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Cold-Adapted Keratinolytic Flavobacterium: A Novel Candidate for Bioremediation of recalcitrant contaminants

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Abstract

Antarctica's extreme conditions harbor cold-adapted microorganisms with remarkable biotechnological potential. This study characterizes two *Flavobacterium* strains (CIN7a and CIN7c), isolated from King George Island, evaluating their specialized capacity to degrade both keratin-rich biomass and multi-xenobiotic industrial pollutants at low temperatures. Fermentation assays revealed that only isolated CIN7a exhibited true keratinolytic activity, achieving feather hydrolysis extent of 83% within 120 h and yielding a specialized keratinase - to- protease ratio of 2:1. While the strain CIN7a also produced anionic biosurfactants with a 52% emulsification index (E24), strain CIN7c demonstrated superior biofilm-forming capacity.

Despite these physiological divergences, both isolates displayed a high efficiency in removing sodium dodecyl sulfate (SDS) and the synthetic azo dyes Reactive Black 5 and Reactive Yellow 84 from simulated effluents, whereas the degradation of Reactive Violet 5 was restricted by structural steric hindrance. These findings highlight the functional versatility and metabolic complementation of these Antarctic *Flavobacterium* strains, positioning them as highly promising candidates for cost-effective, low energy bioremediation strategies in cold environments.

Introduction

The environmental persistence of recalcitrant compounds - defined as structurally novel or naturally occurring substances resistant to microbial degradation - represents a significant challenge for modern waste treatment. While state-of-the-art removal technologies exist, their implementation is often hindered by high costs and operational complexity. Consequently, there is an urgent need to transition toward efficient and sustainable technologies, with a growing emphasis on advanced biological processes (Bardi et al. 2017).

A prime example of this challenge is found in the poultry industry, which generates millions of tons of chicken feathers annually. Despite being a high-protein resource (containing up to 90% keratin and 15% nitrogen), feathers are largely underutilized and typically managed through incineration or landfilling. These conventional disposal methods are increasingly unsustainable due to their high energy consumption and significant environmental risks, including greenhouse gas emissions and the dissemination of pathogens (de-Santos-Casado et al. 2026).

Similarly, the management of industrial wastewater is complicated by the presence of dyes and surfactants. Even in small quantities, dyes are highly visible and often exist within complex mixtures that include heavy metals and organic pollutants. Furthermore, the global production of approximately 7.2 million tons of synthetic surfactants adds another layer of complexity; their unique amphiphilic structure leads to foaming issues in treatment plants and direct toxic impacts on various organisms (Pettersson et al. 2000; Ying 2006).

Currently, the reliance on physicochemical processes to treat these pollutants is limited by cost and the generation of hazardous by-products or sludges that require secure storage. Therefore, exploring advanced biological alternatives is essential to mitigate the environmental impact of these diverse waste streams and promote a more circular and sustainable industrial model.

Given the limitations of current treatment methods, extreme environments such as Antarctica offer a unique opportunity for discovering novel biological solutions. The harsh abiotic conditions of these regions - including extreme cold, high salinity, and high levels of ultraviolet radiation - have shaped the diversity and specialized metabolic capabilities of both marine and continental microbial communities. While access to these environments remains challenging, the bioprospection of psychrophilic and psychrotolerant microorganisms holds significant potential for revealing novel biological processes applicable to industrial challenges (Bezus et al. 2022; Naranjo-Moran et al. 2026).

Within this framework, the aim of this work was to characterize two Antarctic *Flavobacterium* strains isolated from a keratinolytic consortium and evaluate their keratinolytic activity, while simultaneously evaluating their potential for the removal of textile dyes and sodium dodecyl sulfate (SDS) from simulated wastewaters. Also, the capability of producing anionic surfactants and biofilm was evaluated.

Materials and Methods

Sampling sites and isolation of microorganisms

Microorganisms were obtained from a feather-degrading consortium previously isolated from soil collected at Half Three Point (ASPA 125d), King George Island, Antarctica (62°11'4" S, 58°51'7" W) (Fig. 1). Sampling and initial handling were performed under aseptic, cold-preservation conditions as described by Rios et al. (2022).

Consortium enrichment was carried out in feather mineral medium (FMM) using chicken feathers as the sole carbon, nitrogen, and energy source, following the procedure reported by Rios et al. (2022), with incubation at 15°C and rotary agitation. The stabilized keratinolytic community was obtained after consecutive subcultures and preserved at -80°C in glycerol.

Bacterial isolates were purified from the stabilized consortium on Nutrient Agar after incubation at 15°C and selected according to colony morphology. Pure cultures were maintained by routine subculturing at 4°C and preserved at -80°C in glycerol-containing Nutrient Broth or by lyophilization.

Selected strains were deposited in the CINDEFI-CONICET Institute Microbiological Culture Collection and in the Facultad de Química, UdeLar Culture Collection, Montevideo, Uruguay.

Screening of proteolytic and keratinolytic activity

Proteolytic activity was screened on skimmed milk agar, and positive isolates were subsequently evaluated for feather degradation in FMM, following Rios et al. (2022). Cultures were incubated under the same low-temperature conditions used for consortium enrichment, and isolates showing extracellular hydrolysis were selected for further analysis.

Molecular identification

Genomic DNA was obtained by thermal lysis according to Gholoobi et al. (2014). Taxonomic affiliation was assessed by partial 16S rRNA gene sequencing using the universal primers 27F and 1387R, with amplification conditions adapted from Urbieta et al. (2014).

Amplicons were purified and sequenced by Sanger technology at Macrogen Inc. (Seoul, South Korea). Sequences were compared with reference strains from NCBI GenBank using BLASTn, and phylogenetic relationships were inferred in MEGA12 as described by Kumar et al. (2024). Alignments were generated with ClustalW, and Maximum Likelihood analysis was performed using the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985), with bootstrap support estimated according to Felsenstein (1981). The sequences were deposited in GenBank under accession numbers PZ413497 (CIN7a) and PZ413498 (CIN7c).

Growth kinetics at different temperatures

Temperature-dependent growth of *Flavobacterium pygoscelis* CIN7a was evaluated in Nutrient Broth at 16, 18, 24, and 28°C under rotary agitation. Cultures were inoculated to an initial OD_{600} of 0.05 and monitored for 24 h.

Growth was followed spectrophotometrically at 600 nm, and maximum growth rates (μmax) were estimated from batch growth curves using the accumulation balance and Monod equation, as described by Rios et al. (2022).

Culture conditions for keratinases production and enzymatic assays

Keratinase production by *F. pygoscelis* CIN7a was induced in FMM containing chicken feathers, following the submerged fermentation conditions previously described by Rios et al. (2022), with incubation at 24°C and rotary agitation. Culture supernatants were collected over seven days and clarified by centrifugation for enzymatic assays.

Proteolytic activity was determined using azocasein as substrate, following the colorimetric assay described for crude extracellular extracts. Enzyme reactions were carried out in Tris-HCl buffer, stopped with trichloroacetic acid, and quantified at 440 nm. One unit of protease activity was defined as the

enzyme amount required to increase absorbance by 0.01 under the assay conditions; blanks and triplicates were included.

Keratinolytic activity was quantified with azokeratin according to Moridshahi et al. (2021). Reaction mixtures containing azokeratin and diluted enzyme extract were incubated in Tris-HCl buffer, stopped with trichloroacetic acid, clarified by centrifugation, and read at 440 nm. One unit of keratinolytic activity was defined as an absorbance increase of 0.01 under the assay conditions.

Extent of feather degradation

Feather degradation was quantified gravimetrically according to Rios et al. (2022). Residual feathers were recovered after fermentation, washed, dried to constant weight, and used to calculate substrate loss.

Feather degradation (%) was calculated as [(W - F) / W] × 100, where W is the initial feather weight and F is the residual dry weight after fermentation.

All assays were performed in triplicate, and results are reported as mean substrate loss ± standard deviation (SD).

Protein determination

Protein concentration was determined according to the Bradford method using bovine serum albumin (BSA) as a standard (Kielkopf et al. 2020).

SDS-PAGE analysis and Zymography

Protease molecular masses were estimated by SDS-PAGE coupled to casein zymography, adapting the method of García-Carreño et al. (1993). After electrophoresis, gels were treated for enzyme renaturation, incubated with casein-containing buffer, and stained with Coomassie Brilliant Blue G-250; proteolytic bands were visualized as clear zones, as described by Dyballa and Metzger (2009). Molecular mass was estimated using Pierce™ 26610 Unstained Protein MW markers.

Biofilm Formation Assay

Biofilm formation by isolates CIN7a and CIN7c was assessed in 96-well polystyrene microplates using the crystal violet staining method described by Merritt et al. (2011). Overnight cultures were inoculated in LB and incubated at 16, 18, 24, and 28°C. After removal of non-adherent cells, stained biofilms were solubilized and quantified at 595 nm. Assays were performed in quadruplicate in three independent experiments (n = 12), and biofilm-forming capacity was classified according to the ODc criteria proposed by Stepanović et al. (2007).

Screening of biosurfactant production

CTAB-methylene blue agar assay

Anionic biosurfactant production was screened using the CTAB-methylene blue agar assay, as described by Siegmund and Wagner (1991) and Jadhav et al. (2011). Bacterial cultures grown in LB were spotted onto CTAB-methylene blue agar, incubated at 24°C, and monitored for dark blue colony formation, which was considered indicative of anionic biosurfactant production.

Determination of emulsification index (E24)

Emulsifying capacity was determined as the emulsification index after 24 h (E24), following Ali et al. (2021). Culture supernatants were mixed with olive oil, vigorously homogenized, and left to stand before measuring the emulsion layer. E24 was expressed as the percentage of emulsion height relative to the total liquid height, according to Cooper and Goldenberg (1987).

E24 index % = (Height of emulsion / Total height of solution) x 100

Oil-spreading assay

Oil-spreading activity was evaluated using cell-free supernatants from 48 h cultures, following Morikawa et al. (2000). Supernatants were applied onto an oil layer over distilled water, and oil displacement was visually recorded. Distilled water was used as a negative control.

Screening of the capability to degrade recalcitrant compounds

Azo dyes removal screening

The ability of both isolates to remove the azo dyes Reactive Black 5, Reactive Violet 5, and Reactive Yellow 84 was evaluated in liquid Normal Decolorization Medium supplemented with each dye, according to Pajot et al. (2007). Inoculated cultures were incubated at 24°C under rotary agitation, and dye removal was visually recorded after 72 h.

Degradation of SDS by CIN7a and CIN7c

SDS biodegradation was qualitatively assessed using a modified methylene blue active substance (MBAS) assay, following Naimah et al. (2026). Briefly, isolates were grown in minimal salt medium supplemented with SDS as the sole carbon source and trace elements, and degradation was evaluated by the methylene blue/chloroform colorimetric reaction.

Incubated culture supernatants were processed according to the modified MBAS protocol, and SDS degradation was inferred from the color change in the chloroform phase. Non-inoculated SDS-containing minimal medium treated under the same conditions was used as a blank.

Statistical analysis

Data are presented as the mean ± standard deviation derived from three separate experimental trials, where applicable.

Results

Isolation and screening for feather-degrading and keratinase-producing bacteria

In May of 2016 during the Antarkos XXXII expedition to King George Island, several terrestrial samples were collected. The feather- degrading consortium used in this survey was obtained from samples of Half Three Point (ASPA 125d). Three morphologically different culturable bacteria were isolated and among them, only two demonstrated proteolytic activity in milk agar plates and were named CIN7a and CIN7c, respectively (Figure S1). Both bacteria were later submitted to a keratinolytic screening and only the isolate CIN7a showed feather degradation in FMM. After 7 days, a complete degradation of the feather sample was observed (Fig. S2) and it was selected for further studies which included proteolytic and keratinolytic enzymes production, feather hydrolysis extent and SDS-PAGE coupled zymogram. All the other assays were performed for both isolates, in order to test if CIN7c isolate could also be a candidate for removal of other recalcitrant compounds.

Identification of proteolytic bacteriacan be

Molecular analysis of the 16S rRNA gene fragment sequence enabled the taxonomic identification of the isolate CIN7a as *Flavobacterium pygoscelis*. This strain, formally designated as *F. pygoscelis* CIN7a, displayed a sequence identity of 99.90% with the reference strain *Flavobacterium pygoscelis* OL415579. On the other hand, the isolate CIN7c was identified as *Flavobacterium* sp. and named *Flavobacterium* sp. CIN7c. This strain showed 98.16% homology with *F. flabelliforme* P4023 and 97.93% with *Flavobacterium pygoscelis* OL415579. Figure 2 shows the position of our isolates in the phylogenetic tree.

Flavobacterium pygoscelis CIN7a was able to grow at temperatures ranging from 16 to 28°C, with the maximum growth rate occurring at 24°C (μmax = 0.31 h^{-1}). The isolate exhibited reduced growth rates at lower thermal settings, recording a μmax of 0.21 h^{-1} at 16°C and attaining peak cell density by the seventh hour. A comparable developmental pattern for *F. pygoscelis* CIN7a was observed during cultivation at 28°C. At this temperature, its μmax was 0.30 h^{-1}, and the maximum cell density was reached at 8 h (Fig. 3). Based on established microbiological classifications, the observed thermal growth range confirms that the isolate exhibits a psychrotrophic phenotype (Salwan et al. 2010).

Proteolytic and keratinolytic enzymes production - Feather hydrolysis extents

The time course of proteolytic and keratinolytic enzyme production under submerged fermentation with chicken feathers as sole carbon, nitrogen and energy sources is shown in Fig. 4. After 120 h at 24°C, maximum proteolytic (10.25 ± 0.39 U mL^{-1}) and keratinolytic (20.6 ± 0.16 U mL^{-1}) activities were achieved. At this temperature, complete degradation of the substrate was observed, and the extent of hydrolysis was approximately 83 ± 1.2% with 234.6 ug mL^{-1} of soluble protein.

At lower (16 and 18°C) and higher (28°C) temperatures the extent of feather degradation was in concordance with maximum growth rate, e.g., at 16°C the feather substrate loss reached 62.5 ± 1.2% and at 28°C it was 70.4 ± 2.3%, respectively (Fig. S3).

An SDS-PAGE coupled to a casein-zymogram was performed to estimate the molecular weight of the proteases present in CIN7a crude extract (Fig. 5). One band was clearly visualized with a high apparent molecular weight of 116 kDa and at least two more diffuse proteolytic bands with apparent molecular weights between 45 and 35 kDa.

Biofilm Formation Assay

Biofilm formation by strains CIN7a and CIN7c was assessed at four incubation temperatures. According to the classification criteria of Stepanović et al. (2007), both strains were classified as biofilm producers under all tested conditions. Strain CIN7a was categorized as a strong biofilm producer at 16, 24, and 28°C, and as a weak producer at 18°C. Strain CIN7c exhibited strong biofilm production at 16, 18, and 28°C, whereas moderate biofilm formation was recorded at 24°C. These results indicate that biofilm formation is a conserved trait in both isolates across a broad temperature range, although the magnitude of biofilm production varied depending on the strain and incubation temperature.

Screening of biosurfactant production

Biosurfactant production by isolates CIN7a and CIN7c was evaluated using CTAB-methylene blue agar, emulsification index (E24), and oil-spreading assays (Fig. 6). CIN7a yielded a positive reaction in the CTAB-methylene blue agar assay, whereas CIN7c was negative (Fig. 6a). Both isolates exhibited emulsifying activity toward olive oil, with E24 values of 52% and 42% for CIN7a and CIN7c, respectively (Fig. 6b). Positive oil-spreading activity was also observed for both strains (Fig. 6c), indicating the production of surface-active compounds.

Screening of the capability to degrade recalcitrant compounds

Both isolates demonstrated the ability to remove the azo dyes evaluated and showed evidence of SDS degradation (Fig. 7). Dye decolorization was observed for all compounds tested, indicating a broad capacity to transform structurally different azo dyes (Fig. 7a, b and c). Likewise, growth-associated SDS

degradation was detected in both isolates (Fig. 7d), suggesting their potential for the biodegradation of detergent-derived contaminants.

Discussion

Keratinous residues represent some of the most difficult protein substrates to recycle biologically, largely due to their inherent resistance to standard proteolytic cleavage. The recalcitrance of the feather protein matrix is fundamentally linked to its molecular architecture, characterized by tightly packed β-sheet configurations. This structural stability is further reinforced by an extensive network of disulfide cross-links and hydrophobic forces, which render the keratin polymer virtually inaccessible to common proteases such as trypsin, pepsin, and papain (Brandelli et al. 2010). The poultry industry generates large amounts of feather waste every year (Jyothi and Dhanasingh 2025), and only a portion of this material is currently processed through conventional thermochemical methods, which are often energy demanding. In this context, microorganisms able to produce keratinases are of particular interest because these enzymes can efficiently break down the keratin structure and enable the biological conversion of feather waste. While most characterized keratinases originate from mesophilic or thermophilic taxa, enzymes adapted to cold conditions have received significantly less attention. This knowledge gap persists despite the distinct benefits of psychrophilic biocatalysts for industrial applications at low temperatures, such as the potential for substantial energy savings (Joshi and Satyanarayana 2013; Pereira et al. 2014). Antarctic habitats, where keratinous biomass naturally accumulates due to avian populations, serve as promising reservoirs for discovering microorganisms with specialized keratinolytic capabilities.

Consequently, this research aims to characterize two novel isolates (*F. pygoscelis* CIN7a and *Flavobacterium* sp. CIN7c) obtained from a keratin-degrading consortium from King George Island. We assessed their efficacy in hydrolyzing keratin as well as their ability to transform additional recalcitrant environmental pollutants. This comprehensive genomic and metabolic analysis significantly expands the known functional repertoire of the genus *Flavobacterium*, particularly regarding its adaptation to extreme polar environments.

At the time of writing, the genus *Flavobacterium* comprises over 360 validly published species, with more than 30 distinct taxa successfully isolated from pristine Antarctic matrices, including marine sediments, polar lakes, and coastal waters (Nogi et al. 2005; Irgang et al. 2023). While the secretion of generic cold-active proteases is a well-documented trait among psychrotrophic members of this genus - as exemplified by the comprehensive isolation of 28 proteolytic *Flavobacterium* strains from freshwater ecosystems by Matsui et al. (2017) - true keratinolytic activity has remained entirely undocumented within this taxonomic group. Consequently, *F. pygoscelis* CIN7a represents the first documented strain within the genus *Flavobacterium* capable of actively degrading complex keratinous matrices without prior chemical treatments. From an industrial perspective, bioprospecting cold-adapted or psychrotolerant bacteria that combine robust generic proteolysis with a specialized keratinolytic system offers massive advantages for sustainable waste management. Operating enzymatic reactors under low-temperature regimes not only prevents heat-induced denaturing of secondary metabolites but also

yields substantial reductions in operational costs and energy demands when transitioning from bench-scale assays to continuous, large-scale industrial bioremediation (Cao et al. 2012). While several proteolytic *Flavobacterium* species have been reported in Antarctic environments (Nogi et al., 2005; Irgang et al., 2023), keratinolytic activity has not been previously documented within this genus. This makes CIN7a the first *Flavobacterium* strain reported with this specific capability, offering significant advantages for low-energy industrial applications (Cao et al., 2012).

This bioprospection in extreme environments like Antarctica is essential for discovering such novel biocatalysts. To date, only a limited number of Antarctic keratinolytic bacteria has been reported as shown in Table 1.

Table 1
Antarctic keratinolytic microorganisms isolated from environmental samples. Reported isolates originate from diverse keratin- and organic-rich habitats including penguin feathers, bird nests, snow, and terrestrial soils.

Strain	**Genus/Species**	**Source of Isolation**	**Antarctic Location**	**Reference**
A03	*Lysobacter* sp.	Penguin feathers	Elephant Island	Queiroz Pereira et al., 2014
A08	*Arthrobacter* sp.	Penguin feathers	Elephant Island	Queiroz Pereira et al., 2014
A17U	*Chryseobacterium* sp.	Penguin feathers	Elephant Island	Queiroz Pereira et al., 2014
3.14.7	*Pedobacter* sp.	Bird nest material (*Chionis alba*)	King George Island	Rios Cruz et al., 2022
BIM B-1663	*Arthrobacter oryzae*	Antarctic green snow	East Antarctica	Smirnova et al., 2023
CIN 7A	*Flavobacterium pygoscelis* CIN 7A	Terrestrial sample	King George Island	This study

Table 2
Comparison of keratinous substrates degradation and Keratinolytic Activity (Antarctic Isolates)

Strain	Genus/Species	Substrate degradation (%)	Max Keratinolytic Activity (U/ml)	Peak Activity (Time)	Reference
A03	*Lysobacter* sp.	Almost complete	~ 2.50	6 days	Queiroz Pereira et al., 2014
A08	*Arthrobacter* sp.	Efficient	~ 1.0	3 days	Queiroz Pereira et al., 2014
A17U	*Chryseobacterium* sp.	Minor	~ 1.0	4 days	Queiroz Pereira et al., 2014
3.14.7	*Pedobacter* sp.	~ 85%	33.04	10 days	Rios Cruz et al., 2022
BIM B-1663	*Arthrobacter oryzae*	~ 87%	0.094	7 – 2 days	Smirnova et al., 2023
CIN 7a	*Flavobacterium pygoscelis*	83%	20.60	5 days	This study

*Note: Activity values are unified to U/mL and specifically refer to enzymatic action on azokeratin substrate to ensure direct comparability across all Antarctic strains.

The evaluation of *F. pygoscelis* CIN7a under submerged fermentation revealed a remarkable keratinolytic capacity, achieving approximately 83% of raw feather degradation within 120 h. This degradation efficiency aligns closely with other prominent Antarctic isolates, such as *Pedobacter* sp. 3.14.7, and compares favorably with fungal models like *A. oryzae*, which required 12 days to reach 87% utilization using pre-treated feather meal instead of raw substrate (Rios et al. 2022; Smirnova et al. 2023).

A key feature of strain CIN7a is its specific enzymatic profile. At peak production (120 h), the isolate yielded a keratinolytic activity of 20.6 ± 0.16 U mL^{-1} compared to 10.25 ± 0.39 U mL^{-1} of general proteolytic activity. This establishes a keratinase-to-protease ratio of approximately 2:1. According to the criteria established by Gupta et al. (2013), a ratio exceeding 0.5 distinguishes true, specialized keratinases from generic proteases with limited activity against rigid scleroproteins. The substantial ratio exhibited by CIN7a underscores an enzymatic system highly specialized in targeting the dense network of disulfide bonds and hydrophobic interactions that stabilize the β-sheet architecture of keratin.

These results align with the inherent resistance of keratin, a substrate whose structural stability is maintained by a dense network of hydrophobic forces and disulfide linkages. Consequently, its breakdown necessitates specialized enzymes capable of targeting such a robust framework.

It is also noteworthy that all microorganisms in this table are psychrotolerant, capable of growing and degrading keratinous waste at low temperatures (typically 20°C).

The zymographic profiles of the studied Antarctic isolates reveal a wide diversity in their proteolytic systems, ranging from single enzymes to complex proteolytic systems. *Pedobacter* sp. *3.14.7* and *Arthrobacter* sp. *A08* both produce a single monomeric protein band. In contrast, *Flavobacterium pygoscelis* CIN7a presents a complex profile characterized by a prominent high molecular weight band at 116 kDa and at least two additional diffuse proteolytic bands between 35 and 45 kDa. This complexity is even more pronounced in *Lysobacter* sp. *A03*, which stands out with five distinct clear zones of activity. Meanwhile, *Chryseobacterium* sp. *A17U* displays an intermediate system with three identified bands of activity. These varying molecular profiles reflect the specialized and diverse metabolic strategies these psychrotolerant bacteria have evolved to effectively hydrolyze recalcitrant keratinous waste in extreme environments (Pereira et al. 2014; Rios et al. 2022).

The study of biofilm formation is a cornerstone of bioremediation research, as these microbial communities provide significantly higher resilience and metabolic efficiency for pollutant degradation compared to their planktonic counterparts. In Antarctica, the ability to form biofilms represents a critical survival strategy to withstand extreme abiotic conditions, such as freezing temperatures and high ultraviolet radiation. Many strategies for overcoming extremely low temperatures have been reported in bacteria. One of them is the production of biofilm to persist in stressful environmental conditions. Biofilm formation depends on the capacity of bacteria to sense environmental signals such as nutrient availability, pH, temperature, osmolality, fluctuations in oxygen concentration, and the presence of antibiotics (Vásquez-Ponce et al. 2017).

Models such as *Pseudoalteromonas haloplanktis* TAC125 demonstrate remarkable metabolic plasticity, as they can modulate their biofilm architecture and the chemical composition of their extracellular matrix in response to nutrient availability and temperatures as low as 0°C (Ricciardelli et al. 2019). Vásquez-Ponce et al. (2017) demonstrated that the psychrotolerant *Pseudomonas mandelii* 6A1 after 72 h, showed at least six-fold and three-fold more biofilm formation at 4 and 15°C, respectively, than that at 25°C. In our study, both isolates (CIN7a and CIN7c) maintained their biofilm-formation capacity across the entire temperature range tested (16–28°C), demonstrating that this phenotype is not restricted to a narrow thermal window. This broad thermal plasticity reflects an adaptive strategy that enhances persistence under fluctuating environmental conditions characteristic of maritime Antarctic sites where temperature can shift substantially between seasons, allowing cells to maintain metabolic activity despite changes in temperature. The extracellular matrix of biofilms provides a protected microenvironment that buffers cells against abiotic stress while sustaining metabolic activity and conservation of this trait in both isolates suggests that biofilm formation contributes to their ecological fitness. From a biotechnological perspective, this thermal adaptation also positions CIN7a and CIN7c as promising candidates for continuous pollutant removal under low-temperature conditions.

The physiological divergence between the two *Flavobacterium* isolates reveals distinct, yet complementary, ecological adaptation strategies for survival in extreme Antarctic conditions. The production of anionic biosurfactants by *F. pygoscelis* CIN7a, coupled with its emulsification index (E24: 52%) suggests a mechanism aimed at increasing the bioavailability of hydrophobic or complex

substrates. Extracellular surfactants lower surface tension, facilitating the enzymatic attack of the keratinolytic system on raw feathers.

Conversely, the superior biofilm-forming capacity exhibited by strain CIN7c points toward a sessile protection strategy. In cold environments, biofilm matrices act as physical shields against thermal shock, ice crystal damage, and chemical fluctuations, while anchoring the bacteria to potential nutrient sources. This division of metabolic profiles within the original consortium highlights that while CIN7a specializes in active substrate disruption and emulsification, CIN7c excels in spatial persistence, explaining their synergistic coexistence in the original feather-degrading community.

Research on the application of Antarctic microbial communities for treating industrial wastewater remains scarce, with very few studies documented in the literature. Silva et al. (2025) reported the use of Antarctic microbial consortia (bacteria and fungi) and the individual strain as well for the removal of industrial dyes such as Remazol Brilliant Blue R, and two commercial dyes in solid culture medium. Tharek et al. (2017) reported the decolourisation of Reactive Black 5 by *Klebsiella pneumoniae* isolated from Antarctic seawater and Kita et al. (2022) reported the decolourisation of the textile dye sulphur indigo blue by the filamentous fungi *Penicillium cf. oxalicum* recovered from Antarctic marine sediments. This type of studies extends to yeast, where *Candida sake* 41E, *Leucosporidium muscorum* F20A, and *Cystofilobasidium infirmominiatum* F13E demonstrate among a collection of antarctic yeasts the capability to degrade several azodyes including Reactive Orange 16 and Reactive Blue 5 (Ruscasso et al. 2022).

While SDS degradation is well-documented in strains such as *Pseudomonas* sp. and *Bacillus* sp. from contaminated sites (Chaturvedi and Kumar 2011; Shahbazi et al. 2013), research on cold-adapted bacteria remains limited (Halmi et al. 2013; Manogaran et al. 2019). Our study investigates the potential of Antarctic microbes, especially *Flavobacterium* species as novel bioremediation tools for SDS removal - an emerging pollutant whose presence in the Antarctic marine environment was recorded as early as 1998 (George 2002). In this year, Margesin and Schinner (1998) reported the first description of biodegradation of the anionic surfactant sodium dodecyl sulfate (SDS) by cold-adapted diesel oil-degrading microorganisms at low temperatures.

Conclusions

This study successfully characterizes two novel psychrotrophic *Flavobacterium* strains isolated from an Antarctic consortium, demonstrating their remarkable functional versatility and multi-xenobiotic bioremediation potential. Notably, *F. pygoscelis* CIN7a stands out as a unique candidate, as it represents the first documented strain within its genus to possess a specialized keratinolytic system capable of achieving more than 80% degradation of raw feathers at low temperatures without requiring physicochemical pretreatments.

The metabolic complementation observed between the two isolates - where strain CIN7a excels in surface-active emulsification and aggressive enzymatic cleavage, while strain CIN7c prioritizes spatial

persistence through robust biofilm formation - highlights the ecological synergy of this polar consortium. Furthermore, the high efficiency of both strains in removing SDS and synthetic azo dyes reinforces their applicability in designing advanced, cost-effective biological treatments for complex industrial wastewater in cold environments.

Future perspectives will focus on optimizing multi-strain consortia formulations and characterizing the specific cold-adapted enzymes involved, aiming to scale up this sustainable bioremediation platform for industrial-scale applications.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not applicable

Consent to publication

Not applicable

Financial interests

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Author contribution

Beltina León: Data curation, Formal analysis, Investigation, Proofreading the article. Claudia Prieto: Data curation, Formal analysis, Investigation, Visualization, Proofreading the article. Marina Acosta: Investigation, Methodology. Alejandra Bosch: Funding acquisition, Project administration. Silvana Vero: Funding acquisition, Project administration, Proof reading the article. Gustavo Curutchet: Funding acquisition, Project administration. Sebastián Cavalitto: Funding acquisition, Project administration,

Proof reading the article. Ivana Cavello: Data curation, writing original draft, Funding acquisition, Project administration, Supervision, Conceptualization, Methodology, Proofreading the article. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

Data will be made available on request.

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Figures

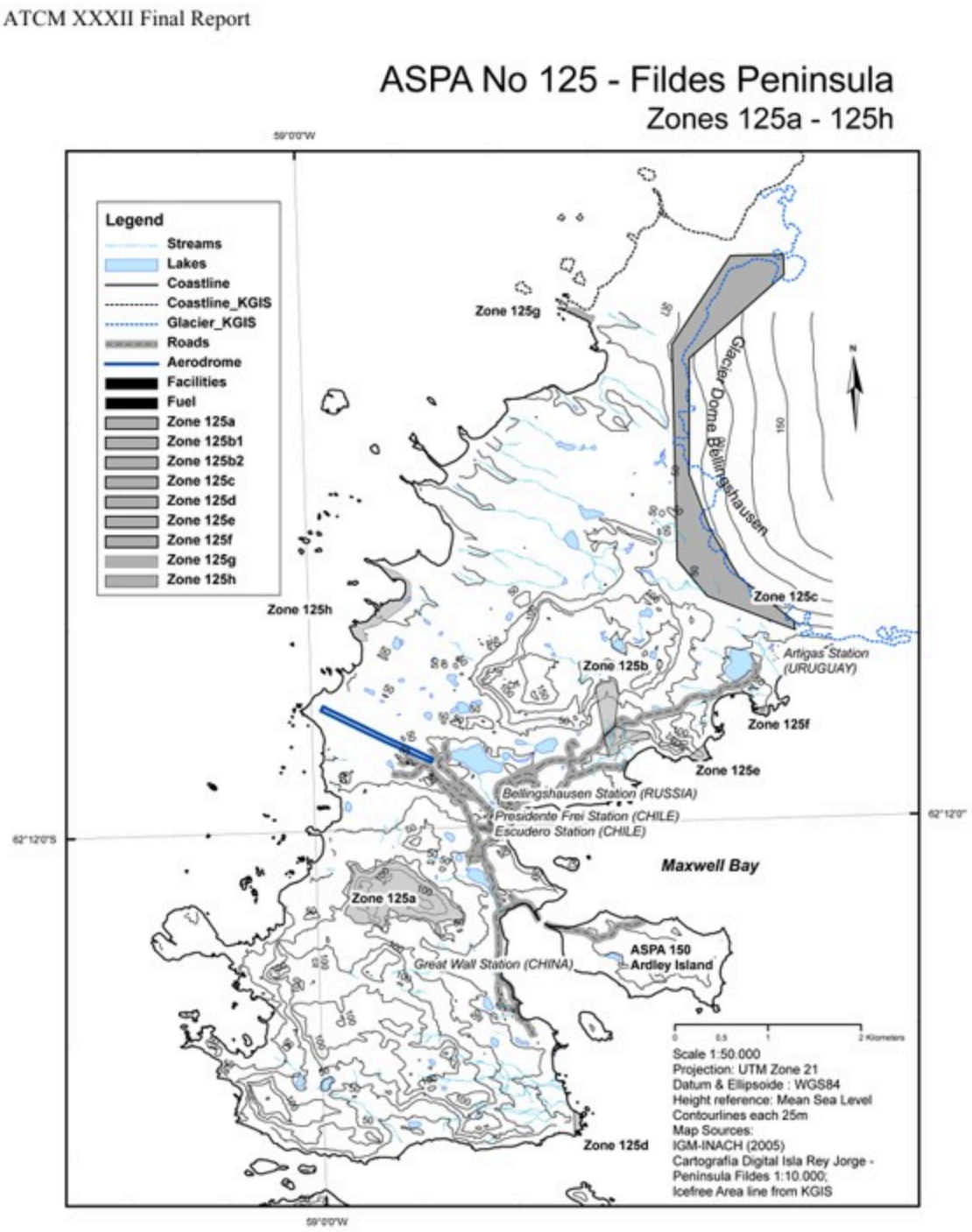


Figure 1

Geographical location of the sampling site. Map showing Half Three Point (ASPA 125d), Fildes Peninsula, King George Island, South Shetland Islands, Antarctica (62°11'4" S, 58°51'7" W), where the keratinolytic consortium was isolated.

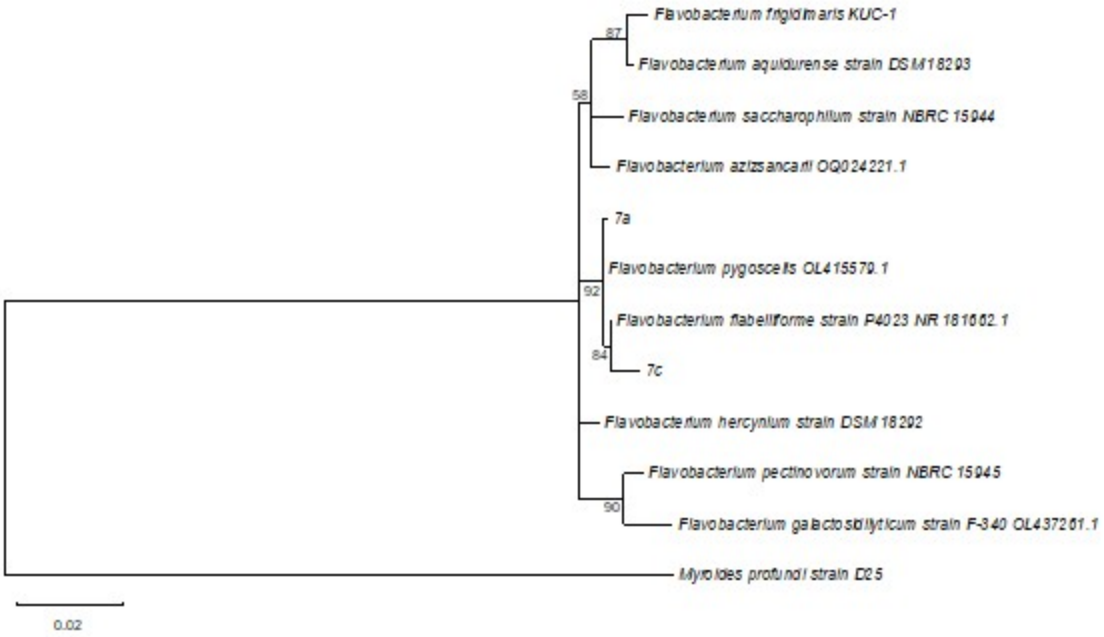


Figure 2

Molecular identification and phylogenetic analysis of Antarctic isolates. Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic position of *Flavobacterium pygoscelis* CIN7a and *Flavobacterium* sp. CIN7c. The tree was constructed using the Hasegawa-Kishino-Yano model with bootstrap values (1000 iterations) indicated at the nodes.

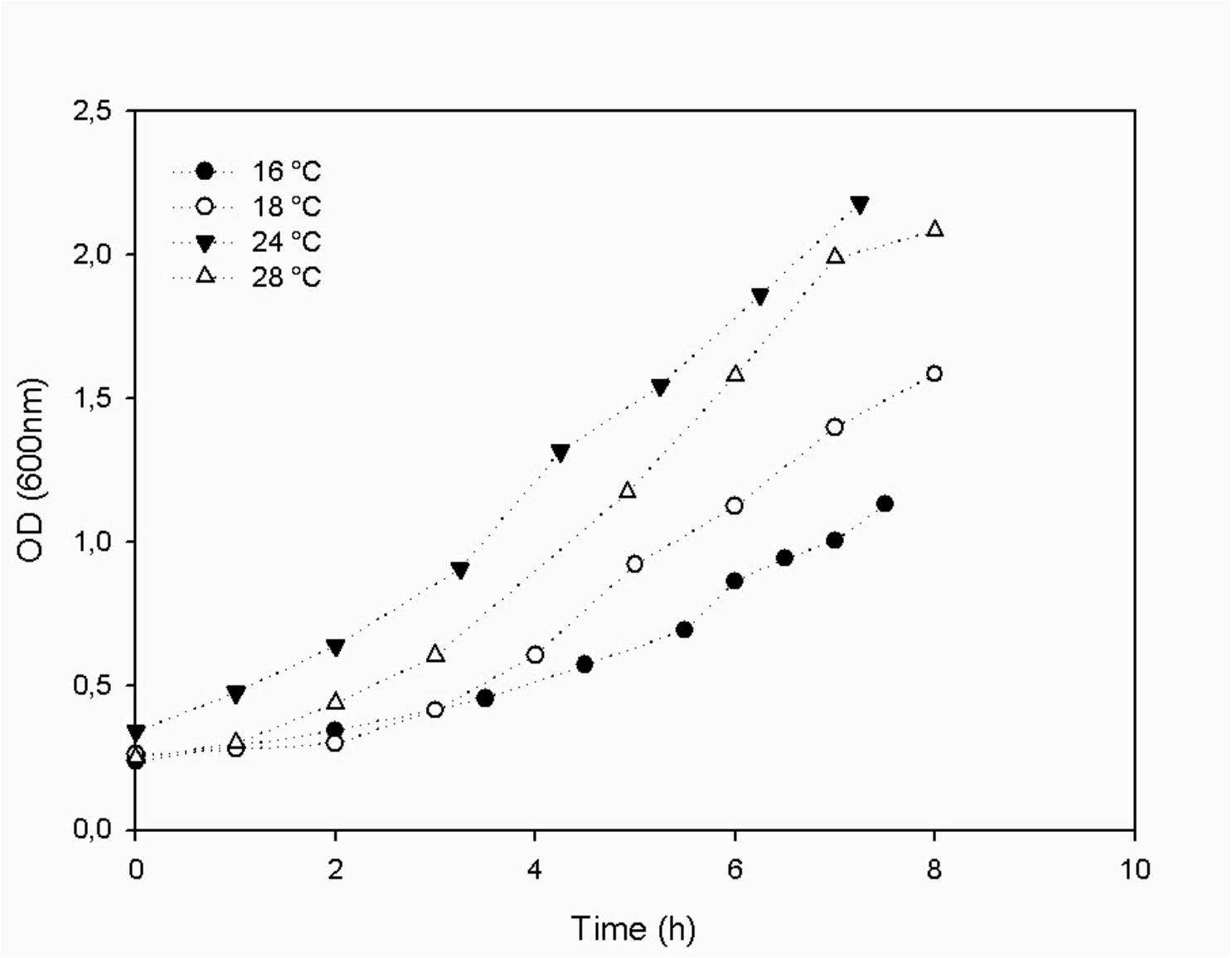


Figure 3

Temperature-dependent growth kinetics of *Flavobacterium pygoscelis* CIN7a. Growth curves monitored by optical density (OD_{600}) in the 16-28 °C range in Nutrient Broth. Data illustrates the psychrotrophic nature of the isolate, with the maximum growth rate (μ_{max}) achieved at 24 °C.

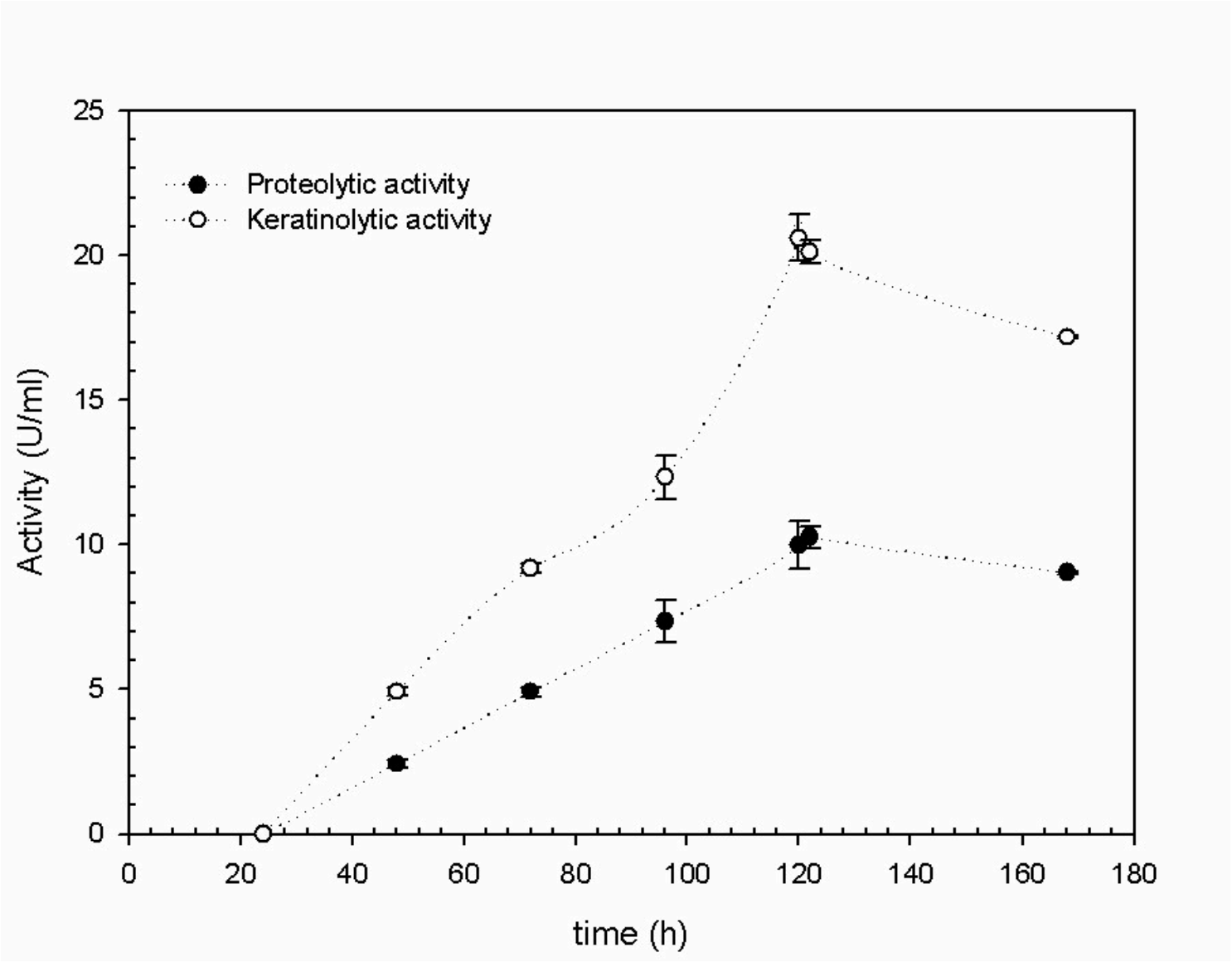


Figure 4

Time course of keratinolytic enzyme production and feather degradation by strain CIN7a. Profiles of proteolytic and keratinolytic activities (U ml^{-1}) during submerged fermentation at 24 °C using chicken feathers as the sole carbon and nitrogen source. Data are expressed as mean ± SD, n=3.

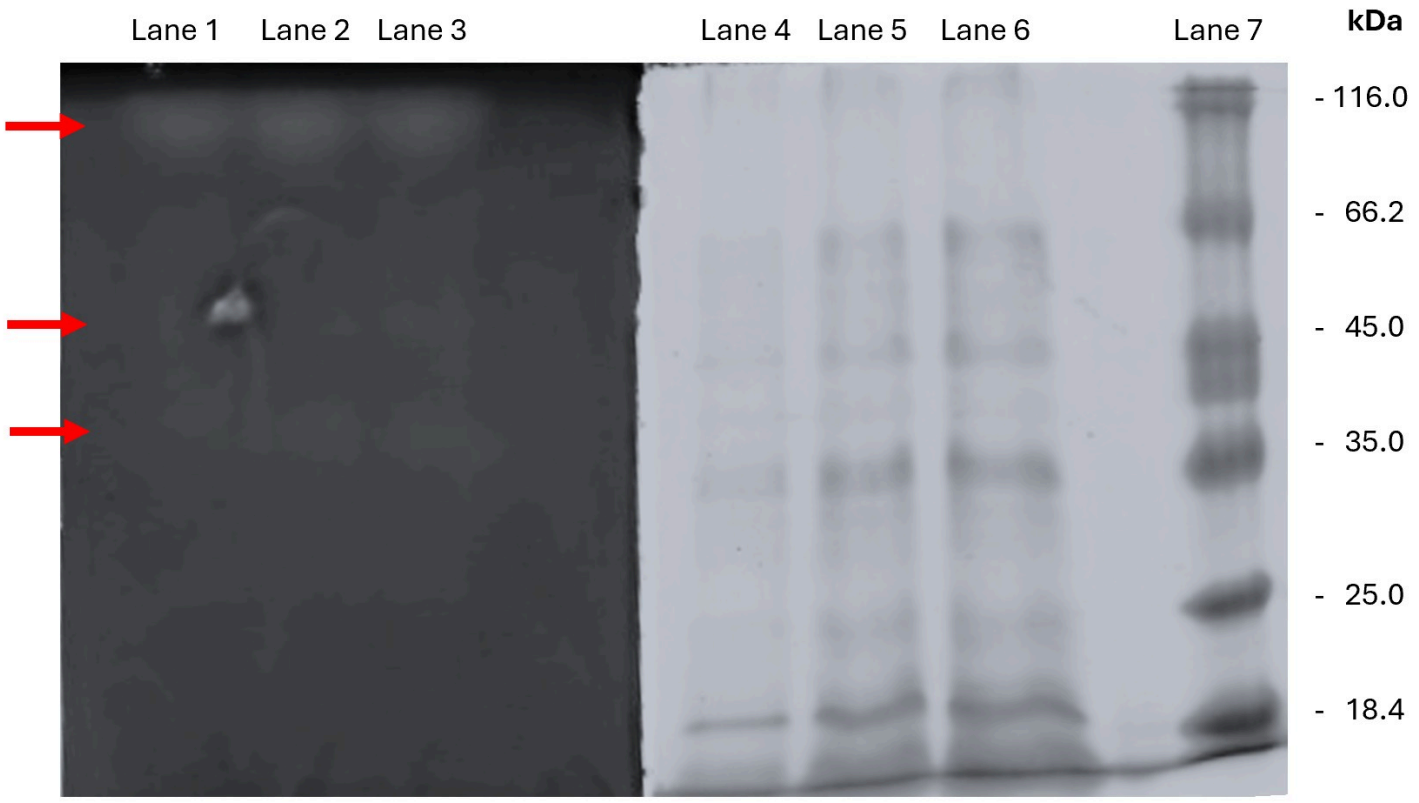


Figure 5

Proteolytic profile of *F. pygoscelis* CIN7a. SDS-PAGE (left) and casein-zymography (right) of the crude enzymatic extract. Lane 1-lane 3: zymogram staining of the protease crude extract; lane 4-lane 6: protein pattern of crude extract. Lane 7: standard pattern of protein markers (kDa, Thermo Scientific™ Pierce™ Unstained Protein 26610). Lanes 1 and 4: 10 ul (1,17 ug soluble protein), lane 2 and 5: 15 ul (1,76 ug soluble protein), lane 3 and 6: 20 ul (2,35 ug soluble protein).

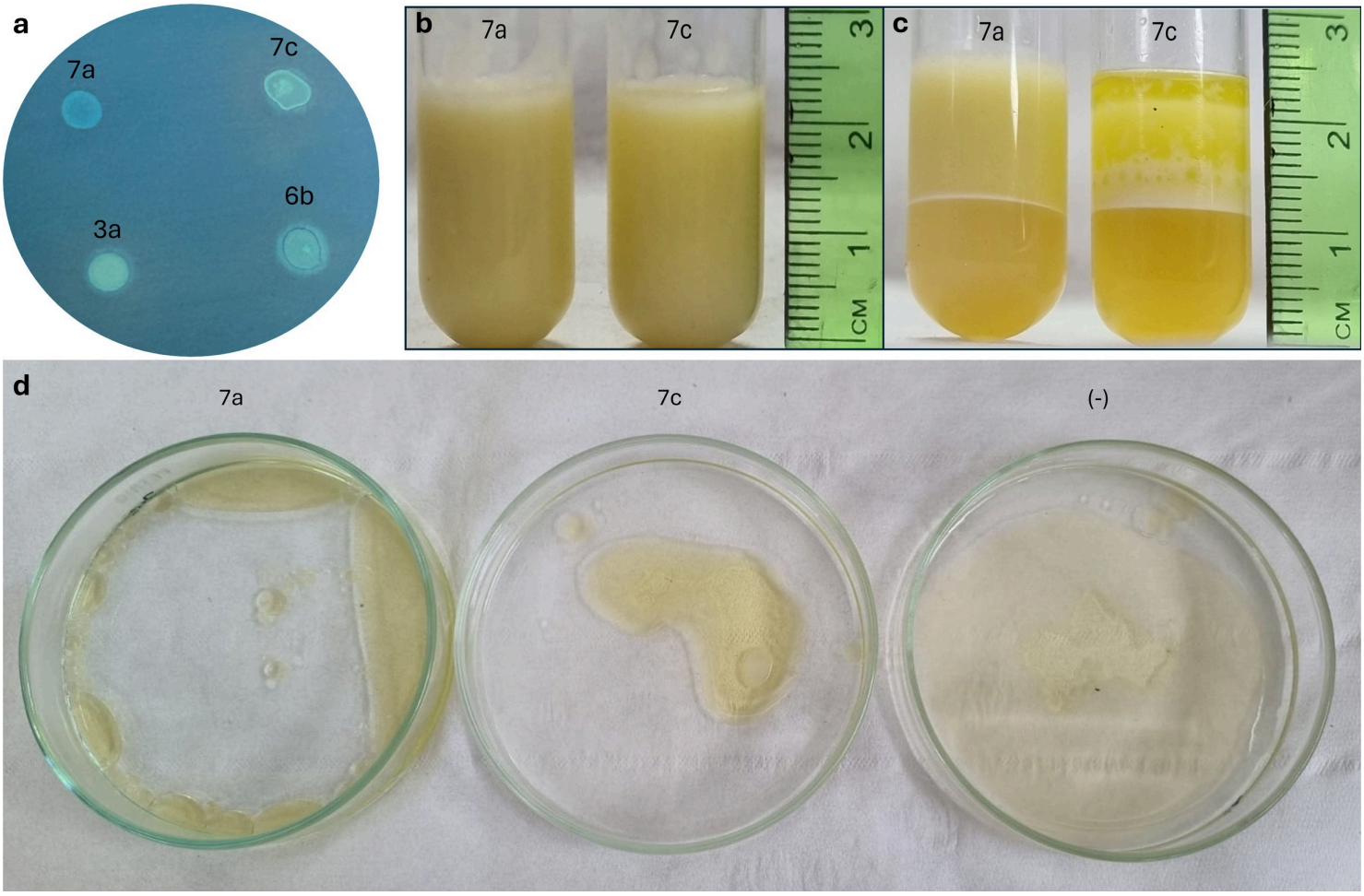


Figure 6

Screening of biosurfactant production by CIN7a and CIN7c isolates. (a) Qualitative CTAB-methylene blue agar assay for anionic biosurfactants. (b) Emulsification index (E24) using olive oil as substrate. (c) Oil-spreading assay showing the displacement of the oil layer by cell-free supernatants. Distilled water was used as a negative control for the assay.

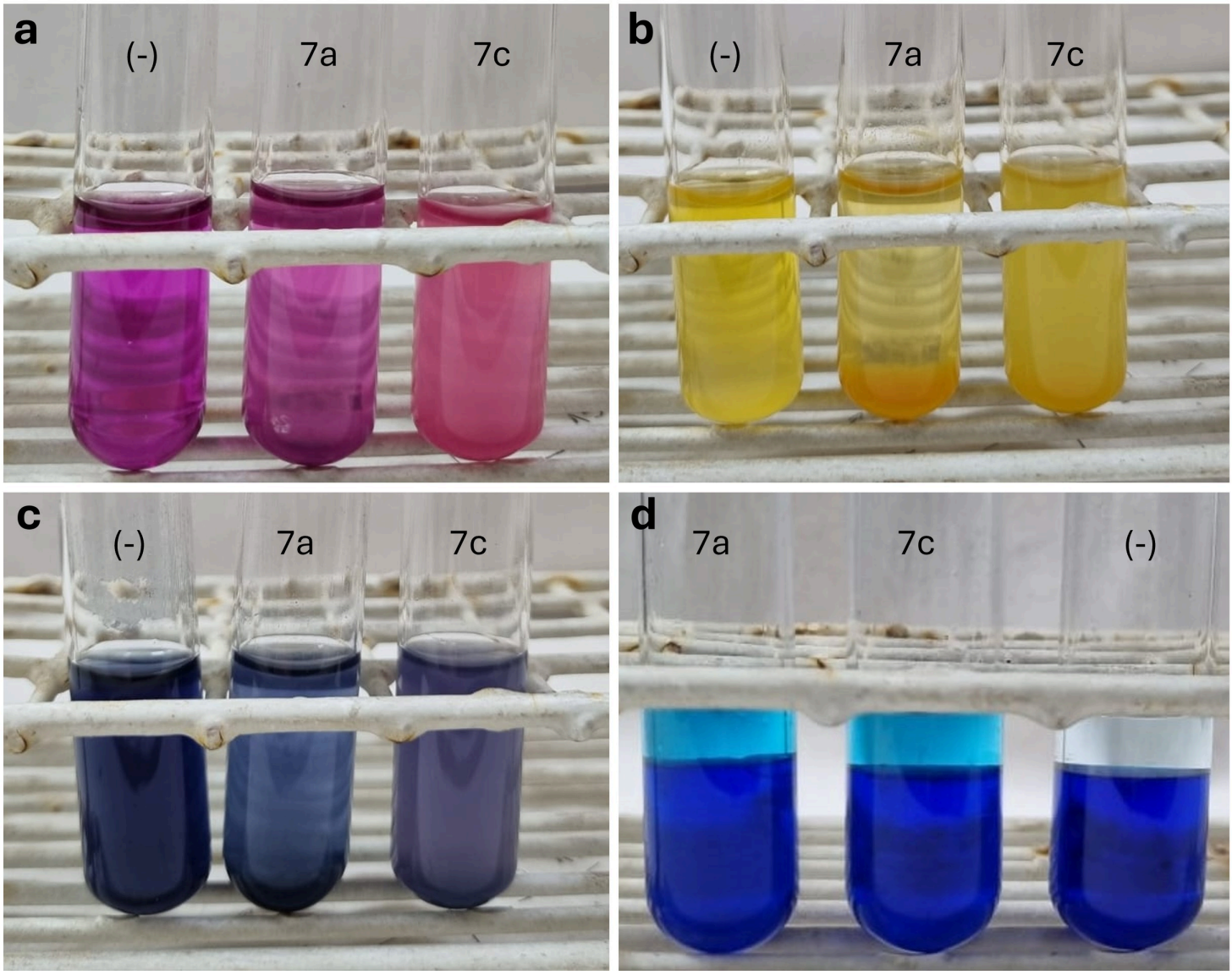


Figure 7

Bioremediation of recalcitrant contaminants. Removal of textile azo dyes and synthetic detergents: (a) Reactive Violet 5, (b) Reactive Yellow 84, (c) Reactive Black 5, and (d) growth-associated degradation of sodium dodecyl sulfate (SDS) as the sole carbon source.

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