Endemic and cosmopolitan bacteria from microbial mats in maritime Antarctica: Antagonism and antibiotic resistance

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Abstract

This is the first study to demonstrate a relationship between antagonistic interactions and antibiotic resistance among bacterial species with different biogeographical ranges, found in freshwater microbial mats in Antarctica. The isolated strains originated from ephemeral freshwater bodies of the periglacial zone of the Ecology Glacier (King George Island, maritime Antarctica). Among the 47 isolates, species with different biogeographical ranges were identified: 22 strains belonging to 12 endemic (EN) bacterial species, and 25 strains belonging to 16 cosmopolitan (CO) species. All the cosmopolitan strains and 82% of the endemic strains produced antimicrobial compounds. The cosmopolitan strains were twice as active antagonistically in relation to the endemic strains, and a reverse relationship was noted in the sensitivity of the cosmopolitan species to antagonism. The endemic strains were twice as sensitive to antagonism as compared to the cosmopolitan strains. The bacterial strains derived from microbial mats exhibited broad resistance to all the antibiotics applied. Most isolates (94%) were resistant to at least one of the 25 used antibiotics. The CO and EN species were characterised by a different spectrum of antibiotic resistance. Among others, strains from the CO

group exhibited greater, statistically significant resistance to antibiotics inhibiting protein synthesis ($p<0.05$). More than half of all the isolates were multidrug-resistant (MDR) strains. Statistically, cosmopolitan MDR strains showed broader resistance ($p>0.05$). A relationship was demonstrated between the antagonistic potential and sensitivity to antagonistic interactions, and antibiotic resistance in cosmopolitan bacterial species. The individual patterns of antagonistic interactions and antibiotic resistance observed among isolates showed a clear relationship with the biogeographical ranges of individual species. Strains of cosmopolitan species characterised by broad antagonism and resistance to antibiotics were identified. These specific "biological contaminants" may be the source and propagator of new traits in endemic populations of polar mat microbiocenoses.

Keywords: Antarctica; antagonistic activity; antibiotic resistance; bacteria; biogeography; microbial mats

Introduction

Antarctica is the most isolated area of the Earth's cryosphere. Glacier ice of continental and maritime Antarctica holds approximately 70% of the freshwater on Earth (Pan et al. 2022). The low availability of water in liquid form in terrestrial areas is a key factor influencing the development of microbiocenoses. In addition, polar areas are characterised by extreme environmental factors, including low temperatures, high UV radiation intensity, low nutrient abundance, and pressure associated with frequent freezing and thawing (Králová et al. 2021). The selectivity of these factors only enables the development of microorganisms with high adaptive capacities. The biodiversity of prokaryotic microbiota developing in polar ecosystems is currently a leading research trend (Kleinteich at al. 2017).

Microbial mats in polar environments are widespread and dominant in the total biomass of terrestrial microbiocenoses (Quesada et al. 2009; Peeters et al. 2012; Jungblut et al. 2016). These

are ecosystems with a complex biocenotic structure consisting mainly of autotrophic and heterotrophic microorganisms belonging to: *Cyanobacteria*, *Chloroflexi*, *Pseudomonadota*, *Actinomycetota*, *Bacteroidota*, *Bacillota* and *Archea* (Prieto-Barajas et al. 2018; Valdespino-Castillo et al. 2018). A characteristic feature of these ecosystems is their self-sufficiency and autonomy, related to the integration of primary and secondary production processes and the regeneration of biogenic compounds (Gomez-Saez 2017).

Among the basic phenomena in microbial conglomerates, there are antagonistic reactions occurring between biotic components (Aguirre-von-Wobeser 2015; Núñez-Montero & Barrientos 2018). The occurrence of both positive and negative interactions on various taxonomic and functional levels is crucial in the microbial mat homeostasis (Long et al. 2013; Slattery & Lesser 2017). Active competition between aggregated microorganisms is determined by different mechanisms, most commonly through the production of bacteriostatic and bactericidal compounds, e.g. toxins, bacteriocins and antibiotics (Clardy et al. 2009; Cotter et al. 2013; Mullis et al. 2019; Peterson et al. 2020). Naturally developing resistance to these compounds determines the dynamic balance of the microbiocenosis. A new phenomenon in polar ecosystems is the acquisition of resistance induced by horizontal transfer of resistance genes from allochthonous microorganisms, and through contact with commercially available antibiotics, which is a consequence of anthropogenic environmental contaminants (Kraemer et al. 2019; Hwengwere et al. 2022; Larsson & Flach 2022). These phenomena are leading to the formation of an extensive antibiotic resistome among polar bacteria, with the strain pool formed serving as a reservoir of resistance genes in the global microbiome (Van Goethem et al. 2018; Morozova et al. 2022; Depta & Niedźwiedzka-Rystwej 2023).

The ongoing discussion on the biogeography of microorganisms and their occurrence in different environments is focused on two aspects: species endemism and cosmopolitism (Walther

et al. 2002; Martiny et al. 2006; Fontaneto et al. 2013). Baas Becking's (1934) classic concept that "everything is everywhere, but the environment selects" is the starting point for a discussion about biodiversity, the origin and formation of microbial consortia. Two aspects are emphasised: the universal nature of microbial cosmopolitism and the role of environmental factors in the selection of prokaryotic strains. The opposing concept assumes the endemicity of microorganisms in isolated environments (Jungblut et al. 2010; Hahn et al. 2015). In the microbiocenoses of circumpolar environments, the unique properties of polar microorganisms, i.e. those originating from frozen and/or periodically freezing ecosystems, are highlighted (Hahn et al. 2015). In an endemic model, the isolation of ecosystems is conducive to the formation of specific ecotaxonomic patterns of autochthonous microorganisms characterised by a limited biogeographical range (Martiny et al. 2006; Nemergut et al. 2011; Fontaneto et al. 2013). Adaptation is indicated as the main mechanism of microbiocenosis formation in extreme polar environments (Kleinteich et al. 2017; Zhang et al. 2021). It is currently assumed that both endemism and cosmopolitanism of polar prokaryotes are traits typical of heterogeneous microbial consortia (Ragon et al. 2012; Jadoon et al. 2013). Global climate change is leading to the weakening and shifting of biogeographical boundaries, which enables the expansion of cosmopolitan prokaryotes into the previously isolated extreme environments (Walther et al. 2002; Kleinteich et al. 2017; Bargagli et al. 2024; Zucconi et al. 2025). As demonstrated by Peeters et al. (2012), over 60% of cosmopolitan microorganism species are found in Antarctic microbial mats. The development of molecular techniques has changed the perception of the biogeographical diversity of microorganisms (Peeters et al. 2012, Kleinteich et al. 2017). Genome plasticity is an important indicator of a species' ability to adapt to extreme environmental conditions (Zhang et al. 2021). The overlapping of the biogeographical ranges of endemic and cosmopolitan species increases the gene pool of the microbial consortia being

jointly formed. Horizontal gene transfer enables the spread of genes responsible for the adaptation to extreme conditions as well as the expansion of metabolic and physiological properties (Vincent 2000; Kleinteich et al. 2017). Contemporary research on microbiocenoses of polar environments lacks reports concerning the biogeography and occurrence of cosmopolitan and endemic bacterial species. This is particularly important in the context of intensifying Global Climate Change and the opening up of the previously isolated environments to colonisation. The expansion of species alien to the polar environment interferes with stable microbiocenotic systems and adversely affects endemic prokaryotic populations (Hughes et al. 2011; Leihy et al. 2023; Zucconi et al. 2025). This may be manifested, e.g., by adverse changes in the structure of the polar microbiome through the reduction in endemic populations and changes in genomic, metabolic and physiological properties (Pessi et al. 2018; Zhang et al. 2021; Abás, et al. 2022).

This paper presents the results of a study into Antarctic strains isolated from freshwater polar microbial mats found in ephemeral waterbodies of the proglacial zone of the Ecology Glacier on the King George Island in maritime Antarctica. The study investigated the resistance of isolates to antibiotics from different functional groups and tested the antagonistic properties of the strains in a cross-inhibition reaction. A hypothesis was formulated that the antagonistic interactions between strains are related to their spectrum of antibiotic resistance, with the nature of these interactions being determined by the biogeographical range of the species.

Materials and Methods

Study site

The study was carried out in the vicinity of Ecology Glacier, situated near the Arctowski Polish Antarctic Station at the western shore of Admiralty Bay, on King George Island, South Shetland Archipelago, Maritime Antarctica. It is subjected to annual surface snow melt like other glaciers in the vicinity (Braun & Gossmann, 2002). Samples were taken during the Antarctic summer

(March/April) of 2019 at twelve sites. Detailed description of research sites, environmental parameters and physicochemical conditions are presented in Górniak et al. (2025).

Research material

A collection of bacterial strains was obtained as part of a large study into the diversity of bacteria in microbial mats from the periglacial zone of the Ecology Glacier. The mats collected for research were also the subject of other analyses, including metagenomic, functional and phycological analyses (Zębek et al. 2021; Górniak et al. 2025). An example of the macroscopic and microscopic (cross-section) structure of the studied microbial mats is shown in Figure 1.

Figure 1. Macroscopic and microscopic (vertical cross section) structure of the microbial mat of the periglacial zone of Ecology Glacier (an example)

Microbial mats sampling and isolation of bacterial strains was detailed described in Górniak et al. (2025). For bacterial taxonomic identification of studied strains the16S rRNA gene-sequencing-based procedure was provided. In the current study, antibiotic resistance and antagonistic interactions of 47 isolates from freshwater microbial mats were examined.

Antibiotic resistance

The disc diffusion method was applied using R2A as a replacement for Mueller-Hinton agar to determine the phenotypic antibiotic resistance of bacterial strains originating from microbial mats (Bauer et al. 1966). The 25 antibiotics with different modes of action, belonging to 12 functional groups, were used (Antimicrobial Susceptibility Discs, Oxoid) (Table 1). The plates were

incubated at 20 ºC for 48-96 hours, depending on the growth rate of strains. Antibiotic resistance was considered to be the absence of a zone of inhibition of the strain's growth around the antibiotic disk. The antibiotic resistance index (ARI) was determined according to Krumperman et al. (1983). Multi-drug resistance (MDR) was determined according to resistance to at least one of each mode of action of antibiotics used (Exner et al. 2017).

Antagonistic interactions

The strains were screened for antimicrobial substance production using the spot-on-lawn method described by Prasad et al. (2011). Each strain was tested against the other strains for cross-inhibition. The cells were washed out from 1 mL of liquid culture of each strain by centrifuging three times (10 min/8000 rpm), each time suspending the cell pellet in sterile physiological saline at 20 °C. The cell pellet was then adjusted to a density of 0.5 McFarland and plated on the R2A medium, treating the inoculated strain as an indicator strain for examining the relationship with other strains. Following this, 10 μL of liquid culture of each of the remaining strains was applied pointwise. The culture was carried out at 20 °C for 7 days. After this time, the formation of a growth inhibition zone of the indicator strain around the spotted strains was considered an antagonistic effect.

Statistics

All data were statistically analysed using Statistica version 13.3 (StatSoft Inc.). The assessment of the significance of differences in the data obtained used a multivariate analysis of variance (ANOVA), and for data that failed to meet the normality test, a non-parametric Kruskal-Wallis test was applied. Linear and nonlinear regression analysis was used to investigate correlational relationships. The antagonistic relationships associated with antibiotic resistance were illustrated in network graphs using the program Cytoscape 3.1.0 (Shannon et al. 2003), and the data were processed in the R Package's in-house scripts.

Table 1. Characteristics of the antibiotics used in the study

Antibiotic	Symbol	Functional group	Mode of action (inhibitors of synthesis)	Disk potency (μg)
Ampicillin	AM	β-lactams	cell-wall	2
Carbenicillin	PY	β-lactams	cell-wall	10
Cefixime	CFM	Modified β-lactams/3rd generation cephalosporine	cell-wall	100
Cefotaxime	CTX	Modified β-lactams/3rd generation cephalosporine	cell-wall	5
Ceftazidime	CAZ	Modified β-lactams/3rd generation cephalosporine	cell-wall	5
Imipenem	IMP	Modified β-lactams/Carbapenem	cell-wall	2
Vancomycin	VA	Glycopeptide	cell-wall	30
Gentamicin	CN	Aminoglycosides	protein	15
Kanamycin	K	Aminoglycosides	protein	15
Streptomycin	S	Aminoglycosides	protein	30
Chloramphenicol	C	Chloramphenicol/ Aminoglycosides	protein	10
Tetracycline	TE	Tetracycline/Polyketide	protein	1.25
Clarithromycin	CLR	Macrolide	protein	10
Erythromycin	E	Macrolide	protein	5
Clindamycin	DA	Lincosamide	protein	15
Lincomycin	L	Lincosamide	protein	30
Nitrofurantoin	F	Nitrofuran Inhibitor of folic acid synthesis	DNA/RNA	5
Novobiocin	NV	Aminocoumarin	DNA/RNA	10
Ciprofloxacin	CIP	Quinolones	DNA/RNA	30
Nalidixic acid	NA	Quinolones	DNA/RNA	5
Metronidazole	MET	Metronidazole/Quinolones	DNA/RNA	100
Rifampicin	RA	Rifamycin	DNA/RNA	25
Trimethoprim	TMP	Trimethoprim	RNA	5
Mupirocin	MUP	Monocarboxylic acid	RNA	5
Cotrimoxazole	SXT	Trimethoprim/Sulfamethoxazole	RNA	5

Results

Characteristics of strains

Among the 47 isolates derived from microbial mats of freshwater bodies in the periglacial zone of the Ecology Glacier, the occurrence of 28 bacterial species belonging to 11 families was found

(Table 2). Twenty-six isolates were classified into 16 cosmopolitan (CO) species. Twenty-two isolates were classified into 12 species belonging to endemic (EN) bacteria.

Table 2. 16S rRNA gene sequence affiliation to the closest phylogenetic neighbours, isolate origin and Gen Bank Accession Number of the bacterial strains isolated from Antarctic freshwater microbial mats (CO – cosmopolitan species; EN – endemic species)

Family	Nearest taxonomic neighbour by BLAST	Biogeographic range	Collection number	Identity [%]	Gen Bank Accession Nr
Carnobacteriaceae	*Carnobacterium maltaromaticum* DSM 20342	CO	P10.M4	97.91	PQ517127
Comamonadaceae	*Rhodoferax ferrireducens* T118	CO	S3.M1	98.83	PQ517124
	Rhodoferax saidenbachensis ED16	CO	S33.M1	96.39	PQ517143
Flavobacteriaceae	*Flavobacterium hydatis* DSM 2063	CO	P45.M8	99.66	OR739452
	Flavobacterium hydatis DSM 2063	CO	P48.M8	99.68	OR739455
	Flavobacterium hydatis DSM 2063	CO	S56.M12	99.76	OR739460
	Flavobacterium hydatis DSM 2063	CO	S57.M12	99.68	OR739461
	Flavobacterium hydatis DSM 2063	CO	S59.M12	99.41	OR739463
	Flavobacterium antarcticum DSM 19726	EN	P5.M4	98.76	OR739435
	Flavobacterium antarcticum DSM 19726	EN	P7.M4	99.1	OR739436
	Flavobacterium antarcticum DSM 19726	EN	P8.M4	99.03	OR739437
	Flavobacterium xanthum NBRC 14972	EN	P14.M8	98.96	OR739440
	Flavobacterium xanthum NBRC 14972	EN	P15.M8	100.00	OR739441
	Flavobacterium xanthum NBRC 14972	EN	S17.M12	99.38	OR739442
	Flavobacterium xanthum NBRC 14972	EN	P39.M4	100.00	OR739448
Microbacteriaceae	*Agreia pratensis* P 229/10	CO	P75.M11	99.68	PQ517155
	Cryobacterium breve TMT4-23	EN	P11.M5	99.8	PQ517128
	Marisediminicola antarctica ZS314	EN	S32.M1	98.97	PQ517142
	Salinibacterium xinjiangense 0543	EN	S1.M1	99.11	PQ517122
	Salinibacterium xinjiangense 0543	EN	P28.M4	99.75	PQ517138
Micrococcaceae	*Arthrobacter methylotrophs* GP3	CO	S67.M9	98.62	PQ517152
	Pseudoarthrobacter oxydans DSM20119	CO	S83.M8	99.22	PQ517158
	Arthrobacter psychrochitiniphilus GP	EN	S18.M9	99.90	PQ517130
	Pseudarthrobacter psychrotolerans YJ56	EN	S24.M1	99.39	PQ517134
Neisseriaceae	*Iodobacter limnosediminis* E1	CO	S22.M1	99.28	PQ517132
	Iodobacter limnosediminis E1	CO	P37.M2	99.39	PQ517144
	Iodobacter limnosediminis E1	CO	P38.M2	99.28	PQ517145
	Iodobacter limnosediminis E1	CO	S73.M9	99.29	PQ517154
	Iodobacter arcticus AsdM4-16	EN	S2.M1	99.16	PQ517123
	Iodobacter arcticus AsdM4-16	EN	P6.M4	99.48	PQ517125
	Iodobacter arcticus AsdM4-16	EN	P16.M8	99.63	PQ517129
	Iodobacter arcticus AsdM4-16	EN	P27.M4	99.56	PQ517137

Nocardiaceae	*Rhodococcus qingshengii* JCM 15477	CO	P26.M4	99.79	PQ517136
	Rhodococcus yunnanensis YIM 70056	CO	P9.M4	99.09	PQ517126
Oxalobacteraceae	*Janthinobacterium lividum* DSM 1522	CO	S29.M9	99.28	PQ517139
	Janthinobacterium lividum DSM 1522	CO	P41.M4	99.63	PQ517146
	Janthinobacterium svalbardensis JA-1	EN	P53.M6	99.12	PQ517149
	Rugamonas violacea P5900	EN	P64.M11	96.09	PQ517150
Pseudomonadaceae	*Pseudomonas caspiana* FBF102	CO	S76.M10	98.82	PQ517156
	Pseudomonas mandelii NBRC 103147	CO	P52.M7	99.90	PQ517148
	Pseudomonas antarctica CMS 35	EN	S30.M8	99.52	PQ517140
	Pseudomonas antarctica CMS 35	EN	S82.M8	99.75	PQ517157
Sphingobacteriaceae	*Pedobacter alluvionis* NWER-II11	CO	S66.M9	99.3	PQ517151
	Pedobacter fastidiosus P8930	EN	S21.M8	99.56	PQ517131
	Sphingomonas faeni MA-olki	CO	S69.M9	98.51	PQ517153
Xanthomonadaceae	*Stenotrophomonas rhizophila* e-p10	CO	S23.M1	99.88	PQ517133
	Stenotrophomonas rhizophila e-p10	CO	S25.M1	99.35	PQ517135

Antibiotic resistance

The bacterial strains derived from microbial mats exhibited broad resistance to all the antibiotics applied (Fig. 3). Ninety-four percent of the isolates exhibited resistance to at least one of the 25 antibiotics. Resistance to 1-5 antibiotics and to 6-10 antibiotics was exhibited by 43% and 17% of the strains, respectively, while 34% of the isolates exhibited resistance to more than 11 antibiotics. Eighty-seven percent and 85% of isolates, respectively, were resistant to antibiotics from the group inhibiting the synthesis of the cell wall (CW) and nucleic acids (NA). More than half of the strains (60%) were resistant to antibiotics from the group inhibiting protein (P) synthesis. The strains classified into the species of the two distinguished biogeographical groups exhibited significantly different resistance to antibiotics (Fig. 2). Among the isolates classified as cosmopolitan species, 84%, 72% and 80% were resistant to at least one antibiotic from the CW, P and NA groups, respectively. In contrast, 91%, 46% and 82%, respectively, of strains classified as endemic species exhibited resistance to these antibiotic groups. In accordance with the adopted criterion, the MDR strains accounted for more than half of all isolates. A statistically significant difference was demonstrated in the MDR strain resistance between the CO and EN species

($p>0.005$). Among the CO isolates, the MDR strains accounted for 56% and were resistant, on average, to 13 antibiotics. However, among the EN isolates, the MDR strains accounted for 45% and were resistant, on average, to 9 antibiotics. Among the MDR strains classified into the CO group, the highest resistance was noted for *Agreia pratensis, Flavobacterium hydatis* and *Iodobacter limnosediminis,* which were resistant to 17 antibiotics. Among the isolates classified into the EN group, only two, *Pseudomonas antarctica* and *Marisediminicola antarctica*, exhibited high resistance to 15 and 14 antibiotics, respectively. More than half (59%) of isolates from the EN group exhibited low resistance to antibiotics (5 or fewer). Among all the strains under study, resistance to the applied antibiotics from the group inhibiting the cell wall (CW) synthesis, the greatest pool comprised the isolates resistant to ß-lactams: ampicillin (64%), 3rd generation cephalosporins – cefixime (57%) and ceftazidime (47%). Only a single strain, *Flavobacterium antarcticum*, was resistant to imipenem. Relatively low resistance to antibiotics inhibiting protein (P) synthesis was found. In total, 43% of isolates were resistant to lincomycin, 38% to erythromycin, 32% to clindamycin, and 23% to chloramphenicol. Among the strains under study, resistance to other antibiotics from this group was very low, not exceeding 4%. In the group of antibiotics inhibiting nucleic acid (NA) synthesis, 86% of strains derived from microbial mats exhibited resistance to at least one antibiotic, and 62% strains were resistant to mupirocin, 57% to metronidazole, and 55% to trimethoprim. Analysis of the results obtained for the isolated CO and EN strain groups showed significant differences in their resistance to antibiotics. In the group of antibiotics inhibiting protein synthesis, the CO strains exhibited markedly higher resistance to clarithromycin, erythromycin, clindamycin and lincomycin, while in the group inhibiting nucleic acid synthesis, to novobiocin, cotrimoxazole, mupirocin and nitrofurantoin. Resistance to antibiotics inhibiting the cell wall synthesis in the two groups of isolates was similar. The average value of the ARI index for the CO and EN strains was 0.35 and

0.24, respectively, with a statistically significantly higher resistance to antibiotics inhibiting protein synthesis in strains from the CO group ($p<0.05$). In the group of strains belonging to endemic species, the average resistance to antibiotics inhibiting protein synthesis was low at 0.12, and did not exceed the ARI threshold value of 0.2, set for multi-antibiotic-resistant strains (Fig. 3).

No.	Species	Collection number	ET	Cell wall inhibitors							Proteins synthesis inhibitors									Nucleid acid inhibitors									NAR	ARI	NA	NS	ITP
				AM	PY	CFM	CTX	CAZ	IMP	VA	CN	K	S	C	TE	CLR	E	DA	L	NV	NA	CIP	SXT	MUP	F	RA	TMP	MET					
1	*Agreia pratensis*	P75.M11	CO																										17	0.68	20	3	PRS
2	*Arthrobacter methylotrophus*	S67.M9	CO																										2	0.08	5	4	PRS
3	*Carnobacterium maltaromaticum*	P10.M4	CO																										2	0.08	5	12	PRS
4	*Flavobacterium hydatis*	P45.M8	CO																										8	0.32	2	0	PR
5	*Flavobacterium hydatis*	P48.M8	CO																										1	0.04	4	12	PRS
6	*Flavobacterium hydatis*	S56.M12	CO																										14	0.56	2	10	PRS
7	*Flavobacterium hydatis*	S57.M12	CO																										17	0.68	16	0	PR
8	*Flavobacterium hydatis*	S59.M12	CO																										12	0.48	17	0	PR
9	*Iodobacter limnosediminis*	S22.M1	CO																										2	0.08	5	9	PRS
10	*Iodobacter limnosediminis*	P37.M2	CO																										1	0.04	3	3	PRS
11	*Iodobacter limnosediminis*	P38.M2	CO																										4	0.16	1	13	PRS
12	*Iodobacter limnosediminis*	S73.M9	CO																										17	0.68	9	0	PR
13	*Janthinobacterium lividum*	S29.M9	CO																										14	0.56	1	0	PR
14	*Janthinobacterium lividum*	P41.M4	CO																										2	0.08	10	3	PRS
15	*Pedobacter alluvionis*	S66.M9	CO																										7	0.28	4	5	PRS
16	*Pseudarthrobacter oxydans*	S83.M8	CO																										13	0.52	21	2	PRS
17	*Pseudomonas caspiana*	S76.M10	CO																										7	0.28	3	13	PRS
18	*Pseudomonas mandelii*	P52.M7	CO																										13	0.52	9	0	PR
19	*Rhodococcus qingshengii*	P26.M4	CO																										7	0.28	7	3	PRS
20	*Rhodococcus yunnanensis*	P9.M4	CO																										0	0	2	4	PRS
21	*Rhodoferax ferrireducens*	S3.M1	CO																										13	0.52	3	0	PR
22	*Rhodoferax saidenbachensis*	S33.M1	CO																										12	0.48	1	0	PR
23	*Sphingomonas faeni*	S69.M9	CO																										2	0.08	1	14	PRS
24	*Stenotrophomonas rhizophila*	S23.M1	CO																										16	0.64	15	0	PR
25	*Stenotrophomonas rhizophila*	S25.M1	CO																										16	0.64	1	0	PR
26	*Arthrobacter psychrochitiniphilus*	S18.M9	EN																										5	0.2	3	0	PR
27	*Cryobacterium breve*	P11.M5	EN																										0	0	2	17	PRS
28	*Flavobacterium antarcticum*	P5.M4	EN																										9	0.36	0	0	PR
29	*Flavobacterium antarcticum*	P7.M4	EN																										3	0.12	0	15	SR
30	*Flavobacterium antarcticum*	P8.M4	EN																										4	0.16	1	0	PR
31	*Flavobacterium xanthum*	P14.M8	EN																										5	0.2	1	12	PRS
32	*Flavobacterium xanthum*	P15.M8	EN																										3	0.12	0	0	R
33	*Flavobacterium xanthum*	S17.M12	EN																										10	0.4	0	3	PRS
34	*Flavobacterium xanthum*	P39.M4	EN																										7	0.28	1	12	PRS
35	*Iodobacter arcticus*	S2.M1	EN																										5	0.2	2	5	PRS
36	*Iodobacter arcticus*	P6.M4	EN																										2	0.08	5	11	PRS
37	*Iodobacter arcticus*	P16.M8	EN																										5	0.2	3	12	PRS
38	*Iodobacter arcticus*	P27.M4	EN																										1	0.04	1	2	PRS
39	*Janthinobacterium svalbardensis*	P53.M6	EN																										13	0.52	13	0	PR
40	*Marisediminicola antarctica*	S32.M1	EN																										14	0.56	3	4	PRS
41	*Pedobacter fastidiosus*	S21.M8	EN																										8	0.32	3	10	PRS
42	*Pseudarthrobacter psychrotolerans*	S24.M1	EN																										1	0.04	5	7	PRS
43	*Pseudomonas antarctica*	S30.M8	EN																										0	0	0	15	SR
44	*Pseudomonas antarctica*	S82.M8	EN																										15	0.6	25	4	PRS
45	*Rugamonas violacea*	P64.M11	EN																										12	0.48	4	7	PRS
46	*Salinibacterium xinjiangense*	S1.M1	EN																										7	0.28	0	1	SR
47	*Salinibacterium xinjiangense*	P28.M4	EN																										4	0.16	11	3	PRS
		Resistance %	Total	64	40	57	47	38	2	36	4	2	4	21	4	23	38	32	43	38	6	6	23	62	38	4	55	57					
			CO	68	44	64	56	40	0	36	4	0	4	24	4	40	56	44	56	52	4	4	36	76	48	4	56	56					
			EN	59	36	50	36	36	5	36	5	5	5	18	5	5	18	18	27	23	9	9	9	45	27	5	55	59					

Figure 2. Map of the antimicrobial resistance profiles (orange – resistant; gray – sensitive) and antagonistic activity data of bacteria isolates from freshwater microbial mats. Abbreviations: ET - ecogeographic type; CO – cosmopolitan species; EN – endemic species; NAR – number of antibiotic resistance; ARI – antibiotic resistance index; ITP – interactive type profile: P – production of antibacterial compounds; R – resistance to antibacterial compounds; S – sensitivity to antibacterial compounds). Characteristic of antibiotics is listed in Table 1.

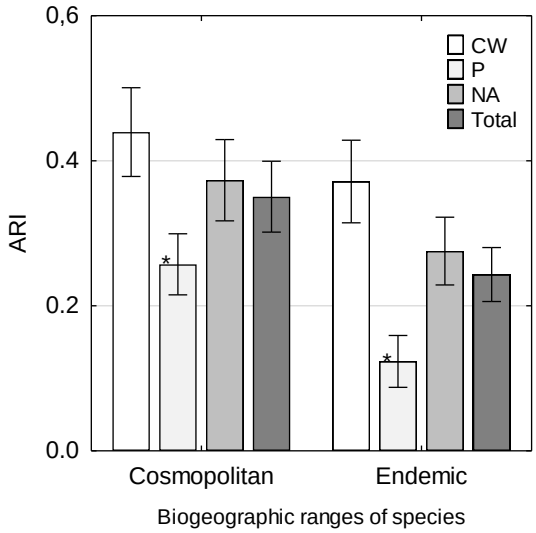

Figure 3. Antibiotic resistance index (ARI) of bacteria to selected group of antibiotics: inhibitors of cell wall (CW), proteins (P) and nucleic acids synthesis (NA); ARI index with $p<0.05$ are indicated (*)

Antagonistic interactions

Compounds inhibiting the development of other strains were produced by 87% of isolates. Various types of interactions occurring between strains derived from freshwater microbial mats were demonstrated. Depending on the nature of the interactions taking place, the occurrence of four groups of strains was noted: **PR** – where the isolate produces antibacterial compounds, but is also resistant to antibacterial compounds produced by other strains; **PRS** – where the isolate is an antagonist, i.e. produces antibacterial compounds, while being resistant and sensitive to one or more strains; **SR** – where the isolate produces no antibacterial compounds, while being sensitive and resistant to antibacterial compounds produced by other strains; **R** – the isolate produces no antibacterial compounds but is resistant to the action of these compounds produced by other strains. Among the isolates classified into species with different biogeographical ranges, significant differences were demonstrated in the antagonistic activity (Fig. 4). All strains belonging to the cosmopolitan (CO) species and 73% of those belonging to the endemic (EN)

species produced antimicrobial compounds. Among the strains classified as CO species, only isolates producing antibacterial compounds, i.e. those exhibiting PR and PRS interaction types, were found. The isolates classified as endemic (EN) species were characterised by a more diverse range of impacts, and the occurrence of PR, PRS, SR and R-type interactions. Statistically significant, higher antagonistic activity was observed among the cosmopolitan species ($p>0.05$) (Fig. 4a). Among the CO species, 24% exhibited high antagonistic properties, i.e. 10 and more reactions inhibiting the growth of other strains, with an average of 6.7 antagonistic reactions per strain observed in this group. Among these, the highest activity was noted for *Agreia pratensis, Flavobacterium hydatis* and *Stenotrophomonas rhizophila.* The antagonistic activity of the EN strains was two-fold lower than that of the CO strains, and amounted to an average of 3.8 reactions per strain. Only 14% of the strains belonging to the EN species exhibited high antagonistic activity, e.g. *Pseudomonas antarctica* and *Salinibacterium xinjiangense*. More than 27% of the EN strains produced no antibacterial compounds (Fig. 2). A significant negative relationship was noted between the antagonistic properties of the strains under study and their sensitivity to antibacterial compounds ($r=-0.50$; $p<0.01$) (Fig. 4b). The strains classified as endemic species were usually characterised by greater susceptibility to the action of antibacterial compounds produced by other strains, and lower antagonistic activity. Such properties were exhibited by e.g. *Cryobacterium breve*, *Flavobacterium antarcticum* and *Pseudomonas antarctica*. Opposing characteristics, i.e. low sensitivity to antagonistic interactions and the production of antibacterial compounds, were exhibited by strains belonging to cosmopolitan species, e.g. *Flavobacterium hydatis*, *Agreia pratensis* or *Stenotrophomonas rhizophila*.

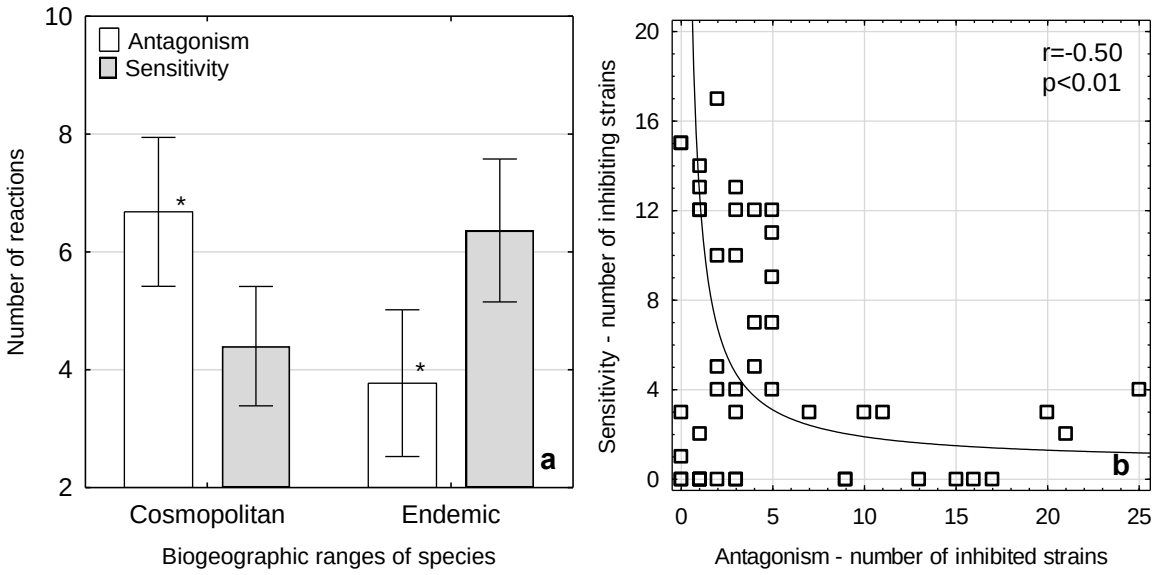

Figure 4. Antagonistic interactions between strains belonging to cosmopolitan and endemic species (**a**), (*) $p<0.05$; the relationship between antagonistic properties and the sensitivity of the strains under study (**b**)

Antagonistic activity and antibiotic resistance interactions

In strains derived from microbial mats, belonging to cosmopolitan and endemic species, a statistically significant correlation was demonstrated between the antagonistic potential, i.e. their production of antibacterial compounds, and their resistance to antibiotics, which was $r=0.46$ ($p<0.05$) and $r=0.49$ ($p<0.05$), respectively (Fig. 5a). In addition, strains with high antagonistic activity were characterised by lower sensitivity to antagonistic interactions, and higher antibiotic resistance. These traits were exhibited by *Flavobacterium hydatis, Agreia pratensis, Iodobacter limnosediminis* and *Stenotrophomonas rhizophila.* In addition, among the strains classified as cosmopolitan species, a negative correlation was noted between their sensitivity to antagonistic interactions and antibiotic resistance $r=-0.6$ ($p<0.005$) (Fig. 5b). These traits were exhibited by *Flavobacterium hydatis, Iodobacter limnosediminis, Agreia pratensis* and *Stenotrophomonas*

rhizophila. Such a statistical relationship was not confirmed among the strains of endemic species. At the same time, broad sensitivity to antagonistic interactions, accompanied by the absence of antibiotic resistance, was revealed among endemic strains, including, e.g., *Cryobacterium breve*, *Flavobacterium antarcticum* and *Pseudomonas antarctica* (Fig. 2).

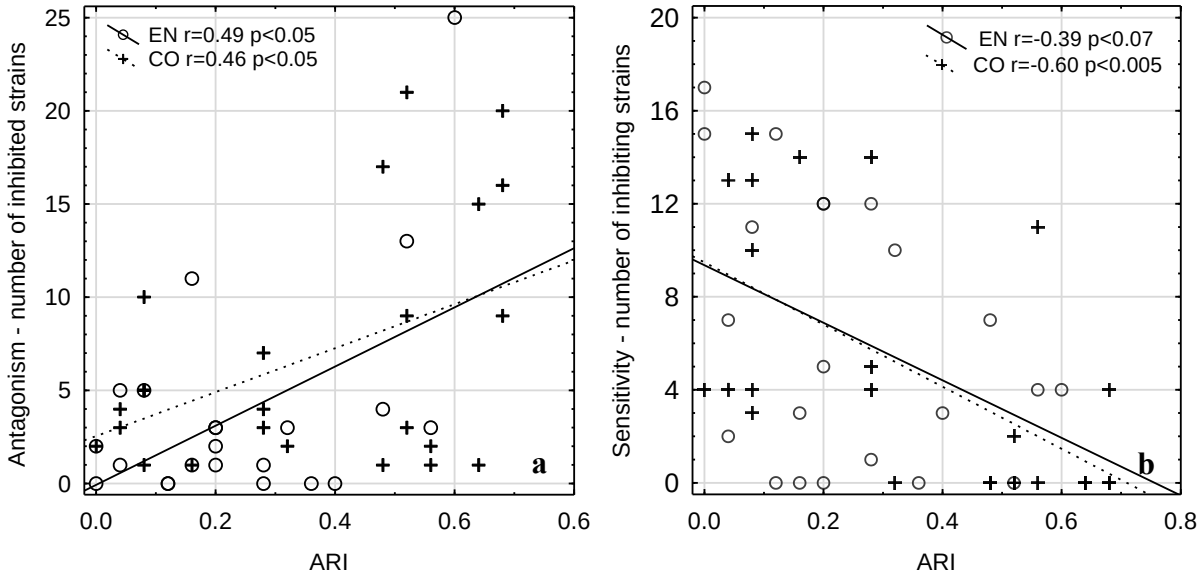

Figure 5. Antagonistic potential (**a**) and sensitivity to antagonistic interactions (**b**) vs the antibiotic resistance of strains derived from microbial mats; CO – cosmopolitan species; EN – endemic species

Analysis of the interactions occurring among the bacteria isolated from microbial mats showed the occurrence of strains with different patterns of intercellular relationships. Strong antagonistic activity accompanied by low sensitivity to antibiotics and lack of sensitivity to antibacterial compounds produced by other strains characterised isolates classified mainly as cosmopolitan species. These included, e.g., *Flavobacterium hydatis*, *Iodobacter limnosediminis*, *Pseudomonas mandelii* and *Stenotrophomonas rhizophila.* Among the strains classified as endemic species, these traits were only exhibited by two isolates, *Janthinobacterium svalbardensis* and

Pseudomonas antarctica. Among the isolates derived from microbial mats, the presence of strains with high sensitivity and low antagonism was also noted. In the CO group, these included: *Sphingomonas faeni, Iodobacter limnosediminis and Pseudomonas caspiana,* while in the EN group: *Cryobacterium breve*, *Iodobacter arcticus* and *Pseudomonas antarctica* (Fig. 6).

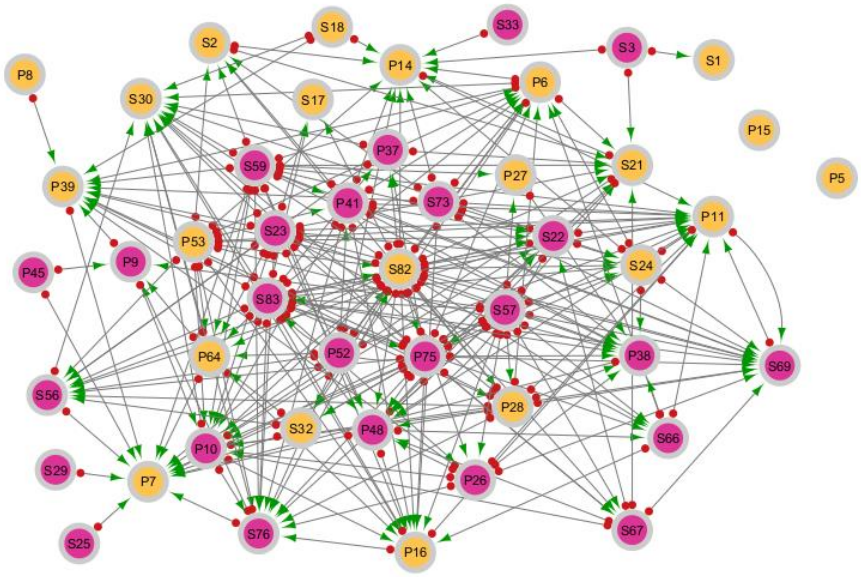

Figure 6. Network analyses of antagonistic interactions and antibiotic resistance among isolated strains. Each node represents bacterial strains. Each line represents an antagonistic interaction from an active strain towards a sensitive strain. Strains with the same type of antagonistic activity (ITP – interactive type profile) have the same fill colour: PR – green, PRS – blue, SR – purple, R – orange. Each line represents an antagonistic interaction from an active strain (red dot) towards to a sensitive strain (arrow). The size of red ring indicate antibiotic resistance-range. The species name of the strains are listed in Figure 2 and Table 2.

Discussion

The set of the authors' strains isolated from freshwater polar microbial mats of the periglacial zone of the Ecology Glacier exhibited significant species heterogenicity typical of microbial mats (Ramos et al. 2017; Prieto-Barajas et al. 2018; Valdespino-Castillo et al. 2018). Most of the

endemic species identified by the authors have previously been described as a component of the microbiota of different Antarctic environments, including, e.g., the soil - *Cryobacterium breve*, *Flavobacterium antarcticum*, *Pseudarthrobacter psychrotolerans*; water bodies - *Pedobacter fastidiosus*, *Rugamonas violacea*; penguin guano - *Arthrobacter psychrochitiniphilus*, *Flavobacterium antarcticum*; microbial mats - *Pseudomonas antarctica*, *Flavobacterium antarcticum*, *Flavobacterium xanthum;* and glaciers - *Janthinobacterium svalbardensis* (Liu et al., 2020; Yi et al. 2005; Shin et al. 2020; Švec et al. 2022; Sedláček et al. 2021; Wang et al. 2009; Reddy et al. 2004; Górniak et al. 2025; Cho et al. 2017). The present study demonstrated, for the first time in Antarctica, the presence of two species previously described as components of the microbiota of other cold environments, including glaciers: Arctic - *Iodobacter arcticus*, and Himalayan - *Salinibacterium xinjiangense* (Zhang et al. 2008; Srinivas et al. 2013). Of the 16 species classified by the authors as cosmopolitan bacteria, eight have already been described in polar environments, including: *Carnobacterium maltaromaticum*, *Flavobacterium hydatis*, *Iodobacter limnosediminis*, *Janthinobacterium lividum*, *Pseudarthrobacter oxydans*, *Rhodococcus qingshengii*, *Rhodococcus yunnanensis* and *Rhodoferax ferrireducens* (Kosiorek et al. 2024; Górniak et al. 2025; Su et al. 2013; Baricz et al. 2018; Morozova et al. 2022; Wang et al. 2025; Smirnova et al. 2021; Ciesielski et al. 2014). The other group of the authors' cosmopolitan strains consisted of species that have not been previously isolated from polar environments, including: *Pedobacter alluvionis*, *Pseudomonas caspiana*, *Pseudomonas mandelii*, *Rhodococcus qingshengii*, *Rhodococcus yunnanensis*, *Rhodoferax saidenbachensis*, *Sphingomonas faeni* and *Stenotrophomonas rhizophila*.

Antagonistic activity

The results of the present study confirmed the adopted hypothesis about the importance of antagonistic interactions in the formation of the structure and the functioning of the

microbiocenoses of polar freshwater microbial mats. The interactions occurring at the species level in consortia such as microbial mats are poorly understood. The literature usually addresses the issue of antagonistic interactions of autochthonous environmental strains in interactions with test pathogenic microorganisms (Kan et al. 2011; Kosiorek et al. 2024). Antimicrobially active strains that produce bioactive substances, such as violacein, are usually sought. Baricz et al. (2018), when testing an Antarctic variant of *Janthinobacterium lividum*, demonstrated over 50% inhibitory activity in relation to 200 pathogenic and environmental MDR strains. Reports of interactions within consortia-forming microbial communities are rare (Prasad et al. 2011). Antagonistic relationships are a fundamental strategy that allows advantage to be gained in competition for trophic resources and ecological niches in aggregated biocenotic systems (Tait et al. 2002; Mangano et al. 2009; Long et al. 2013). The present study revealed a surprisingly high capacity to produce antibacterial substances, with at least one antagonistic reaction being exhibited by the isolates classified as cosmopolitan species, and 77% of strains of the endemic species. Studies by other authors showed a similar range of antagonism, e.g. in saline mats (60%), marine seston (53.5%), and organic oceanic conglomerates (54.1%) (Long & Azam 2001; Grossart et al. 2004; Long et al. 2013). Long et al. (2001) proved that the degree of bacterial cell aggregation is of decisive importance in the antagonistic activity. Planktonic bacteria exhibited very low antagonism towards 5% of strains, compared to over 50% antagonistic activity of strains found in the seston. As demonstrated by the present study, antagonistic interactions in microbial mats are a very complex phenomenon (Górniak et al. 2025). The results obtained revealed the occurrence of different types of interactions, both antagonism and sensitivity to antagonism. Antagonistic relationships occurring at various taxonomic levels affect the structural and functional homeostasis of aggregated microbiocenotic systems. Although this phenomenon is still poorly understood, it has been shown to play a crucial role in the formation of the structure of

aggregated microbial systems (Mangano et al. 2009, Prasad 2011, Long et al. 2013). What should be emphasised is the individualisation of antagonistic relationships in individual isolates forming the consortium, as demonstrated in the course of the study, and the occurrence of traits supporting their competitiveness in the microbiocenosis. The distinct difference in the antagonistic activity of isolates, demonstrated in the present study, appears to be determined by their biogeographical range. The broad antagonism noted over the course of the study, i.e. the inhibition of the growth of many isolates of cosmopolitan strains, is an important trait that promotes the colonisation and adaptation processes in heterogeneous microbial systems. Examples of such bacteria include *Pseudarthrobacter oxydans, Agreia pratensis, Flavobacterium hydatis* or *Iodobacter limnosediminis*, noted for the first time in Antarctica by the authors. The production of antimicrobial substances, even in subthreshold amounts, is crucial in gaining an advantage over other microorganisms forming microbial consortia, and limits the availability of the micro-niche for prokaryotic colonisers (Tait et al. 2002, Rypien et al. 2010).

Antibiotic resistance

Natural antibiotic resistance in native environmental bacteria, similar to other metabolic characteristics, is a common phenomenon and has developed over millions of years (Bell et al. 2013). Microevolutionary processes play a decisive role in the dynamics of antibiotic resistance among bacteria found in natural environments (D'Costa et al. 2006; Hutchings et al. 2019; Paun et al. 2021). Van Goethem et al. (2018) demonstrated that in natural environments isolated from anthropogenic influences, most antibiotic resistance genes originate mainly from ancient antibiotic-producing species. Metagenomic research suggests that the Antarctic microbiota is the source of primary antibiotic resistance genes, but, at the same time, multi-antibiotic-resistant strains are found in areas of high anthropogenic impact (Jara et al. 2020). The high frequency of MDR strains among the isolates derived from microbial mats, shown over the course of the

authors' study, was surprising but not unexpected. As demonstrated by the present study, broad antibiotic resistance among strains derived from microbial mats is a common phenomenon. An increasing number of reports have indicated broad antibiotic resistance occurring among bacteria from different polar environments. Bacteria resistant to multiple antibiotics have been detected in polar terrestrial environments (Tam et al. 2015; Jara et al. 2020; Opazo-Capurro et al. 2020; Marcoleta et al. 2022), glacial ice (Brown & Balkwill 2009), and bottom sediments (De Souza et al. 2006). A significant reservoir of bacterial antibiotic resistance genes is the surface layer of the soil (Králová et al. 2017; 2021; Van Goethem et al. 2018; Marcoleta et al. 2022). The present study indicates that the colonisation of isolated environments by allochthonous, cosmopolitan and frequently, antibiotic-resistant species leads to the transfer of antibiotic resistance genes into the microbiome of the autochthonous microbial community. This phenomenon is common in the microbiocenosis of the microbial mats under study. The different antibiotic resistance profiles of the endemic strains, also belonging to the same species, observed in the present study, indicate the acquired nature of these characteristics. It should be assumed that the extensive resistome of allochthonous cosmopolitan species, demonstrated in the present study, represents a gene pool for the native resistome of endemic strains. The antibiotic resistance observed in numerous endemic strains in the microbial mats under study is a manifestation of specific "biotic contamination" in these microbiocenoses, and represents a significant ecological problem. The degradation of natural endemic polar microbiocenosis through the pressure of allochthonous cosmopolitan species is pointed out by many authors (Vincent et al. 2000; Hughes et al. 2011; Jara et al. 2020; Zucconi et al. 2025).

Summary

The present study is the first attempt to clarify the role of biogeographical ranges of species, which are components of polar microbial mats, in the antagonistic activity and antibiotic

resistance. The study demonstrated the complex nature of the relationships, at both the intra-species and inter-species level, which is crucial in the development of the structure and functioning of mats. The individual patterns of antagonistic interactions and antibiotic resistance observed among isolates showed a clear relationship with the biogeographical ranges of individual species. Strains of cosmopolitan species characterised by broad antagonism and resistance to antibiotics were also identified. These specific "biological contaminants" may be the source and propagator of new traits in endemic populations of polar mat microbiocenoses. The results obtained positively verified the research hypothesis, that the antagonistic interactions between strains are related to their spectrum of antibiotic resistance, with the nature of these interactions being determined by the biogeographical range of the species. As suggested by the present study, cosmopolitan species in polar environments may interfere with the delicate structure of native microbial communities and lead to the elimination of endemic species. A consequence of this phenomenon may be a reduction in the biodiversity of bacteriocenoses and a decline in the number of taxa associated with the processes of regeneration and bioconversion of biogenes. The present study is in line with the discussion on the contemporary model of microbial biogeography, promoting the concept of a "middle model", the coexistence of moderate endemism of polar bacteria, and cosmopolitism of allochthonous species. The taxonomic structure of the authors' collection of strains derived from freshwater microbial mats in maritime Antarctica falls within this definition of biodiversity, especially in relation to global climate change.

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