic material was loaded on a Sephacryl S-400HR column (Sigma, 1 × 110 cm, flow rate 16.2 mL/h, fraction volume 2.5 mL) eluted with ammonium hydrogen carbonate (0.05 M), yielding 1.5 mg of pure CPS.

Chemical analyses

Monosaccharides were analyzed as acetylated methyl glycosides (MGA). The methanolysis and the acetylation were performed as already described (Casillo et al. 2015). The absolute configuration of the glucose was determined by gas chromatography–mass spectrometry (GC–MS) analysis of its acetylated (*S*)-2-octyl glycosides (Leontein et al. 1978). All the derivatives were analyzed using an Agilent Technologies gas chromatograph 7820A equipped with a mass selective detector 5977B and an HP-5 capillary column (Agilent, 30 m × 0.25 mm i.d., flow rate 1 mL/min, He as carrier gas). Acetylated methyl glycosides were

analyzed using the following temperature program: 140 °C for 3 min, 140 °C → 240 °C at 3 °C/min. For the octyl-glycosides the analysis was performed at 150 °C for 5 min, then 150 °C → 240 °C at 6 °C/min, and 240 °C for5 min.

NMR spectroscopy

For the complete structural characterization ^{1}H,^{13}C 1D and 2D NMR spectra were recorded using a Bruker 600 MHz spectrometer. CPS sample was dissolved in 550 µL of D_2O, at 298 K. Two-dimensional correlation spectroscopy (^{1}H–^{1}H COSY), total correlation spectroscopy (^{1}H–^{1}H TOCSY), rotating frame Overhauser enhancement spectroscopy (^{1}H–^{1}H ROESY), distortionless enhancement by polarization transfer-heteronuclear single quantum coherence (^{1}H–^{13}C DEPT-HSQC) and heteronuclear multiple bond correlation (^{1}H–^{13}C HMBC) experiments were performed. Spectra were calibrated with internal acetone-d_6 (^{1}H, at δ 2.225 ppm and ^{13}C at δ 31.45 ppm).

Thermal hysteresis and ice recrystallization inhibition (IRI) measurements

Thermal hysteresis of CPS from *P. arcticus* was measured as previously described (Braslavsky and Drori 2013; Stevens et al. 2015). Briefly, samples were suspended in type B immersion oil as droplets and snap-frozen on a Clifton nanoliter stage using a Newport model 3040 temperature controller. The ice was melted to obtain a single ice crystal. The temperature was decreased below the melting point by 0.005 °C every 4 s. Images and videos were captured using a Panasonic WV-BL200 digital camera.

IRI was performed exactly as described in Graham et al. 2018 in 25 mM Tris–HCl (pH 7.8), 150 mM NaCl and 0.01 mg/mL bovine serum albumin, using sapphire slides with 95 µm deep wells.

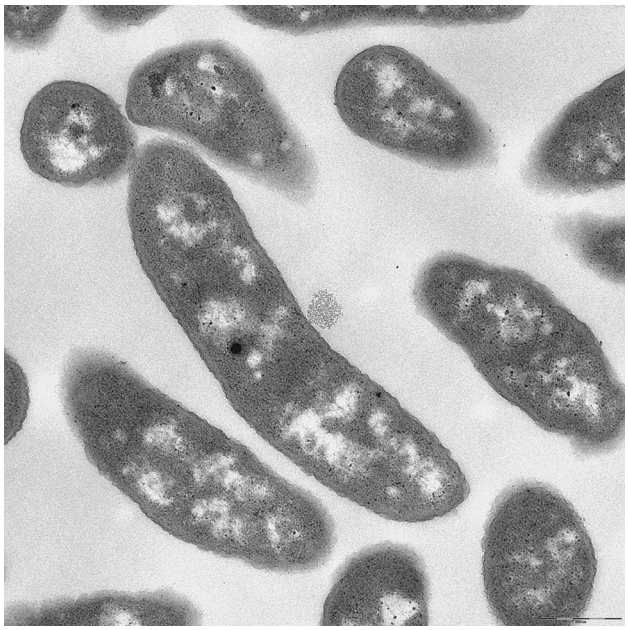


Fig. 1 TEM image of *P. arcticus* grown in NaCl 5%

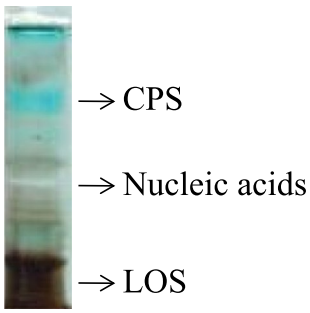


Fig. 2 Analysis of the water extract fraction from *P. arcticus* 273-4 by 14% DOC-PAGE. The sample was stained with Alcian blue dye, followed by silver nitrate staining

Results and discussion

CPS extraction and purification

The presence of a capsular structure around the cells of *P. arcticus* 273-4 when the cells were grown in 16% NaCl was previously reported by Ponder et al. (2008). To detect if the capsule was produced also in the salinity condition used in this work (5% NaCl), a transmission electron microscopy experiment was performed. Results reported in Fig. 1 confirmed the polysaccharide coating in these growth conditions, too.

The cells of *P. arcticus* grown in high salinity conditions (NaCl 5%) were extracted by PCP and then by phenol/water method as already reported (Casillo et al. 2015). The water extract was extensively dialyzed against water, and then lyophilized. The purity of the extracted material was evaluated by means of 14% DOC-PAGE. The analysis revealed the presence of bands corresponding to the lipooligosaccharide (LOS) (Casillo et al. 2015), together with those corresponding to a CPS to and nucleic acids (Fig. 2). The presence of nucleic acids was confirmed by the glycosyl analysis, since the monosaccharide ribose was found. Therefore, the crude extract was digested with DNases, RNases, and proteases, to remove contaminating nucleic acids.

After dialysis against water an aliquot of the digested extract was subjected to the monosaccharide compositional study. The investigation mainly revealed the presence of glucose (Glc), with traces of galactose (Gal), rhamnose (Rha), 2-amino-2-deoxy-glucose (GlcN) and *N*-acetyl-muramic

acid (NAM) belonging to the LOS, confirming its presence in the aqueous extract. The absence of ribose in sugar analysis confirmed the elimination of nucleic acids.

The molecular weights of CPS and LOS species were expected to be very different, based on the DOC-PAGE analysis. Nevertheless, the well-known capability of the LOS to aggregate in aqueous solution did not allow the separation of the two molecular species by size exclusion chromatography. Therefore, the sample was hydrolyzed under mild acidic conditions to cleave the glycosidic linkage between the lipid A and the core oligosaccharides. The obtained supernatant mixture containing the CPS and the core oligosaccharides of the LOS was purified on a Biogel P-10 chromatography column, using pyridinium acetate buffer as eluent. Two fractions were obtained, the first of which, eluted in the void volume, contained the higher molecular weight material. The last was further purified on Sephacryl S-400HR chromatography column, using ammonium hydrogen carbonate as eluent, obtaining a purer fraction of the CPS (Fig. 3).

The CPS was deeply investigated by chemical analysis and NMR spectroscopy. Sugar analysis of the pure compound showed the presence of only D-glucose; the sugar residue 5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid (Pse5Ac7Ac) was identified through the NMR data (see below).

NMR analysis

The assignment of ^{1}H and ^{13}C resonances was obtained from the study of $^{1}H,^{1}H$ and $^{1}H,^{13}C$ two-dimensional NMR experiments COSY, TOCSY, ROESY, DEPT-HSQC

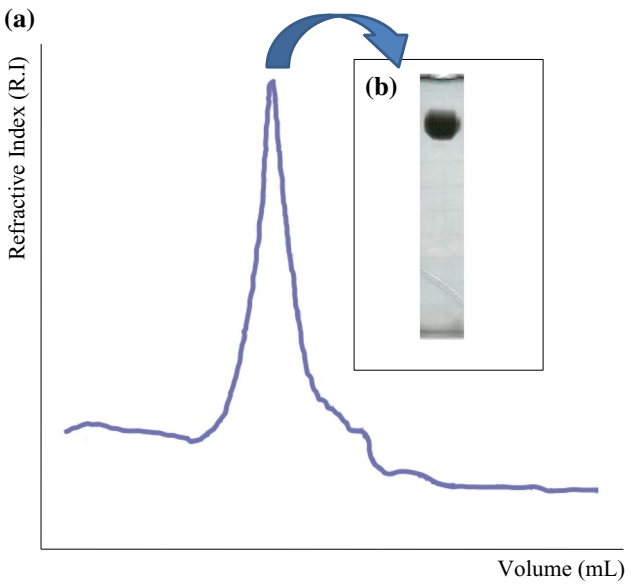


Fig. 3 **a** Elution profile and **b** 14% DOC-PAGE analysis of the purified CPS from *P.arcticus*

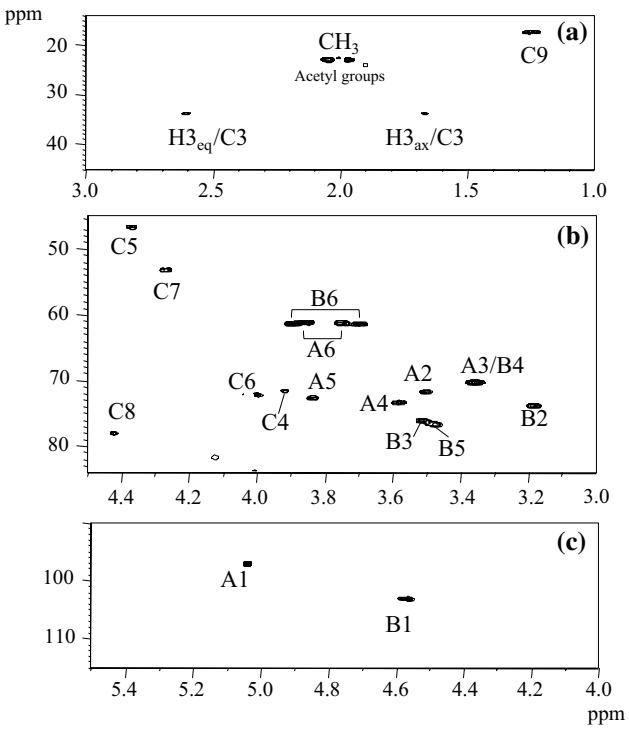


Fig. 4 $^{1}H–^{13}C$ DEPT-HSQC of *P. arcticus* CPS in D_2O at 600 MHz at 298 K: **a** $–CH_3$ methyl, $–CH_3$ acetyl, and $–CH_2$-region; **b** –CHOH and–CHNH-region; **c** anomeric region

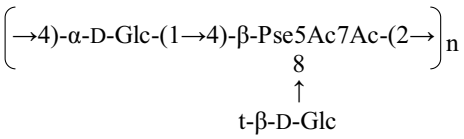
(Fig. 4), and HMBC. The ^{1}H NMR spectrum of the CPS revealed two signals in the region of anomeric protons. The anomeric resonances of residue A at δ 5.05/97.5 ppm and B at δ 4.58/103.4 ppm (Fig. 4) suggested an α-linked residue for A, and a β-linked residue for B. The $^{1}H,^{1}H$ correlations in COSY and TOCSY experiments, together with the ^{13}C chemical shifts, supported the presence of gluco-configured residues. Furthermore, inter-residue $^{1}H,^{1}H$ NOEs were observed from H1 of B to its H3 and H5 resonances. The ^{13}C chemical shifts for residue B revealed a terminal non-reducing Glc. The small glycosylation shifts observed for the C4 resonances (Table 1) of residue A suggested a substitution at this position with a keto sugar (Bock et al. 1994).

NMR spectroscopy indicated that the CPS also includes a derivative of 5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid (*N,N*-diacetyl-pseudaminic acid, Pse5Ac7Ac) in the repeating unit. Indeed, the Pse5Ac7Ac proton and carbon chemical shifts (residue C) were identified starting from the diastereotopic protons H-3ax and H-3eq at δ 1.68 and 2.62 ppm, respectively. The relatively large $J_{3a,3e}$ ~ 14 Hz and $J_{3a,4}$ ~ 13 Hz values, indicated that the OH-4 occupies the equatorial position (Tsvetkov et al. 2001). Furthermore, in the HMBC spectrum these protons showed a cross peak correlation with the carbon at δ 101.8 ppm, thus confirming the presence of 3-deoxy-2-ulosonic acid. The coupling constant values of this residue

Table 1 ^{1}H and ^{13}C NMR assignments of the repeating unit of the CPS from *P. arcticus* strain 273-4. The spectra were recorded at 298 K at 600 MHz

Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-7 C-7	H-8 C-8	H-9 C-9
A	5.05	3.52	3.37	3.59	3.85	3.76,3.85			
4-*α*-D-Glc	97.5	72.1	70.8	73.8	73.2	61.7			
B	4.58	3.20	3.52	3.36	3.49	3.74,3.90			
t-*β*-D-Glc	103.4	74.2	76.6	70.8	77.0	61.9			
C	172.6	101.8	1.68_{ax},2.62_{eq}	3.92	4.38	4.02	4.28	4.43	1.29
4,8-*β*-Pse			33.8	72.0	47.1	72.4	53.6	78.5	16.0

Chemical shifts for NAc: 2.04/23.1 (CH_3), 173.6 (CO) 1.97/22.9 (CH_3), 174.2 (CO)



Scheme 1 Trisaccharide repeating unit of the CPS isolated from *P. arcticus* 273-4

together with its carbon chemical shifts were similar to those reported for pseudaminic acid already published (Tsvetkov et al. 2001; Perepelov et al. 2005), thus confirming the presence of this residue.

Finally, the difference of 0.94 ppm between the $H\text{-}3_{ax}$ and $H\text{-}3_{eq}$ values, together with the chemical shift of its C-6 allowed to establish a β-configuration for the Pse5Ac7Ac (Tsvetkov et al. 2001; Knirel et al. 2003).

^{1}H,^{13}C-13 DEPT-HSQC (Fig. 4) spectrum showed that the H-5 and H-7 signals at δ 4.38 and 4.28 ppm correlated with nitrogen-bearing carbons at δ 47.1 and 53.6, respectively. The H-5 and H-7 downfield chemical shifts suggested the presence of acetyl groups on their corresponding carbons.

The determination of the sequence of the sugar residues in the repeating unit was based on the HMBC experiment. In this experiment, cross peaks between H-1 of A and C-4 of C, H-1 of B and C-8 of C. Since the C-2 of C showed only intra-molecular correlations with H3eq and H3ax, the linkage between residue C and A was deduced on the basis of the glycosylation shift of the C-4 of residue A.

All the above-described data allowed us to establish the repeating unit of the CPS from *P. arcticus* (Scheme 1).

Thermal hysteresis and ice recrystallization inhibition activity

Initially, the structure of the capsular polysaccharide produced by *P. arcticus* attracted our attention for its possible interaction with ice, as already found for other polysaccharides produced by cold-adapted bacteria (Carillo et al. 2015; Casillo et al. 2017a, b). To verify this, thermal hysteresis (freezing point depression) measurements and ice recrystallization inhibition (IRI) activity assays were performed. Ice crystals formed in the presence of 10 mg/mL of CPS formed round discs, similar to those formed in buffer alone, and did not show any facets or crystal shaping typical of antifreeze proteins (Fig. 5a–c). In addition, this disc would grow or shrink with slight changes (±0.002 °C) in temperature, indicating a lack of thermal hysteresis activity.

The IRI activity of the CPS from *P. arcticus* was also tested and compared with the activity of a fish antifreeze protein, used as positive control (Fig. 5d–i). High concentrations (10 mM) of CPS did not show any IRI as recrystallization was comparable to the buffer control. In contrast, antifreeze protein was effective at a concentration three orders of magnitude lower than that of CPS.

Conclusions

In this paper, the structure of the capsular polysaccharide isolated from *Psychrobacter arcticus* 273-4 has been elucidated and reported. The polysaccharide displays a trisaccharide repeating unit, containing two glucose residues and a pseudaminic acid. The pseudaminic acid residue was isolated and characterized for the first time in the 1985, from the O-chain of the LPS of *Pseudomonas aeruginosa* O7 and O9 and *Shigella boydii* type 7 (Knirel et al. 1985). To date, the presence of this sugar is reported for several O-antigens, capsular and extracellular polysaccharides (Perepelov et al. 2005; Senchenkova et al. 2014, 2015; Kaszowska et al. 2016; Lee et al. 2018), mainly from pathogenic Gram-negative bacteria, suggesting a potential role in host–bacterial interactions (Zunk and Kiefel 2014). At the best of our knowledge, this is the first time that this unusual sugar is found as a component of CPS from a cold-adapted microorganism.

Our finding of the crucial role that capsular and medium release polysaccharides play in the cold adaptation mechanisms of psychrophiles has driven the search for new polysaccharide molecules. Unlike the finding for the CPS and EPS isolated from *Colwellia psychrerythraea* 34H, the thermal hysteresis and IRI assays displayed no activity for the *P.*

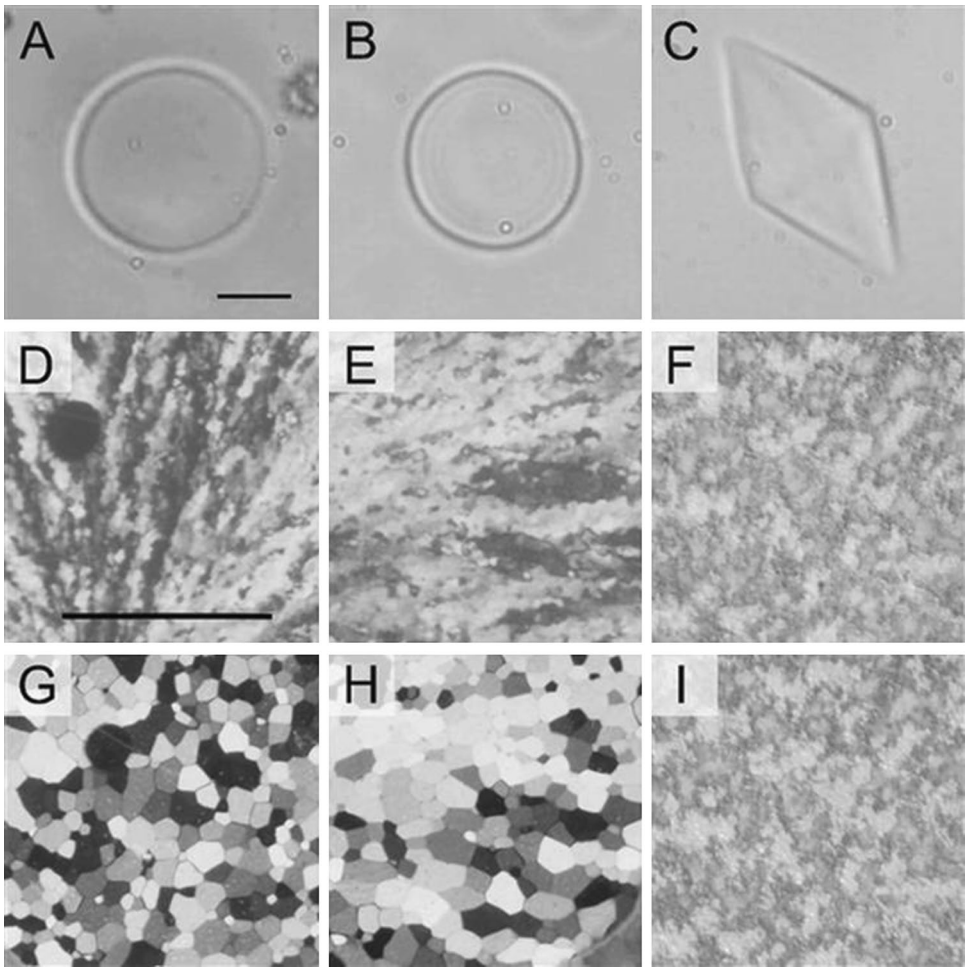


Fig. 5 Ice crystals formed in the presence of 10 mM CPS from *P. arcticus* (**a**, **d**, **g**), buffer (**b**, **e**, **h**) or positive control (**c**, **f**, **i**). The shapes of the ice crystals formed during thermal hysteresis measurements are shown in **a**–**c** with the scale bar representing 10 µm. **d**–**i** Show ice recrystallization (or lack thereof) in a flash-frozen layer of ice at time zero (**d**–**f**) and after 21 h incubation at − 6 °C (**g**–**i**). The scale bar in **d** represents 1 mm. The positive control was type III antifreeze protein, at a concentration of ~ 1 mM (**c**) or 1 µM (**f**, **i**)

arcticus CPS. The lack of activity could be attributed to the absence of the amino acids decorating the active molecules, and the absence of galacto-configured sugars, a feature that could negatively affect the IRI activity. Furthermore, the CPS from *P. arcticus* could display a protective function, since it is exposed to higher temperature fluctuations than microorganisms living in the sea-ice.

Acknowledgements We thank Prof. Peter L. Davies of the Department of Biomedical and Molecular Sciences (Queen's University) for the thermal hysteresis and ice recrystallization inhibition assays. We also thank Dr. Giovanna Benvenuto, Electron Microscopy Facility, Stazione Zoologica Anton Dohrn for TEM experiments.

References

Ayala-del-Rio HL, Chain PS et al (2010) The genome sequence of *Psychrobacter arcticus* 273–4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. Appl Environ Microbiol 76:2304–2312. https://doi.org/10.1128/AEM.02101-09

Bakermans C, Nealson KH (2004) Relationship of critical temperature to macromolecular synthesis and growth yield in *Psychrobacter cryopegella*. J Bacteriol 186:2340–2345. https://doi.org/10.1128/JB.186.8.2340-2345.2004

Bejar V, Calvo C, Moliz J et al (1996) Effect of growth conditions on the rheological properties and chemical composition of *Volcaniella eurihalina* exopolysaccharide. Appl Biochem Biotechnol 59:77. https://doi.org/10.1007/BF02787859

Bock K, Vinogradov E, Holt O et al (1994) Isolation and structural analysis of oligosaccharide phosphates containing the complete carbohydrate chain of the lipopolysaccharide from Vibrio cholerae Strain H11 (Non-O1). Eur J Biochem 225(3):1029–1039. https://doi.org/10.1111/j.1432-1033.1994.1029b.x

Braslavsky I, Drori R (2013) LabVIEW-operated novel nanoliter osmometer for ice binding protein investigations. J Vis Exp 72:e4189. https://doi.org/10.3791/4189

Carillo S, Casillo A, Pieretti G et al (2015) A unique capsular polysaccharide structure from the psychrophilic marine bacterium *Colwellia psychrerythraea* 34H that mimicks antifreeze (glyco) proteins. J Am Chem Soc 137(1):179–189. https://doi.org/10.1021/ja5075954

Caruso C, Rizzo C, Mangano S et al (2018) Extracellular polymeric substances with metal adsorption capacity produced by *Pseudoalteromonas* sp. MER144 from Antarctic seawater. Environ Sci Pollut Res 25:4667. https://doi.org/10.1007/s11356-017-0851-z

Caruso C, Rizzo C, Mangano S et al (2019) Isolation, characterization and optimization of EPSs produced by a cold-adapted *Marinobacter* isolate from Antarctic seawater. Antarct Sci 31(2):69–79. https://doi.org/10.1017/S0954102018000482

Casillo A, Parrilli E, Sannino F et al (2015) Structural investigation of the oligosaccharide portion isolated from the lipooligosaccharide of the permafrost psychrophile *Psychrobacter arcticus* 273-4. Mar Drugs 13:4539–4555. https://doi.org/10.3390/md13074539

Casillo A, Parrilli E, Sannino F et al (2017a) Structure-activity relationship of the exopolysaccharide from a psychrophilic bacterium: a strategy for cryoprotection. Carbohydr Polym 156:364–371. https://doi.org/10.1016/j.carbpol.2016.09.037

Casillo A, Ståhle J, Parrilli E et al (2017b) Structural characterization of an all-aminosugar-containing capsular polysaccharide from *Colwellia psychrerythraea* 34H. AvL 110:1377–1387. https://doi.org/10.1007/s10482-017-0834-6

Casillo A, Lanzetta R, Parrilli M, Corsaro MM (2018a) Exopolysaccharides from marine and marine extremophilic bacteria: structures, properties, ecological roles and applications. Mar Drugs 16:69–103. https://doi.org/10.3390/md16020069

Casillo A, Ziaco M, Lindner B et al (2018b) Lipid A structural characterization from LPS of the Siberian psychrotolerant *Psychrobacter arcticus* grown at low temperatures. Extremophiles 6:955–963. https://doi.org/10.1007/s00792-018-1051-6

Corbett D, Roberts IS (2008) Capsular polysaccharides in *Escherichia coli*. Adv Appl Microbiol 65:1–26

Escalera L, Benvenuto G, Scalco E et al (2014) Ultrastructural features of the benthic dinoflagellate *Ostreopsis* cf. ovata (Dinophyceae). Protist 165(3):260–274. https://doi.org/10.1016/j.protis.2014.03.001

Ewert M, Deming JW (2013) Sea ice microorganisms: environmental constraints and extracellular response. Biology 2:603–628. https://doi.org/10.3390/biology2020603

Galanos C, Lüderitz O, Westphal O (1969) New method for the extraction of R lipopolysaccharides. Eur J Biochem 9(245–249):39. https://doi.org/10.1111/j.1432-1033.1969.tb00601.x

Graham LA, Agrawal P, Oleschuk RD, Davies PL (2018) High-capacity ice-recrystallization endpoint assay employing superhydrophobic coatings that is equivalent to the 'splat' assay. Cryobiology 81:138–144. https://doi.org/10.1016/j.cryobiol.2018.01.011

Kaszowska M, Stojkovic K, Niedziela T, Lugowski C (2016) The O-antigen of *Plesiomonas shigelloides* serotype O36 containing pseudaminic acid. Carbohydr Res 434(3):1–5. https://doi.org/10.1016/j.carres.2016.07.007

Knirel YE, Vinogradov EV, Shashkov AS (1985) Identification of *S* acetamido-3,5,7,9-tetradeoxy-7-[(*R*)-3-hydroxybutyr-amido]-L-glycero-L-manno-nonulosonic acid as a component of bacterial polysaccharides. Carbohydr Res 141:1–3

Knirel YA, Shashkov AS, Tsvetkov YE et al (2003) 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids in bacterial glycopolimers:chemistry and biochemistry. Adv Carbohydr Chem Biochem 58:371–417

Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. PNAS USA 108:3653–3658. https://doi.org/10.1073/pnas.1100701108

Laemmli UK (1970) Most commonly used discontinuous buffer system for SDS electrophoresis. Nature 227:680–685

Lee HK, Chun J, Moon EY et al (2001) *Hahella chejuensis* gen. nov., sp. nov., an extracellular-polysaccharide-producing marine bacterium. Int J Syst Evol Microbiol 51:661–666. https://doi.org/10.1099/00207713-51-2-661

Lee IM, Yang FL, Chen TE et al (2018) Pseudaminic acid on exopolysaccharide of *Acinetobacter baumannii* plays a critical role in phage-assisted preparation of glycoconjugate vaccine with high antigenicity. J Am Chem Soc 140(28):8639–8643. https://doi.org/10.1021/jacs.8b04078

Leontein K, Lindberg B, LoÈnngren L (1978) Assignment of absolute configuration of sugars by g.l.c. of their acetylated glycosides from chiral alcohols. Carbohydr Res 62:359–362

Martínez-Checa F, Toledo FL, El Mabrouki K et al (2007) Characteristics of bioemulsifier V2–7 synthesized in culture media added of hydrocarbons: chemical composition, emulsifying activity and rheological properties. Bioresour Technol 98:3130–3135. https://doi.org/10.1016/j.biortech.2006.10.026

Perepelov AV, Shashkov AS, Torgov VI et al (2005) Structure of an acidic polysaccharide from the agar-decomposing marine bacterium *Pseudoalteromonas atlantica* strain IAM 14165 containing 5,7-diacetamido-3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid. Carbohydr Res 340(1):69–74. https://doi.org/10.1016/j.carres.2004.11.007

Ponder MA, Thomashow MF, Tiedje JM et al (2008) Metabolic activity of Siberian permafrost isolates, *Psychrobacter arcticus* and *Exiguobacterium sibiricum*, at low water activities. Extremophiles 12(4):481–490. https://doi.org/10.1007/s00792-008-0151-0

Ricciardelli A, Casillo A, Vergara A et al (2019) Environmental conditions shape the biofilm of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC 125. Microbiol Res 218:66–75. https://doi.org/10.1016/j.micres.2018.09.010

Roca C, Lehmann M, Torres CAV et al (2016) Exopolysaccharide production by a marine *Pseudoalteromonas* sp. strain isolated from Madeira Archipelago ocean sediments. New Biotechnol 33:460–466. https://doi.org/10.1016/j.nbt.2016.02.005

Senchenkova SN, Shashkov AS, Shneider MM et al (2014) Structure of the capsular polysaccharide of *Acinetobacter baumannii* ACICU containing di-*N*-acetylpseudaminic acid. Carbohydr Res 391(4):89–92. https://doi.org/10.1016/j.carres.2014.04.002

Senchenkova SN, Popova AV, Shashkov AS et al (2015) Structure of a new pseudaminic acid-containing capsular polysaccharide of *Acinetobacter baumannii* LUH5550 having the KL42 capsule biosynthesis locus. Carbohydr Res 407(30):154–157. https://doi.org/10.1016/j.carres.2015.02.006

Stevens CA, Drori R, Zalis S et al (2015) Dendrimer-linked antifreeze proteins have superior activity and thermal recovery. Bioconjugate Chem 26(9):1908–1915. https://doi.org/10.1021/acs.bioconjchem.5b00290

Sun ML, Zhao F, Shi M et al (2015) Characterization and biotechnological potential analysis of a new exopolysaccharide from the Arctic marine bacterium *Polaribacter* sp. SM1127. Sci Rep 5:18435. https://doi.org/10.1038/srep18435

Sutherland IW (1972) The exopolysaccharides of *Klebsiella* serotype 2 strains as substrates for phage-induced polysaccharide depolymerases. J Gen Microbiol 70:331–338. https://doi.org/10.1099/00221287-70-2-331

Tsai CM, Frasch CE (1982) A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal Biochem 119:115–119

Tsvetkov YE, Shashkov AS, Knirel YA (2001) Synthesis and NMR spectroscopy of nine stereoisomeric 5,7-diacetamido-3, 5, 7, 9-tetradeoxynon-2-ulosonic acids. Carbohydr Res 335(4):221–243. https://doi.org/10.1016/S0008-6215(01)00235-X

Westphal O, Jann K (1965) Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure. Methods Carbohydr Chem 5:83–91

Yim JH, Kim SJ, Aan SH, Lee HK (2004) Physicochemical and rheological properties of a novel emulsifier, EPS-R, produced by the marine bacterium *Hahella chejuensis*. Biotechnol Bioprocess Eng 9:405. https://doi.org/10.1007/BF02933066

Zhang Z, Cai R, Zhang W et al (2010) A novel exopolysaccharide with metal adsorption capacity produced by a marine bacterium *Alteromonas* sp. JL2810. Mar Drugs 15:175. https://doi.org/10.3390/md15060175

Zhang Z, Chen Y, Wang R et al (2015) The fate of marine bacterial exopolysaccharide in natural marine microbial communities. PLoS One 10:e0142690. https://doi.org/10.1371/journal.pone.0142690

Zunk M, Kiefel MJ (2014) The occurrence and biological significance of the a-keto-sugars pseudaminic acid and legionaminic acid within pathogenic bacteria. RSC Adv 4:3413–3421. https://doi.org/10.1039/c3ra44924f