Psychrophilic and cryophilic bacterial copiotrophs from a Trans-Himalayan lake-desert ecosystem as biogeothermometers of warming, mitigators of perturbation, and candidates for biodegradation

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ABSTRACT

A Trans-Himalayan lake-desert ecosystem (Tso Moriri, Changthang; Ladakh) was explored for the culturable diversity, and extreme-temperature adaptations, of psychrophilic/cryophilic bacterial chemoorganoheterotrophs. 27 species, under 15 genera across Actinomycetota, Bacillota, Bacteroidota, and Pseudomonadota, were isolated from the lake-sediment, lake-water, and fine-talus covering a lake-side rocky mountain. In Luria broth, all isolates grew at 4°C and 15°C; 13 grew, and 14 retained 19-99% cells in metabolically-active state, at -10°C; 23, 11, and none grew at 28°C, 37°C, and 42°C respectively. A *Cryobacterium* was most heat-susceptible (0.24% cells metabolically-active at 28°C); some cryophilic *Arthrobacter*, *Microbacterium*, *Paenarthrobacter*, *Pseudomonas* and *Streptomyces* were most warming-resilient (growth at 37°C; 7-58% cells active at 42°C). Using different simple/complex organic compounds, all isolates accomplished low/moderate/high growths at 4°C, 20 performed small growths at -10°C. Taxonomy/phylogeny did not govern the isolates' psychrophilic/cryophilic potentials or warming resilience. Lake-water and rock-dust isolates had the widest, while lake-sediment-dwellers had the narrowest, temperature-window for growth/survival. Most actinobacterial isolates could eradicate mesophilic bacteria from other warmer ecosystems, alongside non-actinobacteria and actinobacteria from their native/adjoining Tso Moriri habitats. Implications are discussed for negative feedback control of warming, microbiome protection from infiltration amidst climate-warming, niche partitioning within cold/frigid microbiomes, and cryophilic copiotrophs as constituents of cold-customized bioreactors.

INTRODUCTION

Microbial life at low temperatures is constrained by a number of biophysical and biochemical adversities (Cavicchioli et al. 2000; Öquist et al. 2009). However, across the seasonally or perpetually frozen alpine/polar territories of the Earth's biosphere, barren soils, outwardly life-less rocky terrains, and most conspicuously, numerous limnic and fluvioglacial features, harbor considerably diversified microbiomes (Cary et al. 2010; Choe et al. 2021) that in turn sustain highly climate-sensitive ecosystems via biogeochemical cycling of carbon and other elements within the cold/frigid realm (Finlay et al. 2010; van der Valk 2012; Clow et al. 2015).

Throughout the high-latitude and high-altitude areas of the globe, copious endolithic and chasmolithic microbial communities colonize the rocky terrains and contribute actively or passively to the weathering of rocks into dust and scree, thereby promoting sediment and soil formation (Archer et al. 2017; Samuels et. al., 2020). Supraglacial and subglacial microbiomes drive hectic biogeochemical activities (Hodson et al. 2008; Boetius et al. 2015; Yarzábal et al. 2021), with the former types accelerating the melting of glaciers (Cook et al. 2020; Williamson et al. 2020), and the latter generating copious greenhouse gases such as carbon dioxide and methane that normally remain trapped under the englacial cover but are released to the atmosphere upon deglaciation (Ma et al. 2018; Lamarche-Gagnon et al. 2019; Zhang et al. 2021). Microbiomes thriving in young as well as matured soils, including those associated with permafrosts, and biocrusts covering deglaciated moraines and rock surfaces, render temporally slow but spatially extensive, fixation and remineralization of carbon (Drotz et al. 2010; Nikrad et al. 2016). Furthermore, approximately, 25 million lacustrine bodies (Verpoorter et al. 2014), together with the vast expanses of thermokarst landforms, sequester huge amount of organic carbon by remaining frozen for a substantial part of the year, and emit enormous volume of carbon dioxide and methane upon thawing (Kessler et al. 2012; MacIntyre et al. 2018; Serikova et al. 2019; Guo et al. 2020; Johnston et al. 2020).

In all temporally or permanently frozen ecosystems, microbial activities and proliferation apparently remain subdued as long as the temperature remains below or around 0°C (Mohn and Stewart, 2000; Nikrad et al. 2016). However, hectic organotrophic growth, and thereby remineralization of carbon and emission of copious greenhouse gases, starts once the temperature rises and cryoturbation takes place on a seasonal or climatic scale (Graham et al. 2012; Aschenbach et al. 2013; Ernakovich et al. 2015; Nikrad et al. 2016; Walz et al. 2017). Greenhouse effect, at any spatiotemporal level, is thought to enhance microbial activities and growth, which in turn induces further thawing of the environment (Zimov et al. 2006; Nikrad et al. 2016). Such positive feedback cycles (Walter et al. 2006; Graham et al. 2012; Schneider von Deimling et al. 2012) can, in the long run, alter the structures and functions of microbiomes, and thereby entire ecosystems, within the cold/frigid realm.

To understand the functioning of cold/frigid ecosystems, and in order to manage them in a sustainable way, we require comprehensive information on the scopes of carbon cycling and

sequestration across the psychrophilic and cryophilic temperature ranges. For that purpose, chemoorganoheterotrophic capabilities of autochthonous microorganisms need to be delineated at near-zero and sub-zero degree Celsius, via extensive pure-culture-based investigations. Furthermore, in the context of global warming in the present era, it is imperative to appreciate the homeostatic vulnerabilities of alpine/polar microbiomes alongside their potential intrinsic resilience against perturbations. This would subsequently help mitigate impending microbiome (and habitat) alterations triggered by climate change across the global cryosphere. Towards that end, we require comprehensive data on how psychrophilic and cryophilic microorganisms (Cavicchioli, 2006, 2016; Raymond-Bouchard et al. 2018) respond to different levels of warming in terms of population-level growth or survival. Concurrently, in relation to plausible microbiome transformation within cold/frigid ecosystems due to global warming, we need to know whether psychrophilic/cryophilic microbiotas have any indigenous safeguard against infiltration of organisms from warmer territories (Hubert et al. 2009; Pearce et al. 2009).

In this study of pure-culture microbiology we explore three adjacent ecological systems within a Trans-Himalayan lake-desert continuum centered on the massive fresh-to-brackish water body called Tso Moriri (vernacular meaning: lake amid the mountains) that is situated on the cold arid Changthang plateau of eastern Ladakh, India. We isolate and characterize phylogenetically diverse chemoorganoheterotrophic bacterial psychrophiles and cryophiles from Tso Moriri's sediments, as well as water that remains frozen for approximately one third of a year (Biggs et al. 2015); we also do the same from the weathered rock dust (fine talus and scree particles) that covers the hill overlooking the western bank of the lake. It is investigated whether the copiotrophic microbial species isolated after subjecting the samples to iterative cycles of freezing and thawing in the laboratory, can grow or survive at the population-level by catabolizing simple or complex organic substrates at near-zero and sub-zero degree Celsius. We further explore how the growth and activity of the isolates are affected by different levels of warming, and whether natural populations of some of them can act as "biogeothermometers" chronicling *in situ* temperatures over time and space. From the contemporary perspective of heightened thawing of the cold/frigid realm, we examine what potentials the Tso Moriri microflora, especially the warming-resilient psychrophiles and cryophiles, have for resisting incursion of microorganisms from warmer climatic territories. For this purpose we test the antibiosis potentials of the new isolates against higher-temperature-adapted bacteria retrieved previously from distant warm ecosystems, situated within the same geographical province. Cold/frigid ecosystems being exceedingly vulnerable to burgeoning anthropogenic intrusion (Forbes et al. 2001; Aronson et al. 2011; Elser et al. 2020; Geoffroy et al. 2023), possibilities are deliberated for using the climate-resilient copiotrophic psychrophiles and cryophiles in bioreactors designed to degrade organic wastes at low temperatures (Gupta et al. 2020).

MATERIALS AND METHODS

Study site

The Himalayas and Trans-Himalayas encompass the largest reservoir of snow and ice on Earth outside the Arctic Circle and Antarctica (Meena and Kukreja, 2025). Within the cold arid vastness of the Himalayan rain shadow, the territory of Ladakh features an extensive high-altitude plateau, where multifaceted microbial life thrives to support homeostatically fragile ecosystems harboring unique multi-stress-adapted plants and animals (Dvorský et al. 2011, 2013; Aschenbach et al. 2013; Janatková et al. 2013; Angel et al. 2016). Covering a large swathe of eastern Ladakh (Fig. 1a) sprawls the Changthang desert dotted by several small to large lacustrine bodies among which Tso Moriri is one of the most prominent (Fig. 1b). This gigantic water body (Figs. 1b-c), located at an altitude of 4522 m, and GPS coordinates 32°54′ N and 78°18′ E, is flanked on all sides by barren hills (Figs. 1d-e) that rise either from a distance or very close to the bank of the lake (Fig. 1d). Small patches of lush green meadows, marshes, and wetlands hem only the northern and southwestern shores of Tso Moriri where two big glacial streams pour their water into the lake. Rest of the cold-desert habitat around the lake is characterized by a bleak topography and highly inhospitable physicochemical conditions that are paralleled only by the extreme multi-stressor environments of a few high-altitude areas of the Chilean and Argentinean Andes (Albarracin et al. 2015, 2016).

Sampling

On 28 October 2021, water (Fig. 1f) and sediment (Fig. 1g) samples were collected from near the western shore of Tso Moriri, together with samples of weathered rock dust from the barren slope of a hill overlooking the western bank of the lake (Fig. 1e). The entire hill slope, rising from ~100 m off the Tso Moriri shore, was devoid of any vegetative cover; visibly, not a single blade of grass grew on it.

In order to arrest the aquatic microbiota, as described previously (Mondal et al. 2024), five different batches of 500 mL water were sampled from five discrete sites located at intervals of 1 m, and within 1 m from the shore of the lake. Each batch of 500 mL water was aspirated from within 1 cm of the lake surface using a sterile syringe (Tarsons Products Limited, India), and passed through an autoclaved, Swinnex holder-mounted (Merck KGaA, Germany), sterile, mixed cellulose ester membrane filter (Merck Life Science Private Limited, India) having a mesh size of 0.22 μm and diameter of 47 mm. Subsequent to filtration, each membrane corresponding to the cell residue of 500 mL lake-water was dislodged from the Swinnex holder, folded using autoclaved forceps, and inserted into a 7.5 mL autoclaved cryovial that contained 5 mL of 15% (v/v) glycerol supplemented with 0.9% (w/v) NaCl (Roy et al. 2016).

From the same five locations where lake-water was sampled, sediments were collected in approximately equal quantities, and pooled inside a single 250 mL polypropylene bottle. Inside the polypropylene bottle the sediment fractions were mixed thoroughly to give rise to a bulk sample which was used for all downstream investigations. At each sampling point, soft deposits were scraped carefully from

the sediment-water interface using a flat and wide sterile spatula without disturbing layers deeper than 1 cm from the sediment-surface.

Dry and loose, weathered rock dust samples (fine talus and scree particles) were collected in approximately equal quantities from five distinct points located within a few meters of each other, on the lake-facing slope of the aforesaid hill. At each sampling site, fine powdery materials were scraped cautiously from the surface without disturbing layers deeper than the top 1 cm. The five sample fractions were pooled inside a 250 mL polypropylene bottle, and mixed thoroughly with a sterile spatula to yield a bulk sample that was used for all subsequent investigations.

After the insertion of a cell-precipitated membrane, or sediment / rock-dust sample, each cryovial or polypropylene bottle was capped tightly, sealed with parafilm (Tarsons Products Limited, India), and put inside a polyethylene bag, which eventually was packed in a heat-insulated ice box and shipped by air to the laboratory. All samples were investigated immediately upon their arrival at the laboratory.

Enrichment and isolation of psychrophilic and cryophilic chemoorganoheterotrophs

In order to enrich copiotrophic psychrophiles and cryophiles from Tso Moriri's water, each 0.22 μm cellulose acetate filter, which contained microbial cell residue from 500 mL lake-water, was shredded with sterile scissors inside the same vial in which it was inserted on-field. The vial was whirled for 15 minutes; after that the filter-shreds were allowed to settle at the bottom; finally, the supernatant was collected in a fresh sterilized vial without disturbing the debris. This process was repeated for all the five filter-containing cryovials involved in lake-water sampling, and the supernatants recovered from each of them were pooled to get an approximately 22 mL cell-suspension in NaCl-glycerol, which corresponded to 2.5 L of bulked lake-water. This ~22 mL cell-suspension was added to 80 mL Luria broth (LB) prepared in such a way that the actual LB concentration was achieved after the mixing. The inoculum-medium mixture was subjected to three consecutive cycles of "7 day freezing at -10°C, followed by 7 day thawing at 4°C", after which pure cultures were isolated at an incubation temperature of 4°C by means of dilution plating, picking of visibly-distinct single colonies, and repeated dilution streaking on Luria agar (LA) plates.

To enrich psychrophilic and cryophilic copiotrophs from the lake-sediment or rock-dust sample, 1 g of the corresponding material was added to 100 mL LB, and the suspension was subjected to three cycles of "7 day freezing at -10°C, followed by 7 day thawing at 4°C". After the third round of thawing, pure culture strains were isolated at 4°C in the same way as described above.

All the new isolates were maintained in LA slants with a standard transfer interval of 15 days. For routine growth in LB, or fortnightly transfer in LA, all isolates were grown at 4°C. The strains were classified, as described previously (Saha et al. 2019), up to the lowest taxonomic rank that was ascribable based on 16S rRNA gene sequence similarities with validly-published species curated in the List of Prokaryotic names with Standing in Nomenclature (Parte et al. 2020).

Determining the temperature window for growth

The temperature range over which the new isolates could, or could not, grow was delineated by recording the extent to which the density of colony forming units (CFU mL^{-1}) increased, or decreased, in the individual LB cultures of the strains after incubation at -10°C, 4°C, 15°C, 28°C, 37°C, and 42°C. For a given experiment, a seed culture of the strain tested was prepared by transferring a loopful of cell mass from a 7 day old LA slant culture to fresh 20 mL LB medium kept in a 50 mL Erlenmeyer flask, which was then incubated aerobically under shaking condition (120 rpm) at 15°C until when the culture attained its mid-log stage. From this 20 mL seed culture, cells were harvested, washed two times with 0.9% NaCl and finally resuspended in the same. The cleaned cell suspension was finally added to fresh 20 mL LB medium to set up the test culture, which typically had an initial (0 h) CFU density between 10^3 mL^{-1} and 10^5 mL^{-1} (experiments checking growth at 4-42°C were carried out in 50 mL Erlenmeyer flask, whereas those carried out at -10°C involved 50 mL polypropylene tubes).

CFU density was recorded at daily intervals for the experimental cultures incubated at -10°C, 4°C, 15°C, 28°C, and 37°C, whereas for the 42°C sets CFU density was recorded at intervals of 12 h. To record CFU density at any time point, 1 mL of the experimental culture concerned was serially diluted and plated on LA in triplicates (in case of -10°C incubations, the frozen cultures were first liquefied via thawing at 4°C, and then subjected to dilution plating). Subsequently, the LA plates were incubated at 15°C, and colonies appearing on them were counted after 2-3 days depending on the growth rate of the culture in question. Finally, the CFU density was calculated by first multiplying the colony-counts of the individual dilution-plates by their corresponding dilution factors, and subsequently adding and averaging the values across the dilution grades and replica plates available.

The above experiments were repeated for the comparator organism *Escherichia coli* by recording the increases or decreases in CFU density that the LB cultures of strain K-12 underwent after incubation at -10°C, 4°C, 15°C, 28°C, 37°C, and 42°C. *E. coli* seed cultures were prepared in the same as those prepared for the new isolates except for the fact that incubations were carried out at 28°C for 12 h. Experimental cultures too were subjected to the same procedure as above, while CFU densities for K-12 were recorded by incubating the colony-counting LA plates at 28°C for two days.

When the final CFU density of an experimental culture recorded after the stipulated period of incubation was higher than its initial (0 hour) CFU density, the growth rate of the concerned isolate, at the temperature in question, was denoted as the percentage change that was recorded in the CFU density over time (percentage of the initial CFU mL^{-1} culture that increased day^{-1} incubation).

$$\text{Growth rate} = \frac{(\text{Final CFU density} - \text{Initial CFU density}) \times 100}{\text{Initial CFU density}} \times \frac{1}{\text{Total days of incubation}}$$

Determining the temperature window for survival

When the final CFU density of an experimental culture recorded after the stipulated period of incubation was lower than its initial (0 hour) CFU density, the survival frequency of the concerned isolate's cells at the temperature in question was delineated in terms of what percentage of cells remained metabolically active in the culture. Proportion of metabolically-active cells in a given culture was determined by testing the cells' ability to take in the nontoxic and nonfluorescent molecule called fluorescein diacetate (FDA), and subsequently hydrolyze FDA to the tracer compound fluorescein by means of esterase activity (Battin, 1997). Fluorescein-stained cells, eventually, were detected with the help of flow cytometry, as described previously (Samaddar et al. 2016; Mondal et al. 2022).

The procedure of fluorescence-activated cell sorting (FACS) is described below in a nutshell. Cell-pellet was harvested via cold (4°C) centrifugation for 20 minutes at 6000 *g*, and then resuspended in 2 mL of a 0.9% NaCl solution. From a 0.5% (w/v) FDA (Sigma, USA) solution in dimethyl sulfoxide, 4 µl was added to the cell suspension and incubated at 37°C for 15 minutes. The cells were then washed and resuspended again in 500 µl of 0.9% NaCl. Ultimately, with the help of a BD FACSVerse flow cytometer (Becton, Dickinson and Company, USA), 10000 cells were analyzed randomly for their ability to fluoresce through 475-495 nm excitation and 520-530 nm emission. The BD FACSuite software package (Becton, Dickinson and Company) was used to present the data in the form of a dot plot depicting the level of fluorescence recorded for each cell as a function of its (photodiode-array-detected) ability to forward scatter light having a wave length of 488 nm. Positions of the experiment-specific quadrant gates separating the metabolically-active cells from their inactive counterparts were determined by analyzing an unstained version of the sample.

Testing chemoorganoheterotrophic growth on complex carbon compounds

The new isolates were tested for psychrophilic (at 4°C) and cryophilic (-10°C) growth on various simple to complex organic compounds as sole sources of energy, electron, and carbon. For this purpose, each strain was cultured, as described above, on modified basal and mineral salts (MS) solution (Ghosh and Roy, 2006) supplemented with any one of the following organic compounds (HiMedia Laboratories, India) at one particular instance (L^{-1} double distilled Milli-Q water): acetate (10 mM), agar (2 g), benzoate (5 mM), cellulose (5 g), chitin (10 g), n-hexadecane (0.5 mL), pectin (10 g), water-soluble starch (4g) and xylan (10 g). For testing psychrophilic and cryophilic growth on single chemoorganoheterotrophic substrates isolates were incubated for 14 and 28 days respectively.

Testing antibiosis in the new isolates

Members of the phylum Actinomycetota are recognized for their exceptional proficiency in antibiotic production (Barka et al. 2016; Ebrahimi-Zarandi et al. 2022). All actinobacterial species currently isolated from the three different environments of the Tso Moriri area (TMA) were tested by agar overlay assay (Berberov et al. 2025) for their antibiosis potentials against higher-temperature-adapted, mesophilic, Gram

negative and Gram positive bacteria that had been isolated previously from distantly located warmer habitats within the Western-Himalayan and Trans-Himalayan territory. The target organisms included the temperate soil isolate *Advenella kashmirensis* WT001 (Ghosh et al. 2005, 2013), the Puga Valley hydrothermal vent isolate *Paracoccus* SMMA_5 (Roy et al. 2016; Mondal et al. 2022), and the Chumathang hydrothermal vent isolates *Bacillus subtilis* SC_1, *Bacillus licheniformis* PAMA2_SD1, and *Lysinibacillus fusiformis* LAPE1_SD1 (Dutta et al. unpublished). The actinobacterial isolates were also tested for their antibiosis capabilities against other isolates obtained from the TMA, including fellow Actinomycetota members.

The potential antagonists (actinobacterial isolates) were first grown in LB, at 15°C for 48 h, to generate their seed cultures. 10 µL inocula from the individual seed cultures were spotted on LA plates at minimum mutual distances of 3 cm, following which the plates were incubated at 15°C for 48 h. After fully grown colonies had appeared for all the potential antagonists investigated, 100 µL of a mid-log-phase culture of the target organism against which antibiosis was to be tested, was mixed with 900 µL molten agar (0.4% w/v) having 37°C temperature, and poured uniformly on the LA plates that were already dotted by incumbent colonies of the potential antagonists. Seed culture of the incoming bacterium (target of potential antibiosis) was grown at 28°C or 15°C, according as the organism was a mesophile from a foreign habitat or a current isolate from the TMA. Eventually, these test plates were incubated for 48 h (at 28°C or 15°C, according as the incoming bacterium was a mesophile from a foreign habitat or a TMA isolate) and checked for the development of uninhibited lawns of growth, or cleared zones of eradication, for the incoming organism, around the pre-established colonies of the incumbent actinobacteria (potential antagonists).

RESULTS

Cold-adapted copiotrophs from Tso Moriri lake-desert ecosystem

A sum total of 61 aerobic, copiotrophic, and freeze-thaw resilient bacterial pure-cultures, apparently dissimilar with regard to colony morphology, were enriched and isolated in Luria broth, from the lake-water, lake-sediment, and rock-dust samples (Table 1). 22 of these were obtained from the weathered rock dust of the lake-side hill, while 5 and 34 were from the lake's water and sediments respectively. 16S rRNA gene sequence similarities clustered the 61 isolates into 27 species level entities that in turn were ascribable to the genera *Acinetobacter*, *Aeromonas*, *Ancylobacter*, *Arthrobacter*, *Cryobacterium*, *Flavobacterium*, *Microbacterium*, *Mycetocola*, *Paenarthrobacter*, *Pseudarthrobacter*, *Pseudomonas*, *Psychrobacter*, *Sanguibacter*, *Streptomyces* and *Trichococcus*. Furthermore, the 15 genera identified in this way were distributed over the phyla Actinomycetota, Bacillota, Bacteroidota and Pseudomonadota; the five genera classified under Pseudomonadota were affiliated to the two major classes Alphaproteobacteria and Gammaproteobacteria.

13 out of the total 15 genera isolated from the TMA appeared to have an environmental preferentiality. Whereas *Microbacterium* strains were isolated from the lake-water as well as weathered rock dust of the lake-side hill, and *Pseudarthrobacter* were retrieved from the lake-sediment and rock-dust samples, *Arthrobacter*, *Mycetocola*, *Paenarthrobacter* and *Streptomyces* were restricted to the rock-dust; *Acinetobacter* and *Ancylobacter* were obtained only from the lake-water, and *Aeromonas*, *Cryobacterium*, *Flavobacterium*, *Pseudomonas*, *Psychrobacter*, *Sanguibacter* and *Trichococcus* were isolated from the lake-sediment only. Eventually, one representative strain from each species level cluster was selected for further characterization (Table 1).

TMA psychrophiles as cryophilic and cryotolerant copiotrophs

In LB medium, all the 27 species retrieved from the Tso Moriri area could grow at 4°C (Figs. 2c-d) and 15°C (Figs. 2e-f), whereas only 13 could grow at -10°C (Figs. 2a-b). In terms of the percentages of the starting (initial) CFU densities that were existent in the different cultures after the stipulated periods of incubation (Tables S1-S3), the extents of growth recorded at -10°C (109% - 3285%) were far lower than those recorded at 4°C (244% - 376552%) and 15°C (1000% - 809524%). After 14 days at 4°C, the cultures of *Flavobacterium* TS_SC_2 and *Arthrobacter* TRD_SC_3 showed the lowest and highest increases in CFU density respectively. After two days at 15°C, lowest and highest increases in CFU density were exhibited by the cultures of *Flavobacterium* TS_SC_2 and *Arthrobacter* TRD_SC_8 respectively. After 28 days at -10°C, *Pseudarthrobacter* TRD_SC_9 and *Arthrobacter* TRD_SC_6 had the lowest and highest increases in CFU density respectively.

Notably, the 14 species, which could not grow in LB at -10°C, managed to maintain considerable proportions of their cell populations in metabolically-active (as evidenced by FDA-staining followed by flow cytometry, Figs. 2g and 3) and/or divisible (as evident from CFU data, Figs. 2a-b) states. After 28 days at this temperature, *Acinetobacter* TW_SC_4 and *Aeromonas salmonicida* TS_SC_11 retained the lowest and highest proportions (2% and 75%) of the initial CFU density respectively, while *Pseudarthrobacter* TS_SC_4 had the lowest (19%), and *Cryobacterium* TS_SC_7 and *Sanguibacter* TS_SC_8 had the highest (99%), proportions of cell stained with FDA. Furthermore, for every bacterium lacking growth at -10°C, the percentage of metabolically-active cells exceeded the percentage of cells retaining their divisibility after 28 days of incubation at this temperature, even though no significant correlation existed between the proportions of divisible and metabolically-active cells across the 14 species incapable of growing at -10°C (Fig. S1a).

Psychrophilic and cryophilic growth on one simple/complex carbon compound at a time

After 14 days of incubation at 4°C, all the bacterial species isolated from the TMA rendered at least a little growth by utilizing no less than three out of the 10, simple or complex, organic compounds tested as single chemoorganoheterotrophic substrates (Fig. 4a, Tables S4-S13). The extent of growth differed across the isolates in terms of the percentage increase in CFU density that was attributable to the

utilization of the carbon compound in question (Table S14). 10 TMA isolates were found to accomplish low, moderate, or high growth on all the 10 compounds tested; these were *Arthrobacter* TRD_SC_3, *Aeromonas salmonicida* TS_SC_11, *Cryobacterium* TS_SC_7, *Flavobacterium* TS_SC_2, *Flavobacterium* TS_SC_5, *Microbacterium* TRD_SC_10, *Sanguibacter* TS_SC_8, *Pseudomonas* TS_SC_12, *Pseudomonas* TS_SC_13 and *Psychrobacter* TS_SC_6. Although unable to use acetate, *Streptomyces* TRD_SC_5 could accomplish high growth on maximum number of complex carbon compounds, namely cellulose, chitin, pectin, starch and xylan; this organism also rendered moderate growth on agar, albumin and hexadecane, while its growth on benzoate was low. In contrast, *Pseudomonas* TS_SC_3, which could only render moderate growth on cellulose, and low growth on albumin and starch, was the least efficient TMA isolate in terms of chemoorganoheterotrophic utilization of the carbon substrates tested. Overall, highest (3200%) increase in CFU density, attributable to pectin utilization, was recorded for *Pseudarthrobacter* TRD_SC_9, whereas the lowest (0.7%) increase in CFU density, attributable to albumin utilization, was recorded for *Microbacterium* TW_SC_2.

After 28 days of incubation at -10°C, a sum total of 20 TMA isolates were found to render small but definite growth on at least one of the 10 carbon compounds tested (Fig. 4b, Tables S15-S24), even though in LB medium, only 13 species had exhibited low to high growth at -10°C (Figs. 2a-b; Table S3). Of the 14 TMA isolates that had failed to render cryophilic growth in LB, seven – namely, *Aeromonas salmonicida* TS_SC_11 (benzoate and cellulose), *Ancylobacter* TW_SC_1 (agar and chitin), *Cryobacterium* TS_SC_7 (acetate, albumin, cellulose and pectin), *Flavobacterium* TS_SC_5 (agar), *Microbacterium* TRD_SC_10 (agar, albumin, benzoate, chitin and starch), *Pseudarthrobacter* TS_SC_4 (agar and starch), and *Pseudomonas* TS_SC_10 (agar) – could render a little growth attributable to the use of at least one carbon compound (Table S25). Overall, *Arthrobacter* TRD_SC_3 accomplished small growth on all the 10 compounds tested, while *Streptomyces* TRD_SC_5 rendered small growth on all the substrates except acetate, and *Mycetocola* TRD_SC_2 could do the same on all the carbon compounds except acetate, albumin and benzoate. Of the remaining 24 isolates, 17 could grow on a few, or just one, of the 10 organic substrates, whereas seven could grow on none of them. The highest (95%) increase in CFU density, attributable to agar utilization, was recorded for *Arthrobacter* TRD_SC_3, wheres the lowest (1.11%) increase in CFU density, attributable to starch utilization, was recorded for *Arthrobacter* TRD_SC_8.

Extreme oligotrophy in the psychrophilic and cryophilic temperature range

In the above experiments, growth of an isolate on a given organic substrate was defined exclusively by a positive remainder value obtained after subtracting the percentage increase in CFU density recorded for the isolate in MS solution from the percentage increase in CFU density recorded in MS plus the substrate in question (furthermore, for the two the percentage terms in question, only positive values were taken into consideration). A separate scrutiny of the CFU density values obtained for the different isolates after incubation in MS solution at 4°C and -10°C revealed their extreme oligotrophic potentials.

At 4°C, after 14 days of incubation in MS solution (Table S26), CFU densities of *Microbacterium* TW_SC_2, and *Pseudomonas* strains TS_SC_1 and TS_SC_10, increased by >200% of the initial levels, whereas those of *Acinetobacter* TW_SC_4, *Arthrobacter* TRD_SC_1 and TRD_SC_6, *Microbacterium* TW_SC_3, *Mycetocola* TRD_SC_2, *Paenarthrobacter* TRD_SC_7 and *Trichococcus* TS_SC_9 increased by 6-72%. Under these conditions, CFU densities of *Arthrobacter* TRD_SC_3, *Microbacterium* TRD_SC_10, and *Streptomyces* TRD_SC_5 remained unchanged, while those of the remaining 14 strains decreased by 3-90% of the initial levels.

At -10°C, after 28 days in MS (Table S27), CFU densities of *Arthrobacter* strains TRD_SC_1 and TRD_SC_6, *Microbacterium* strains TW_SC_2 and TW_SC_3, *Mycetocola* TRD_SC_2, and *Paenarthrobacter* TRD_SC_7 increased by 10-50%, whereas those of the remaining species decreased by 2-98%, of the corresponding initial levels.

Progressive thermal susceptibility of the TMA isolates

In Luria broth, 23 out of the 27 TMA isolates studied could grow at 28°C (Figs. 5a-b; Table S28), whereas only 11 could grow at 37°C (Figs. 5c-d; Table S29), and none at 42°C (Figs. 5e-f; Table S30). In terms of the percentages of the starting CFU densities that were there in the different cultures after the specified incubation periods, extents of growth recorded at 28°C (149% - 482143%) were much higher than those recorded at 37°C (131% - 14750%). After four days of incubation at 28°C, *Sanguibacter* TS_SC_8 and *Microbacterium* TW_SC_2 had the lowest and highest increases in CFU density. Likewise, after two days at 37°C, *Arthrobacter* TRD_SC_6 and *Ancylobacter* TW_SC_1 exhibited lowest and highest increases in CFU density.

Of the four species incapable of growing in LB at 28°C, three did not retain any CFU, while only *Arthrobacter* TRD_SC_4 retained 28% of the initial CFU density, after four days of incubation at this temperature. The three organisms having no CFU left after four days at 28°C had 0.2-22% cells, whereas *Arthrobacter* TRD_SC_4 had 58% cells, stained with FDA after this incubation (Figs. 5g and 6a). In this way, after four days of incubation at 28°C, *Arthrobacter* TRD_SC_4 had the maximum proportions of cells in divisible as well as metabolically-active conditions, whereas *Cryobacterium* TS_SC_7 had zero and near-zero cells in the two states, respectively.

Overall 16 TMA isolates failed to grow in LB at 37°C. The lone *Cryobacterium* and the two *Flavobacterium* species, which had already failed to retain any CFU at 28°C, could not do so at 37°C either. Predictably, they had lower proportions of cells stained with FDA after two days at 37°C (Figs. 5h and 6b), compared with their respective proportions of FDA-stained cells recorded after four days at 28°C (Figs. 5g and 6a). Additionally, after two days at 37°C, retention of CFU density went down to near-zero (0.01-0.04) percent for *Arthrobacter* TRD_SC_4, *Psychrobacter* TS_SC_6 and *Sanguibacter* TS_SC_8 (these three species, however, had 4-39% cells stained with FDA, after this incubation). For the remaining 10 TMA isolates incapable of growing at 37°C, 0.4-48% of the initial CFU densities were recorded in the

cultures, while 4.5-76% of the cells were found to take FDA stain, after two days of incubation at this temperature.

None of the 27 species retrieved from the Tso Moriri area could grow in LB at 42°C. Expectedly, the six species which had retained zero or near-zero CFU density at 28°C and/or 37°C could not retain any CFU at 42°C; they also had lower proportions of cells stained with FDA after one day at 42°C (Figs. 5i and 7), compared with the corresponding percentages recorded after two days at 37°C (Figs. 5h and 6b). Furthermore, after one day at 42°C, retention of CFU density went down to zero or near-zero percent of the initial level for 13 other species, which however had 0.06-58% cells stained with FDA. For the remaining eight TMA isolates, 0.4-33% of the starting CFU densities were recorded in the cultures, while 7-87% of the cells were found to take FDA stain, after one day at 42°C.

In the context of growth or survival above the psychrophilic range, it was noteworthy that for all the 4, 16, and 27 strains lacking growth in LB at 28°C, 37°C, and 42°C, respectively, the percentage of cells remaining metabolically active at a given temperature exceeded the percentage of cells which retained their divisibility at that temperature (Figs. 5g-i). Furthermore, for any strain susceptible to different levels of high temperature, retention of CFUs, as well as the proportion of FDA-staining of cells, after the stipulated period of incubation, decreased with increase in temperature. That said, no significant correlation was observed between the proportions of divisible and metabolically-active cells across the 4, 16, and 27 species incapable of growth at 28°C, 37°C, and 42°C, respectively (Figs. S1b-d).

Differential temperature windows for growth and survival

At every incubation temperature tested for growth in LB medium, different TMA isolates exhibited different rates of increase or decrease in CFU density (Tables S31-36 show the data for the six individual temperatures). On the flip side, temperature windows for growth (increase in CFU density) and survival (retention of >1% cells in metabolically-active state), over the tested range, varied across the isolates (Fig. 8). *Arthrobacter* TRD_SC_3 and TRD_SC_8, *Microbacterium* TW_SC_2 and TW_SC_3, *Paenarthrobacter* TRD_SC_7, and Streptomyces TRD_SC_5, shared the widest temperature range over which growth was recorded (-10°C to 37°C), whereas *Flavobacterium* TS_SC_5 and *Cryobacterium* TS_SC_7 had the narrowest growth window (4°C to 15°C).

For all those 4°C-growing psychrophiles whose growth in LB ceased at -10°C, more than 1% cells remained metabolically active at this freezing temperature (Fig. 2g); the -10°C-growing cryophiles, on the other hand, were likely to possess resilience against even lower temperatures. Above the psychrophilic range, all the TMA isolates, except *Arthrobacter* TRD_SC_6 and *Cryobacterium* TS_SC_7, exhibited survival at temperatures above the points where their growth was last recorded (Fig. 9a). For TRD_SC_6 and TS_SC_7, growth in LB was last recorded at 37°C and 15°C respectively, but their population-level survival dropped below the 1% threshold at the immediately higher temperature points tested, i.e. at 42°C and 28°C respectively (Figs. 2 and 5).

So far as the most suitable temperature for LB growth was concerned (Fig. 8), 22 TMA isolates had

their highest rates of increase in CFU density at 15°C (for all these, except *Microbacterium* TRD_SC_10, growth rate at 15°C exceeded the same at 4°C by orders of 10). For *Microbacterium* TW_SC_2, *Streptomyces* TRD_SC_5, *Ancylobacter* TW_SC_1 and *Acinetobacter* TW_SC_4, maximum growth rate was recorded at 28°C, whereas for *Mycetocola* TRD_SC_2 alone, maximum growth rate was recorded at 4°C (notably the growth rate of TRD_SC_2 at 15°C was only 4% less than that at 4°C).

Antibiosis in the TMA actinobacteria

Of the 15 actinobacterial species isolated from the Tso Moriri area, 11 had the ability to eradicate at least one of the six mesophilic foreign bacteria against which antibiosis was tested (Fig. 10). Only the four *Arthrobacter* species represented by the strains TRD_SC_1, TRD_SC_3, TRD_SC_4 and TRD_SC_8 had no antagonistic activity against any of these six higher-temperature-adapted organisms. In contrast, fully grown colonies of both *Pseudarthrobacter* TRD_SC_9 and *Streptomyces* TRD_SC_5 disallowed the growth or colonization of all the six Gram negative or Gram positive bacteria targeted, while *Paenarthrobacter* TRD_SC_7 abolished all but *Advenella kashmirensis* WT001; *Arthrobacter* TRD_SC_6 could deter only one target mesophile, namely *Lysinibacillus fusiformis* LAPE1_SD1. On the flip side of the above data, most number of TMA actinobacteria (nine each) were active against the Gram positive targets *Lysinibacillus fusiformis* LAPE1_SD1 and *Bacillus licheniformis* PAMA2_SD1, followed by the Gram negative target *Escherichia coli* K-12 that was deterred by seven TMA actinobacteria. Five, four, and three TMA actinobacteria were found to abolish the growth of *Bacillus subtilis* SC_1, *Advenella kashmirensis* WT001, and *Paracoccus* SMMA_5, respectively.

The same 11 actinobacterial isolates, which had abolished the growth of foreign mesophilic bacteria, also eradicated at least one or more non-actinobacterial TMA isolates (Fig. 10). The lake-water isolate *Microbacterium* TW_SC_2 could eradicate most number of TMA bacteria (nine) outside Actinomycetota, including those from the lake-water, lake-sediment, and rock-dust. In contrast, the rock-dust isolate *Arthrobacter* TRD_SC_6 could abolish only one TMA bacterium (the lake-sediment isolate *Psychrobacter* TS_SC_6). From the reverse perspective, *Aeromonas salmonicida* TS_SC_11, followed by *Trichococcus* TS_SC_9 and *Pseudomonas* TS_SC_1, all isolated from the lake's sediments, was the most vulnerable TMA bacteria outside Actinomycetota. Growth of these three organisms was abolished by 10, eight, and seven TMA actinobacteria, respectively. In contrast, the lake-water isolate *Ancylobacter* TW_SC_1, and the three lake-sediment isolates *Flavobacterium* TS_SC_2, *Pseudomonas* TS_SC_12 and *Pseudomonas* TS_SC_13, were least prone to antibiosis as they were susceptible to only three actinobacterial species.

Out of the 15 TMA bacteria belonging to Actinomycetota, eight could abolish the growth of fellow actinobacterial isolates from their native, as well as adjoining, habitats (Fig. 10). Whereas five of these eight actinobacteria-destroying actinobacteria were themselves eradicated by one or more actinobacteria, (Fig. 10), three of them, namely *Microbacterium* TRD_SC_10, *Paenarthrobacter*

TRD_SC_7, and *Pseudarthrobacter* TRD_SC_9, were resistant to any antagonism by any of the TMA actinobacteria. Overall, the rock-dust isolates *Microbacterium* TRD_SC_10 and *Pseudarthrobacter* TRD_SC_9 (Table 1) could eliminate most number of actinobacteria (six each) from across the three Tso Moriri environments explored. In contrast, the rock-dust isolate *Arthrobacter* TRD_SC_6, and the lake-water isolate *Microbacterium* TW_SC_2, abolished only one TMA actinobacteria each, namely, the two rock-dust isolates *Arthrobacter* TRD_SC_3 and TRD_SC_6 (Fig. 10; Table 1). From the opposite point of view, *Arthrobacter* TRD_SC_8 (abolished by eight TMA actinobacteria) - followed by *Arthrobacter* TRD_SC_6, *Arthrobacter* TRD_SC_4 and *Pseudarthrobacter* TS_SC_4 (each abolished by four TMA actinobacteria) - was the most vulnerable TMA isolates belonging to Actinomycetota. In contrast, the lake-water isolate *Microbacterium* TW_SC_3, and the three rock-dust isolates *Pseudarthrobacter* TRD_SC_9, *Paenarthrobacter* TRD_SC_7 and *Microbacterium* TRD_SC_10, were not prone to any of the fellow actinobacterial species (Fig. 10; Table 1).

DISCUSSION

Habitat as the key driver of adaptation to extremes of temperature

All the genera under which the TMA isolates were classified, excepting *Ancylobacter* and *Paenarthrobacter*, encompassed multiple members retrieved previously from other natural or artificial cold/frigid environments (Table S37; also see Supplementary References). Remarkably, each of them also encompassed at least one member retrieved previously from a mesic/hot habitat (Table S38; also see Supplementary References). Consistent with these trends of wide thermal adaptation and ecological amplitude, different TMA isolates belonging to the same genus exhibited differential growth/survival responses to different levels of temperature. For instance, four conspicuous patterns of responses across temperatures were recorded for the five species of *Arthrobacter*, and two distinctive patterns were identifiable for the two species of *Pseudarthrobacter*, isolated from the TMA (Fig. 9a). Finer differences were also recorded between the temperature response patterns exhibited by the two *Flavobacterium* and three *Microbacterium* species retrieved from this area (Figs. 2 and 5).

A number of present findings further suggested that the native environment of microorganisms, rather than their phylogenetic background, exerted the most crucial influence on adaptation to extremes of temperature. For all the genera isolated in this study, except *Paenarthrobacter*, member strains from other parts of the world, especially mesic/hot environments, are known to grow at temperatures higher than the maxima recorded for the TMA variants (Fig.11; Table S39; also see Supplementary References). As for *Paenarthrobacter*, it was intriguing as to why the growth temperature maxima of its strains reported earlier from warmer habitats, and that of the current isolate from cold/frigid environment, converged at 37°C. It was, however, understandable that the growth temperature minimum of *Paenarthrobacter* was pegged thus far at 4°C (Table S39; Kotoučková et al. 2004; Busse et al. 2016) because no member of this genus had been isolated from any cold habitat before this study. With regard to variable cold adaptation in

closely-related bacteria, it was further noteworthy that none of the genera isolated from Tso Moriri area, except *Cryobacterium*, *Pseudomonas*, *Psychrobacter* and *Sanguibacter* (Molin et al. 1986; Bakermans et al. 2006; Liu et al. 2012; Pikuta et al. 2017), encompassed any such strain from any other part of the world which possessed growth temperature minimum lower than its TMA counterpart (Fig. 11; Table S39).

While taxonomic affiliation of the TMA isolates appeared to have no bearing on their growth or survival potentials at different temperatures, certain habitat-dependent adaptive inclinations seemed to unify majority of strains isolated from a given environment within the ecosystem (Fig. 9b).

(1) The bacteria from Tso Moriri's water accomplished growth or survival through the entire range of incubation temperatures tested (i.e. -10°C to 42°C). Specifically, all but one were cryophilic, one was cryotolerant, and all were thermotolerant.

(2) All the bacteria isolated from the weathered rock dust of the lake-adjoining hill, with the exception of only *Arthrobacter* TRD_SC_4 and TRD_SC_6, also grew or survived through the entire temperature range tested. Specifically, all were cryophilic, while eight out of the total 10 (TRD_SC_4 and TRD_SC_6 being the exceptions) were thermotolerant, in nature.

(3) In contrast to the lake-water or rock-dust bacteria, most strains isolated from Tso Moriri's sediments had growth across a narrow range of incubation temperature. For most of them, growth at -10°C and 37°C was absent; furthermore, for six out of the 13 lake-sediment bacteria, population-level survival dropped below the 1% threshold at 42°C, or at even lower temperatures.

Cold desert biomes within the Trans-Himalayas, including the climatically-harsh Changthang Plateau, are constrained by multiple environmental stressors that collectively lead to an overall low biodiversity in these territories. However, microbial life flourishes amid the low atmospheric pressure (that translates into low oxygen availability for respiration), high ultra-violet radiation, and extreme aridity, which characterize these areas over and above the central biophysical stressor prevailing in the form of wide seasonal and diurnal variation of ambient temperature (from -40°C during the winter to 35°C during the summer; Dar et al. 2024). Tso Moriri's surficial water freezes in the winter and thaws in the summer, while the rocky slopes of the adjoining hills get covered by snow in the winter and the same melts through the summer, exposing the surface to extreme heating and cooling on a diurnal basis. Thus, the possession of wider growth/survival temperature windows by bacteria from these two environments (Fig. 9b) could be reflective of a general linkage between adaptation to the extremes of temperature and the variability of the thermal condition prevailing in the habitat. By the same reasoning, it is also quite natural for the bacteria from Tso Moriri's subsurface (presently sediments, but this can also be true for bacteria from the deeper water-levels) to have narrower temperature windows for growth and survival than their surficial relatives (Fig. 9b). The subsurface being shielded from the atmosphere experiences slenderer temperature fluctuations, so the bacteria residing in these insulated habitats tend not to develop very wide-ranging adaptation to the extremes of temperature.

To elucidate the biochemical and biophysical drivers of differential cold-adaptation and heat-resilience in taxonomically close psychrophilic/cryophilic entities, a new line of comparative multi-omics

investigation needs to be undertaken for the TMA isolates of *Arthrobacter*, *Flavobacterium*, *Microbacterium* and *Pseudarthrobacter*, alongside their global phylogenetic relatives (Mandakovic et al. 2020; Shen et al. 2021; Gushgari-Doyle et al. 2022; Kim et al. 2023; Ghimire et al. 2024). Simultaneously, findings of such future studies need to be interpreted in the context of the habitats' comprehensive physical and chemical conditions so as to understand the environmental dependencies of cold-adaptation as well as heat-resilience in psychrophilic and cryophilic microorganisms. Furthermore, in the context of environment as a key driver of low temperature adaptation, it is noteworthy that for *Acinetobacter*, *Aeromonas*, *Microbacterium*, *Paernarthrobcter*, *Pseudarthrobacter* and *Trichococcus*, strains known to grow at genus-specific minimum temperatures before this study (Fig. 11; Table S39) were all isolated from mesic or hot environments (Krovacek et al. 1991; Kotoučková et al. 2004; Pikuta et al. 2006; Yamahira et al. 2008; Kallimanis et al. 2009; Paściak et al. 2024), even though other strains of *Acinetobacter*, *Aeromonas*, *Microbacterium*, *Pseudarthrobacter* and *Trichococcus* had been isolated from natural and/or artificial cold/frigid environments (Table S37). On the flip side, strains of *Aeromonas* and *Mycetocola* that were known to grow at genus-specific maximum temperatures before this study (Fig. 11; Table S39) were all isolated from natural or artificial cold/frigid environments (Rouf and Rigney, 1971; Zhu et al. 2013), even though other strains of these genera had been isolated from mesic or hot environments (Table S38). These ecological idiosyncrasies could be reflective of some hitherto unappreciated metabolic convergence between microbial adaptations to high and low temperatures. Such a counterintuitive idea is reinforced by the facts that out of the 20 cryophilic bacteria detected in the TMA, 16 and 10 could grow at 28°C and 37°C respectively (Fig. 9a). Furthermore, after 1 day at 42°C, only eight TMA isolates had >0.1% CFU left in the culture (Figs. 5f and 5i), of which again six were cryophiles (Fig. 2b). Notably, these thermotolerant cryophiles included *Arthrobacter* TRD_SC_8, which, among all TMA isolates, retained the highest proportion of CFUs after 1 day at 42°C.

Autochthonous populations of heat-susceptible psychrophiles and cryophiles as biogeothermometers of *in situ* warming

The TMA isolates, irrespective of their phylogenetic background, exhibited different levels of thermal susceptibility. For instance, almost all cells of *Cryobacterium* TS_SC_7 lost their divisibility, as well as metabolic activity, after a four-day exposure to 28°C (Figs. 5b and 5g), so their viable presence in the lake's sediments indicated that the temperature of this under-water substratum either does not reach 28°C, or even if it does so, it does not remain incessantly at that level for several days. On the flip side, elimination of such bacteria from environments where they had been prevalent previously would indicate protracted *in situ* warming above the psychrophilic range. Population dynamics of TS_SC_7-like bacteria can, therefore, be construed and contrived as spatiotemporal biogeothermometers of cold/frigid environments that are indisposed to prolonged physical monitoring.

Almost all cells of *Flavobacterium* TS_SC_5 and TS_SC_2 lost their metabolic activity after a full day at 42°C (Figs. 5f and 5i), while their divisibility was already abolished after a single day's exposure to

37°C (Fig. 5c), or even after a two-day exposure to 28°C (Fig. 5a). Likewise, almost all cells of *Arthrobacter* TRD_SC_4 lost their activity after a full day at 42°C (Figs. 5f and 5i), while their divisibility was already abolished after a two-day exposure to 37°C (Figs. 5d and 5h), and only a quarter of the cell population retained their divisibility after four days at 28°C (Figs. 5b and 5g). Thus, a progressive decline in the population of such *Flavobacterium* species over time and/or space can be correlated with a continuing temporal and/or spatial escalation of environmental temperature to 28°C and above. The same for *Arthrobacter* TRD_SC_4-like populations can, in turn, corroborate ongoing spatiotemporal increases of *in situ* temperatures to 37°C and above. In the same vein, population dynamics of bacteria like *Arthrobacter* TRD_SC_6 and *Trichococcus* TS_SC_9, within cold/frigid ecosystems, can be used as real-time bellwethers signaling shifts of *in situ* temperature towards 42°C.

Thermal susceptibility of autochthonous organotrophs can usher negative feedback control of warming in cold/frigid ecosystems

Within cold/frigid ecosystems vulnerable to global warming and anthropogenic perturbations (Forbes et al. 2001; Aronson et al. 2011; Elser et al. 2020; Geoffroy et al. 2023), microbial activity is thought to remain minimal as long as temperature remains below or around 0°C. However, hectic carbon cycling, and so copious greenhouse gas emission, starts once the temperature rises and cryoturbation takes place due to season and/or climate change (Knoblauch et al. 2018; Schuur et al. 2022). Greenhouse effect of heightened microbial activity stimulates *in situ* biotic processes all the more, and that in turn enhances further thawing. In this way, a positive feedback loop of cyclical warming is operationalized in the ecosystem (Walter et al. 2006; Graham et al. 2012; Schneider von Deimling et al. 2012).

In case of the TMA microflora, vigorous organotrophic growth was recorded for all the isolates at 4°C, while slow but sizeable growth also took place for ~75% of them at -10°C (Figs. 2 and 4). How these *in vitro* potentials for psychrophilic and cryophilic catabolism translate (under normal climatic conditions) into community-level actions *in situ* is central to the delineation of baseline carbon cycling and sequestration in cold/frigid ecosystems. However, elucidation of the actual biogeochemical implications of cold-adapted copiotrophs - in terms of their contribution to carbon remineralization (conversion of organic matter to carbon dioxide) and subsequent facilitation of methanogenesis, at different levels of freezing and thawing of the environment - would require multi-omics information on the microflora and geochemical knowledge of the ecosystem, integrated to microcosm experiments involving pure-culture isolates.

The psychrophilic (4-15°C) growth proficiencies of the TMA isolates, in juxtaposition with their progressive susceptibilities to temperatures ≥28°C, corroborated that native-microbes-mediated positive ecological feedback abet environmental warming most effectively within the 4°C to 28°C range. On the other hand, as the temperature approaches 28°C, and rises further, step-by-step cessation of organotrophic growth, and metabolic activity, across the cold-adapted microflora (Fig. 5), putatively cuts back on the *in situ* production of simple fatty acids and carbon dioxide, the central substrates for

methanogenesis which is positioned at the terminal end of the syntrophic chain that governs the carbon cycle (Hedderich and Whitman, 2013). Curb on the generation of greenhouse gases by the microflora can trigger a negative feedback control of warming, and usher over time, a course reversal in the vicious cycle of “warming - microbial growth - and further warming”.

On the part of a cold/frigid ecosystem, exercise of negative feedback control (termination of growth of autochthonous bacteria) towards the restoration of homeostatic balance, is fraught with the danger of thermally-better-adapted “microbial weeds” (Cray et al. 2013; Cowan et al. 2014) from warmer regions infiltrating and colonizing organotrophic niches within the perturbed habitat. Such incursions can rapidly transform microbiome architectures if not abated reasonably below the critical high temperature (Kosaka et al. 2019) where most cells of most species of the native microflora lose their growth as well as metabolic activity. Furthermore, microbiome transformations can, in the long run, alter overall ecosystem functions drastically, and in doing so tilt the equilibrium irreversibly in favour of the positive feedback mechanism which promotes warming (Walter et al. 2006; Graham et al. 2012; Schneider von Deimling et al. 2012). At such critical ecological crossroads, prospects of microbiome, and habitat, preservation across alpine/polar territories hinge not only on the warming-resilience of native psychrophiles and cryophiles but also on their inherent ability to resist foreign microbial invasion.

Heat-enduring actinobacterial psychrophiles and cryophiles as defenders of cold/frigid microbiomes

Varied levels of thermotolerance were exhibited by all the TMA isolates, except *Cryobacterium* TS_SC_7, in the 28-42°C temperature range (Figs. 5 and 9a). 23 species could grow, while three could retain >1% cells in metabolically-active condition, at 28°C. 11 grew, while 15 retained >1% cells in active state, at 37°C. Whereas none could grow at 42°C, 19 retained >1% cells in active state at this temperature. This staggered resilience of the microflora against warming might be adequate to revive their *in situ* populations, and restore community structure, as long as warming remains limited in time, space (across microenvironments), and degree of heating. However, as the different temperature thresholds withstood by autochthonous psychrophiles and cryophiles are surpassed over time and/or space, population size recovery becomes more and more difficult, even after warming trends are reversed. Moreover, with rising temperature, native cold-adapted populations not only stop growing but their proportion of active cells also decline critically (Fig. 5). Consequently, they run the risk of being removed from the habitat, and their ecological niches become susceptible to take-over by more-thermotolerant intruders from discrete geographical territories, via long or short range dispersal (Hubert et al. 2009; Pearce et al. 2009). In such scenarios of imminent microbiome alteration, antibiosis potentials of heat-enduring actinobacteria native to the ecosystem can deter foreign microbes from colonizing the habitat and apprehending the ecological niches of the indigenous psychrophiles and cryophiles. In the long run, this kind of niche safeguard enhances the chances of rejuvenation for the autochthonous cold-adapted populations upon reversal of thawing.

Actinomycetota members are known for their extraordinary abilities to produce secondary metabolites including antibiotics, which confer them adaptive fitness against diverse environmental challenges, resulting in the high ecological amplitude of the phylum (Claverías et al. 2015; Barka et al. 2016; Lewin et al. 2016; Ebrahimi-Zarandi et al. 2022). Moreover, they are also known to be abundant in cryospheric microbiomes (Voytsekhovskaya et al. 2018), and protect indigenous microbial communities from external invaders in other critical ecosystems (Salazar-Hamm et al. 2025). So far as the 15 TMA actinobacteria were concerned, a majority of them were substantially thermotolerant. While 13 and eight could grow, one and six could retain >1% cells in active state, at 28°C and 37°C, respectively. Albeit none could grow at 42°C, 11 retained >1% cells in active condition at this temperature (Figs. 5 and 9a). In addition to this, 11 TMA actinobacteria were capable of eradicating Gram positive and/or Gram negative bacteria from discrete higher-temperature ecosystems at 28°C (Fig. 10), which is lower than the critical high temperature for most of the TMA isolates (Fig. 5). Furthermore, of the 11 actinobacterial species having antibiosis potentials against foreign bacteria, six were isolated from the rock-dust sample, while three and two were from the lake's sediments and water respectively (Fig. 10). This distribution suggested that microbiome protection (thwarting possible incursion by foreign microbes from warmer territories) by antibiosis-enabled heat-enduring actinobacterial psychrophiles and cryophiles could be widespread across physicochemically distinct cold/frigid habitats. That said, authenticity of this hypothesis remains to be verified by multi-omics-based analyses of *in situ* population dynamics within geographically diverse cold/frigid ecosystems, followed by *in vitro* experiments designed in the context of complex community-/trophic-level interactions (Liu et al. 2022).

In relation to the overall ecological implications of antibiosis it was noteworthy that a number of TMA actinobacteria eliminated not only foreign microorganisms, but also co-inhabitants of their own environment as well as natives of their adjacent habitats (these included fellow actinobacteria also). These findings brought to the fore extensive niche partitioning (Dussud et al. 2018; Shaiber et al. 2020) as a central stratagem of microbiome functioning in cold/frigid ecosystems. Presumably, the intricacies of minute niche differentiation were not revealed in the present results because bulk sampling had already smudged their imprints. To reveal the actual ecological importance of niche partitioning, plus macro- and micro-environmental factors (Cowan et al. 2020), in the functioning of microbiomes within cold/frigid ecosystems, very-finely-resolved spatial studies of *in situ* population dynamics are needed alongside those aimed at revealing community heterogeneity and inter-specific interactions.

Using TMA bacteria to solve the concerns of biodegradation in cold/frigid localities

Besides global warming, burgeoning human activities have, in recent times, brought about unprecedented perturbation in the delicately balanced ecosystems across high-altitude as well as high-latitude territories of the world. One of the major factors detrimental to these pristine ecosystems is the accretion of anthropogenic wastes. The pace of microbial growth (Mohn et al. 2000; Nikrad et al. 2016; also see Figs. 2 and 8), and thereby degradation of organic matter (Mohn et al. 2000; Si-Zhong et al. 2009; Gupta et al.

2020), being slow in the cryophilic as well as psychrophilic range, waste-treatment systems (bioreactors) operated in alpine and polar territories require external energy input for optimum-temperature maintenance (Dhaked et al. 2010); consequently, these contrivances are neither eco-friendly nor cost effective. Even bioreactors employing microbes isolated from cold/frigid habitats also sometimes fail to deliver efficient results because psychrophiles and cryophiles, owing to their residence in and adaptation to organic-carbon-scarce habitats (Zimov et al. 2006; Schuur et al. 2015), are often oligotrophic (Gilichinsky et al. 2008; Zhang et al. 2013), and so unfit for proliferation in the nutrient-rich environments of bioreactors. Moreover, tens of degree-Celsius thawing above the freezing point of water runs the risk to making natural cold-adapted bacterial populations uncompetitive against the thermotolerant, and mostly copiotrophic, microorganisms that find their way into the bioreactors along with the waste materials introduced for degradation (García-Descalzo et al. 2022; Piercy et al. 2025). Towards solving these concerns of biodegradation in alpine and polar localities, microbes such as the TMA psychrophiles and cryophiles – by virtue of their abilities to (i) grow via catabolism of simple to complex carbon compounds over a wide range of temperature, (ii) grow under low as well as high concentrations of organic matter, and (iii) thwart the proliferation of thermotolerant microbes from other warmer habitats – can become ideal constituents of bioreactors designed to perform through zero and sub-zero degree Celsius temperatures. To successfully materialize such *ex situ* objectives, however, in-depth information is needed on their syntrophic requirements and contributions (especially in relation to autochthonous cold-adapted methanogens that can augment the internal heat content of bioreactors; Cavicchioli, 2006, 2016; Menzel et al. 2020), niche overlaps and separations, antagonisms and cooperations, not only in the context of co-culture consortia, but also under the aegis of the biogeochemical conditions prevailing in their natural habitats.

SUPPLEMENTARY DATA

Supplementary information and data are available online in the form of a Word file named Supplimentary_Information.docx, and an Excel file named Supplimentary_Dataset.xlsx.

DATA AVAILABILITY

GenBank accession numbers for the 16S rRNA genes of the new isolates are as follows:
PV789631 (*Arthrobacter* sp. TRD_SC_1), PV789699 (*Arthrobacter* sp. TRD_SC_3), PV789726 (*Arthrobacter* sp. TRD_SC_4); PV789747 (*Arthrobacter* sp. TRD_SC_6); PV789751 (*Arthrobacter* sp. TRD_SC_8); PV793422 (*Cryobacterium* sp. TS_SC_7); PV790046 (*Microbacterium* sp. TRD_SC_10); PV793448 (*Microbacterium* sp. TW_SC_2); PV793500 (*Microbacterium* sp. TW_SC_3); PV789771 (*Mycetocola* sp. TRD_SC_2); PV682789 (*Paenarthrobacter* sp. TRD_SC_7); PV790021 (*Pseudarthrobacter* sp. TRD_SC_9); PV793432 (*Pseudarthrobacter* sp. TS_SC_4); PV793425 (*Sanguibacter* sp. TS_SC_8); PV789776 (*Streptomyces* sp. TRD_SC_5); PV793427 (*Trichococcus* sp.

TS_SC_9); PV793436 (*Flavobacterium* sp. TS_SC_2); PV793440 (*Flavobacterium* sp. TS_SC_5); PV790452 (*Ancylobacter* sp. TW_SC_1); PV793444 (*Acinetobacter* sp. TW_SC_4); PV793419 (*Aeromonas salmonicida* TS_SC_11); PV793374 (*Pseudomonas* sp. TS_SC_1); PV793375 (*Pseudomonas* sp. TS_SC_3); PV793377 (*Pseudomonas* sp. TS_SC_10); PV793378 (*Pseudomonas* sp. TS_SC_12); PV793393 (*Pseudomonas* sp. TS_SC_13); PV793413 (*Psychrobacter* sp. TS_SC_6).

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AUTHOR CONTRIBUTIONS

W.G. conceived the study, designed the experiments, interpreted the results, and wrote the paper. S.C. anchored the program, planned and performed the experiments, analyzed and curated the data, and also composed the paper. S.D. analyzed the data, while S.D., J.G., S.S., M.M., J.S., and N.M. performed the experiments. All authors read and approved the manuscript.

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COMPETING INTEREST

The authors declare no competing interest.

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LEGENDS

Fig. 1. Geographical location of the Tso Moriri lake, topography of the explored area, and position of the sampling sites. (**a**) Map of northern India showing the location of the Tso Moriri lake. (**b**) Satellite view of the lake shown in the context of the adjoining areas of the Changthang desert (location of the study area is indicated by a yellow square). (**c**) Satellite view of the west bank of Tso Moriri where the study sites (indicated by cyan arrows) were located. (**d**) Panoramic view of the portion of the lake and the adjoining hill from where samples were collected. (**e**) The barren hill slope where weathered rock dust (fine talus and scree) was sampled for the upper 1 cm from the surface. (**f**) Shallow coastal water of Tso Moriri that was sampled from within a depth of 1 cm. (**g**) Shallow coastal sediments of Tso Moriri that were sampled for the top 1 cm.

Fig. 2. Growth, survival, or abolition of cell populations documented for the TMA isolates in LB medium at psychrophilic and cryophilic temperatures. Growth of individual isolates, or the lack of it, was recorded at (**a** and **b**) -10°C, (**c** and **d**) 4°C, or (**e** and **f**) 15°C, and expressed as increase, or decrease, in CFU density over time (final CFU count mL^{-1} of the culture as a percentage of the initial value). Every number given here is an arithmetic mean of data acquired from three different experiments, where standard deviation always remained <2% of the mean. Numerical values of the CFU densities underlying the data shown in panels (**a** and **b**), (**c** and **d**), and (**e** and **f**) can be found in Tables S1, S2, and S3 respectively. (**g**) Percentage of the initial CFU density retained by each isolate lacking growth at -10°C is tabulated alongside the percentage of cells remaining metabolically active (capable of taking FDA stain) in the same culture. Refer to Fig. 3 for the flow cytometry-derived dot plots depicting what proportions of cells were stained by FDA in the 14 different isolates incapable of growing at -10°C.

Fig. 3. Flow cytometry-derived dot plots depicting what proportions of cells were stained by FDA (after 28 days of incubation) in the LB cultures of the 14 TMA isolates incapable of growing at -10°C. In every dot plot, the levels of fluorescence recorded for 10000 randomly chosen cells have been shown as the functions of the corresponding forward scattering data, with green and red points representing FDA stained and unstained cells respectively. Each FACS experiment was repeated two more times, with deviation from the result (percentages of FDA stained and unstained cells) shown here being <2% on every occasion.

Fig. 4. Extent of (**a**) psychrophilic or (**b**) cryophilic growth (or the lack of it) recorded for the different TMA isolates, in MS medium supplemented with one simple or complex carbon compound at a time. After incubation for (**a**) 14 days at 4°C, or (**b**) 28 days at -10°C, growth due to the utilization of a given carbon compound was delineated exclusively as a positive remainder value obtained after subtracting the percentage increase in CFU density recorded for the same organism in MS (here also, only positive

values were considered) from the percentage increase in CFU density recorded in MS supplemented with the carbon compound in question (here too, only positive values were considered). Numerical values for the percentage increases in CFU-density that were attributable to the utilization of the different carbon compounds at 4°C and -10°C have been given in Tables S14 and S25, while all CFU density values underlying the data shown in Tables S14 and S25 have been presented in Tables S4-S13 and S15-S24, respectively.

Fig. 5. Growth, survival, or abolition of cell populations documented for the TMA isolates in LB medium at temperatures above the psychrophilic range. Growth of the individual isolates, or the lack of it, was recorded at (**a** and **b**) 28°C, (**c** and **d**) 37°C, or (**e** and **f**) 42°C, and expressed as increase, or decrease, in CFU density over time (final CFU count mL^{-1} of the culture as a percentage of the initial value). All the numbers given in this figure are arithmetic means of the data acquired from three different experiments, with standard deviations being always <2% of the corresponding means. Numerical values of the CFU densities underlying the data shown in panels (**a** and **b**), (**c** and **d**), and (**e** and **f**) can be found in Tables S28, S29, and S30 respectively. (**g**, **h**, and **i**) Percentage of the initial CFU density retained by each isolate lacking growth at 28°C, 37°C, and 42°C respectively, is tabulated alongside the percentage of cells remaining metabolically active (capable of taking FDA stain) in the same culture. Refer to Figs. 6a, 6b, and 7 for the flow cytometry-derived dot plots depicting what proportions of cells were stained by FDA in the 4, 16, and 27 different isolates incapable of growing at 28°C, 37°C, and 42°C respectively.

Fig. 6. Flow cytometry-derived dot plots depicting what proportions of cells were stained by FDA in the LB cultures of the isolates incapable of growing after (**a**) four days of incubation at 28°C, and (**b**) two days of incubation at 37°C. In each dot plot, the fluorescence levels recorded for 10000 randomly chosen cells have been shown as the functions of the corresponding forward scattering data, with green and red points representing FDA stained and unstained cells respectively. Each FACS experiment was repeated two more times, with deviation from the result (percentages of FDA stained and unstained cells) shown here being <2% on every occasion.

Fig. 7. Flow cytometry-derived dot plots depicting what proportions of cells were stained by FDA in the LB cultures of the 27 TMA isolates after 24 h incubation at 42°C**.** In each plot, the fluorescence levels recorded for 10000 randomly chosen cells have been shown as the functions of the corresponding forward scattering data, with green and red points representing FDA stained and unstained cells respectively. Each FACS experiment was repeated two more times, with deviation from the result (percentages of FDA stained and unstained cells) shown here being <2% on every occasion.

Fig. 8. Growth rates, or absence of growth, recorded for the individual TMA isolates in Luria broth at -10°C, 4°C, 15°C, 28°C, 37°C, and 42°C. Growth rates, plotted using isolate-specific symbols, were

expressed as those percentages of the initial CFU densities which increased per day of incubation at the different temperatures. Green bar indicates an increase recorded in the CFU density over time. Blue bar indicates no increase in CFU density over time, but >1% cells of the culture getting stained with FDA at the end of the incubation. Red bar indicates no increase in CFU density over time, and <1% cells of the culture getting stained with FDA at the end of the incubation. Numerical values for all the CFU densities underlying this graphical representation can be found in Tables S31-S36. FACS data for the proportions of FDA-stained cells can be found in Figs. 3, 6 and 7.

Fig. 9. Graphic representation of growth, survival, or abolition of cell population for different TMA isolates after incubation in Luria broth for 28, 14, 2, 4, 2, and 1 days, at -10°C, 4°C, 15°C, 28°C, 37°C, and 42°C, respectively. (**a**) TMA isolates classified based on the patterns of their responses to different incubation temperatures in terms of growth, survival, or abolition of cell population. (**b**) TMA isolates clustered on the basis of their isolation source.

Fig. 10. A heatmap illustrating the bactericidal activities (antibiosis potentials) of the actinobacterial species isolated from the Tso Moriri area. Green cells indicate growth, while red cells indicate eradication, of the target organisms.

Fig. 11. Growth temperature windows of the bacterial genera under which the different TMA isolates were classified. Data obtained for the present isolates have been depicted in the context of the data available in the literature for their global relatives (see Table S39, and Supplementary References, for the detailed information).

Table 1. Chemoorganoheterotrophic bacteria isolated from three different environments (habitats) within the Tso Moriri area, viz. lake-water, lake-sediment, and weathered rock dust of a lake-side hill.

Phylum-level affiliation of the isolate(s)	Name of the isolate	Isolation source	Number of sibling strains isolated from the different habitats explored			Closest bacterial species in the LPSN* (16S rRNA gene sequence similarity and alignment, in percentages)
			Weathered rock dust	Lake-water	Lake-sediment	
Actinomycetota (formerly Actinobacteria)	*Arthrobacter* sp. TRD_SC_1	Weathered rock dust	1	0	0	*Arthrobacter oryzae* KV-651^{T} (98.84, 100) *Arthrobacter pascens* DSM 20545^{T} (98.47, 100) *Arthrobacter humicola* KV-653^{T} (98.68, 99)
	Arthrobacter sp. TRD_SC_3	Weathered rock dust	2	0	0	*Arthrobacter cheniae* Hz2^{T} (99.64, 100) *Arthrobacter bussei* KR32^{T} (99,56, 100) *Arthrobacter agilis* DSM 20550^{T} (99.49, 100)
	Arthrobacter sp. TRD_SC_4	Weathered rock dust	1	0	0	*Arthrobacter ruber* MDB1-42^{T} (98.38, 100) *Arthrobacter bussei* KR32^{T} (98.30, 100) *Arthrobacter cheniae* Hz2^{T} (98.23, 100)
	Arthrobacter sp. TRD_SC_6	Weathered rock dust	1	0	0	*Arthrobacter tumbae* LMG 19501^{T} (99.22, 100) *Arthrobacter parietis* LMG 22281^{T} (99.15, 100) *Arthrobacter subterraneus* CH7^{T} (98.76, 99)
	Arthrobacter sp. TRD_SC_8	Weathered rock dust	1	0	0	*Arthrobacter citreus* DSM 20133^{T} (99.28, 100) *Arthrobacter sunyaminii* zg-ZUI122^{T} (98.99, 100) *Arthrobacter jiangjiafuii* zg-ZUI227^{T} (98.85, 100)
	Cryobacterium sp. TS_SC_7	Lake-sediment	0	0	1	*Cryobacterium breve* TMT4-23 (99.14, 100) *Cryobacterium psychrotolerans* 0549 (98.55, 99) *Cryobacterium tepidiphilum* NEAU-85 (97.9, 99)
	Microbacterium sp. TRD_SC_10	Weathered rock dust	2	0	0	*Microbacterium paraoxydans* CF36 (99.06,100) *Microbacterium algeriense* G1 (98.84, 100) *Microbacterium oxydans* DSM 20578 (98.77, 100)
	Microbacterium sp. TW_SC_2	Lake-water	0	1	0	*Microbacterium algeriense* G1 (99.63, 100) *Microbacterium oxydans* DSM 20578 (99.56, 100) *Microbacterium paraoxydans* CF36 (99.56, 100)
	Microbacterium sp. TW_SC_3	Lake-water	0	1	0	*Microbacterium lacus* A5E-52 (99.05, 100) *Microbacterium invictum* DC-200 (98.83, 100) *Microbacterium aoyamense* KV-492 (98.76, 100)
	Mycetocola sp. TRD_SC_2	Weathered rock dust	5	0	0	*Mycetocola manganoxydans* MB1-14^{T} (99.48, 100) *Mycetocola miduiensis* MD-T1-10-2^{T} (99.47, 98) *Mycetocola zhadangensis* ZD1-4^{T} (98.22, 100)
	Paenarthrobacter sp. TRD_SC_7	Weathered rock dust	6	0	0	*Paenarthrobacter nitroguajacolicus* G2-1 (99.18, 100) *Paenarthrobacter aurescens* DSM 20116 (98.81, 100) *Paenarthrobacter ilicis* DSM 20138 (98.44, 100)
	Pseudarthrobacter sp. TRD_SC_9	Weathered rock dust	1	0	0	*Pseudarthrobacter psychrotolerans* YJ56 (98.60, 99) *Pseudarthrobacter phenanthrenivorans* Sphe3 (98.10, 100) *Arthrobacter oryzae* KV-651 (98.18, 100)
	Pseudarthrobacter sp. TS_SC_4	Lake-sediment	0	0	2	*Pseudarthrobacter psychrotolerans* YJ56 (99.13, 99) *Pseudarthrobacter siccitolerans* 4J27 (99.06, 100) *Pseudarthrobacter oxydans* DSM 20119 (98.91, 100)
	Sanguibacter sp. TS_SC_8	Lake-sediment	0	0	1	*Sanguibacter gelidistatuariae* ISLP-3 (99.06, 100) *Sanguibacter suarezii* ST26 (98.70, 100) *Sanguibacter bilivelldensis* 4.1 (98.32, 99)
	Streptomyces sp. TRD_SC_5	Weathered rock dust	2	0	0	*Streptomyces anulatus* NBRC 13369 (98.04, 100) *Streptomyces pratensis* ch24 (98.04, 100) *Streptomyces spiroverticillatus* NBRC 12821 (98.04, 100)
Bacillota (formerly Firmicutes)	*Trichococcus* sp. TS_SC_9	Lake-sediment	0	0	1	*Trichococcus paludicola* B7-2 (99.72, 100) *Trichococcus pasteurii* KoTa2 (99.72, 100) *Trichococcus flocculiformis* DSM 2094 (99.65, 100)
Bacteroidota (formerly Bacteroidetes)	*Flavobacterium* sp. TS_SC_2	Lake-sediment	0	0	2	*Flavobacterium psychrolimnae* NBRC 102679 (99.63, 100) *Flavobacterium petrolei* Kopri-42 (99.20, 100) *Flavobacterium sandaracinum* LB-D12 (98.90, 100)
	Flavobacterium sp. TS_SC_5	Lake-sediment	0	0	2	*Flavobacterium caseinilyticum* AT-3-2 (99.27,100) *Flavobacterium psychrolimnae* NBRC 102679 (98.98,100) *Flavobacterium limicola* ST-82 (98.54, 100)
Pseudomonadota (class Alphaproteobacteria)	*Ancylobacter* sp. TW_SC_1	Lake-water	0	2	0	*Ancylobacter aquaticus* NBRC 102453 (98.37, 100) *Ancylobacter novellus* DSM 506 (98.37, 100) *Ancylobacter koreensis* NBRC 100963 (98.15, 100)
Pseudomonadota (class Gammaproteobacteria)	*Acinetobacter* sp. TW_SC_4	Lake-water	0	1	0	*Acinetobacter variabilis* NIPH 2171 (99.43, 100) *Acinetobacter refrigeratoris* WB1 (99.28,100) *Acinetobacter kanungonis* PS-1 (96.64, 100)
	Aeromonas salmonicida TS_SC_11	Lake-sediment	0	0	10	*Aeromonas salmonicida* CECT 894 (100, 100)
	Pseudomonas sp. TS_SC_1	Lake-sediment	0	0	1	*Pseudomonas mandelii* NBRC 103147 (99.86, 100) *Pseudomonas silesiensis* A3 (99.86, 100) *Pseudomonas svalbardensis* S025 (99.42, 100)
	Pseudomonas sp. TS_SC_3	Lake-sediment	0	0	1	*Pseudomonas guineae* M8 (99.78, 99) *Pseudomonas leptonychotis* CCM 8849 (99.57, 100) *Pseudomonas peli* R-20805 (99.57, 100)
	Pseudomonas sp. TS_SC_10	Lake-sediment	0	0	2	*Pseudomonas crudilactis* UCMA 17988 (98.92, 100) *Pseudomonas helmanticensis* OHA11 (98.70, 100) *Pseudomonas baetica* a390 (98.63, 100) *Pseudomonas germanica* FIT28 (98.63, 100)
	Pseudomonas sp. TS_SC_12	Lake-sediment	0	0	3	*Pseudomonas cuatrocienegasensis* 1N (99.56, 100) *Pseudomonas borbori* R-20821 (98.62, 100) *Pseudomonas paeninsulae* IT1137 (98.46, 99)
	Pseudomonas sp. TS_SC_13	Lake-sediment	0	0	1	*Pseudomonas cannabina* CFBP 2341 (98.50,100) *Pseudomonas congelans* P 538/23 (98.50,100) *Pseudomonas coronafaciens* NCPPB 600 (98.50, 100) *Pseudomonas syringae* ICMP 3023 (98.50, 100)
	Psychrobacter sp. TS_SC_6	Lake-sediment	0	0	7	*Psychrobacter cryohalolentis* K5 (99.78, 100) *Psychrobacter glaciei* BIc20019 (99.21, 100) *Psychrobacter arcticus 273-4* (99.14, 100)

* List of Prokaryotic names with Standing in Nomenclature (Parte et al. 2020).

a
National Park
Bokhtar
Hotan
Prefecture
Skardu
Kabul
LADAKH
0
200.41 km
Peshawar
Srinagar
Leh
JAMMU AND
KASHMIR
Tso Moriri
Jammu
Sargodha
HIMACHAL
PRADESH
Lahore
Jalandhar
CHANDIGARH
Multan
PUNJAB
UTTARAKHAND
Pakistan
Bahawalpur
HARYANA
New Delhi
Rahim
Yar Khan
Nepal
Sukkur
UTTAR
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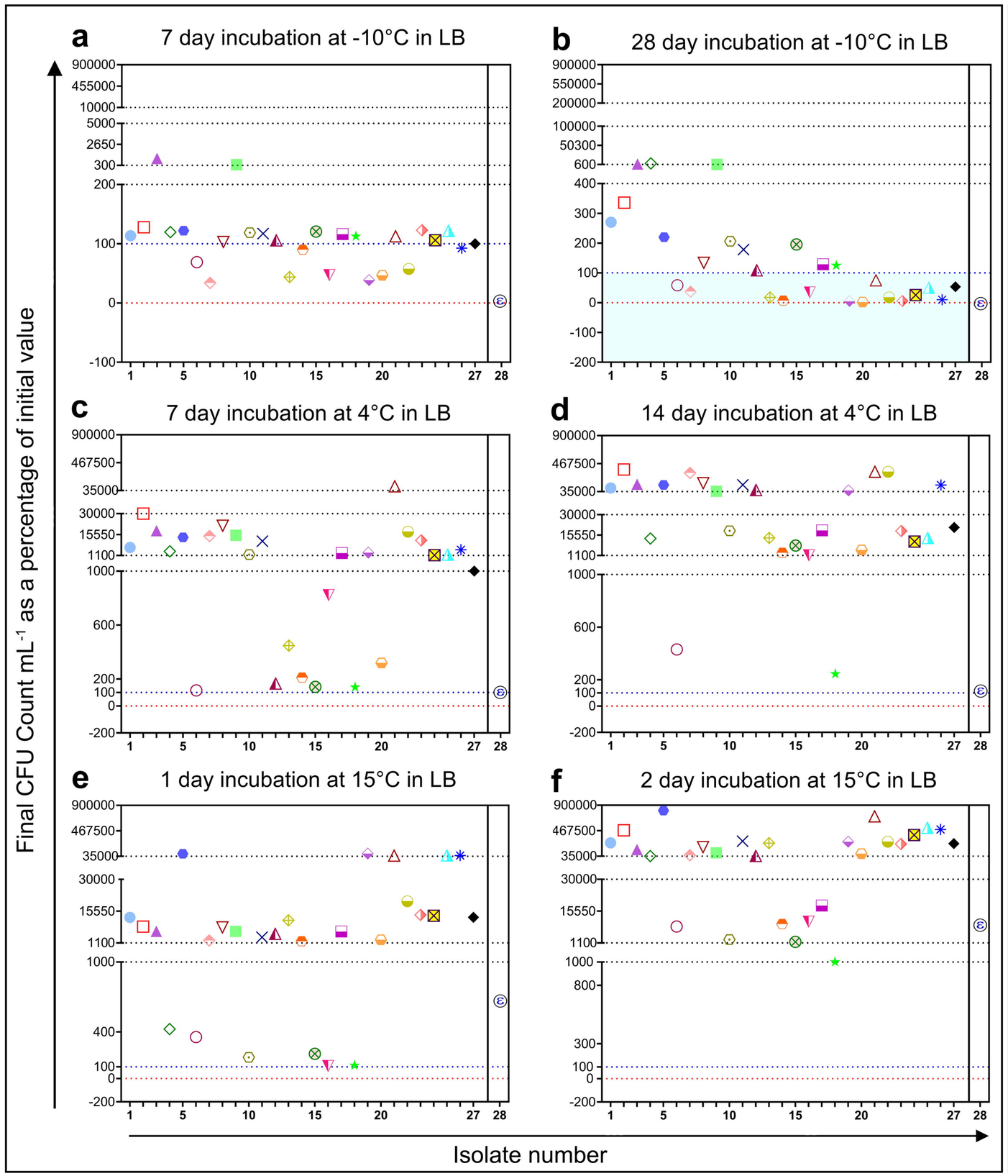
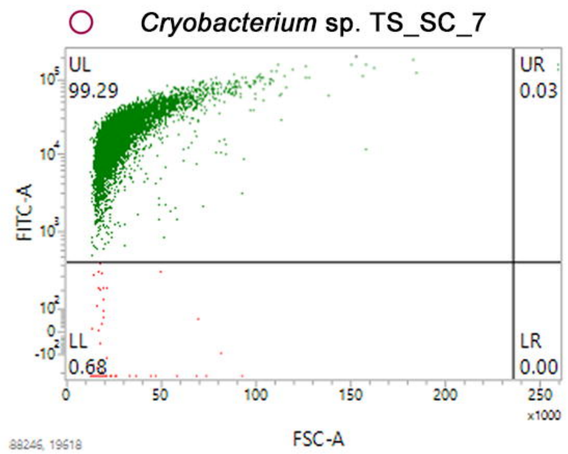


g Phenotypes of the strains lacking growth at -10°C

Strain name	Percentage of CFUs retained after 28 days at -10°C	Percentage of cells remaining metabolically active after 28 days at -10°C
Cryobacterium sp. TS_SC_7	58.52	99.32
Microbacterium sp. TRD_SC_10	37.14	95.75
Pseudarthrobacter sp. TS_SC_4	17.77	18.75
Sanguibacter sp. TS_SC_8	7.00	99.16
Ancylobacter sp. TW_SC_1	34.72	87.21
Flavobacterium sp. TS_SC_5	4.93	93.11
Acinetobacter sp. TW_SC_4	2.00	75.71
Aeromonas salmonicida TS_SC_11	75.00	95.46
Pseudomonas sp. TS_SC_1	17.37	87.72
Pseudomonas sp. TS_SC_3	5.71	35.07
Pseudomonas sp. TS_SC_10	25.49	46.82
Pseudomonas sp. TS_SC_12	50.00	67.03
Pseudomonas sp. TS_SC_13	10.00	81.66
Psychrobacter sp. TS_SC_6	53.49	88.97

1) *Arthrobacter* sp. TRD_SC_1
2) *Arthrobacter* sp. TRD_SC_3
3) *Arthrobacter* sp. TRD_SC_4
4) *Arthrobacter* sp. TRD_SC_6
5) *Arthrobacter* sp. TRD_SC_8
6) *Cryobacterium* sp. TS_SC_7
7) *Microbacterium* sp. TRD_SC_10
8) *Microbacterium* sp. TW_SC_2
9) *Microbacterium* sp. TW_SC_3
10) *Mycetocola* sp. TRD_SC_2
11) *Paenarthrobacter* sp. TRD_SC_7
12) *Pseudarthrobacter* sp. TRD_SC_9
13) *Pseudarthrobacter* sp. TS_SC_4
14) *Sanguibacter* sp. TS_SC_8
15) *Streptomyces* sp. TRD_SC_5
16) *Ancylobacter* sp. TW_SC_1
17) *Trichococcus* sp. TS_SC_9
18) *Flavobacterium* sp. TS_SC_2
19) *Flavobacterium* sp. TS_SC_5
20) *Acinetobacter* sp. TW_SC_4
21) *Aeromonas salmonicida* TS_SC_11
22) *Pseudomonas* sp. TS_SC_1
23) *Pseudomonas* sp. TS_SC_3
24) *Pseudomonas* sp. TS_SC_10
25) *Pseudomonas* sp. TS_SC_12
26) *Pseudomonas* sp. TS_SC_13
27) *Psychrobacter* sp. TS_SC_6
28) *Escherichia coli* K-12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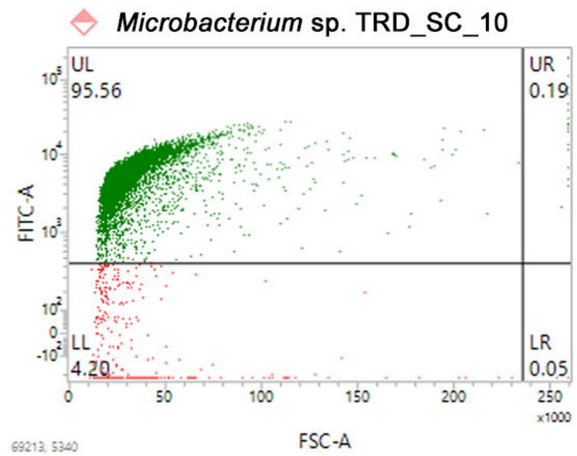
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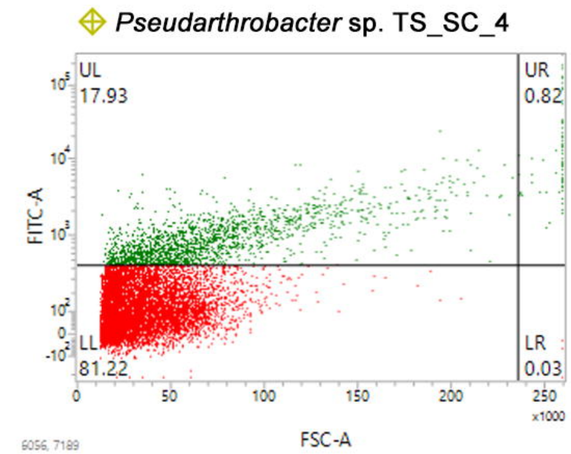
FSC-A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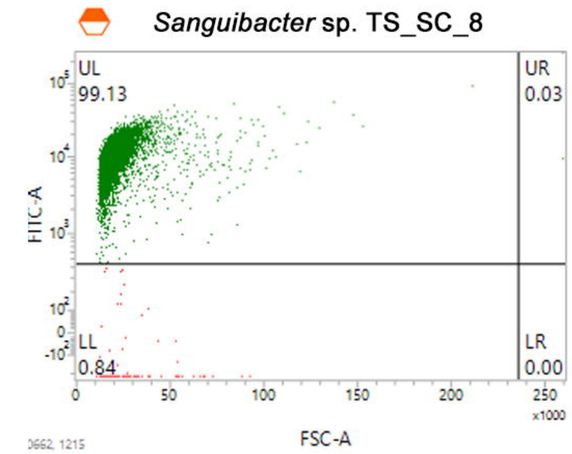
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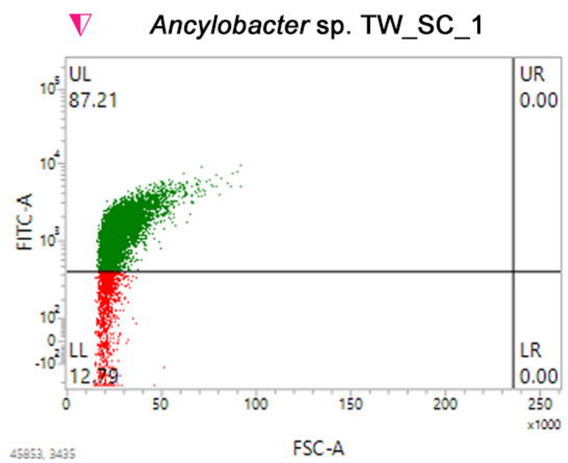
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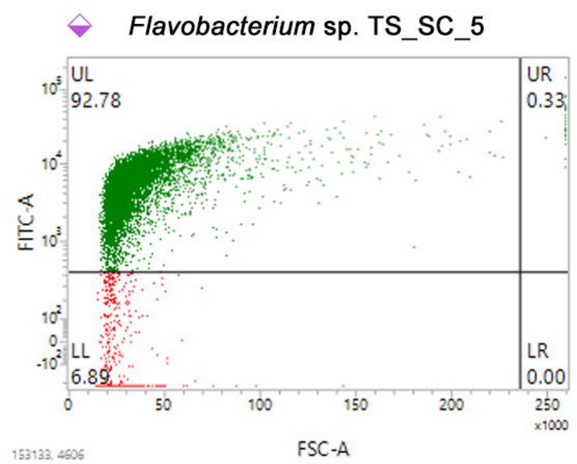
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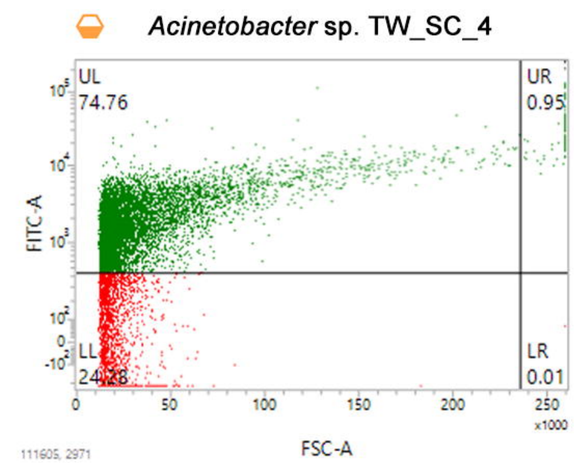
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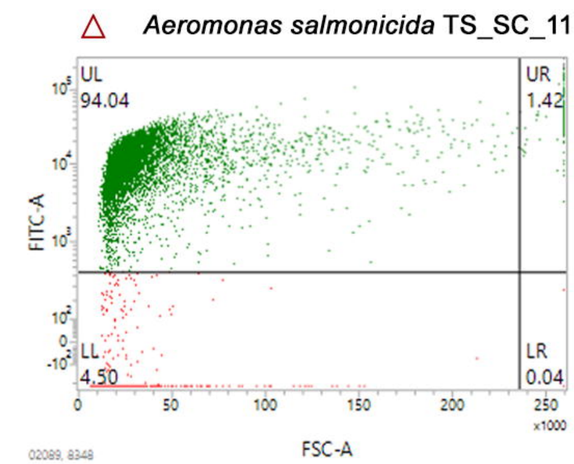
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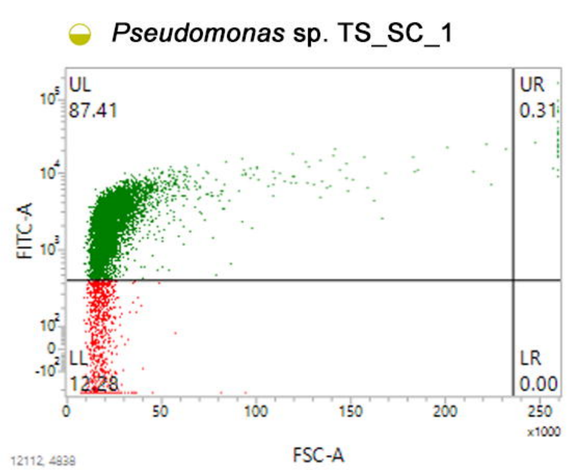
FSC-A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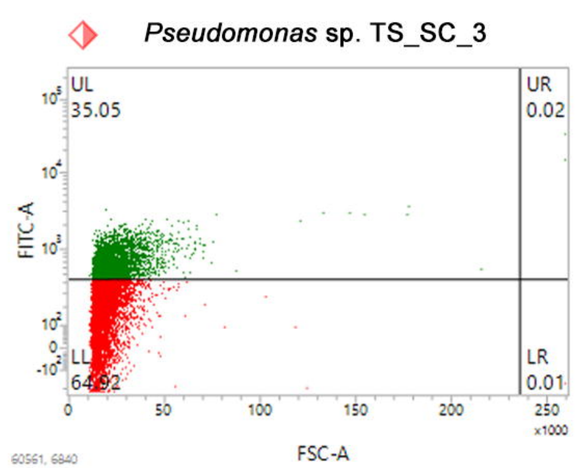
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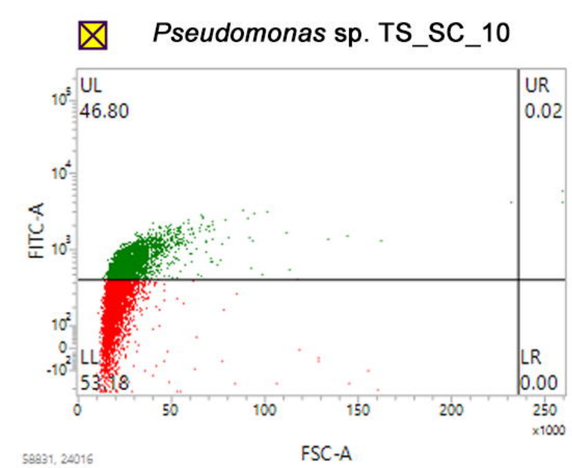
FSC-A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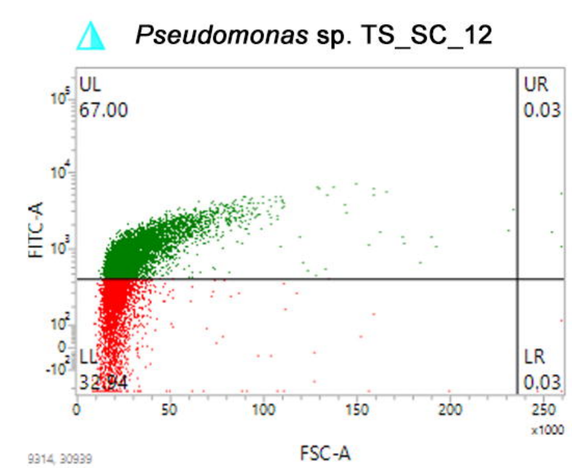
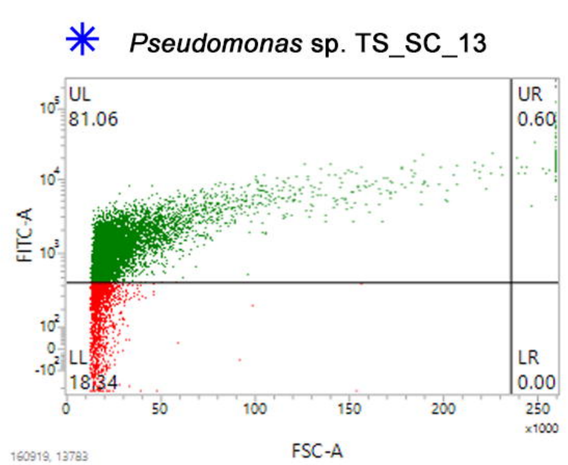
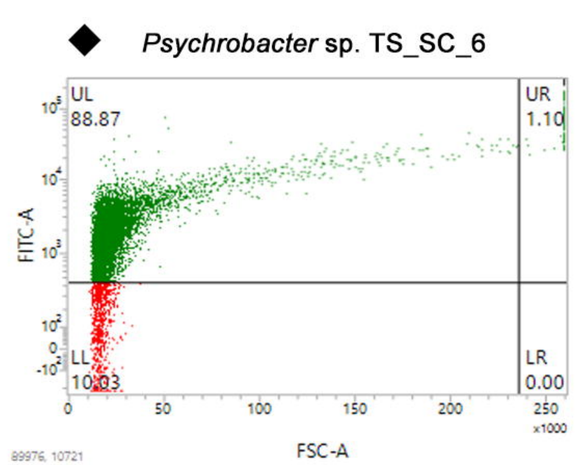
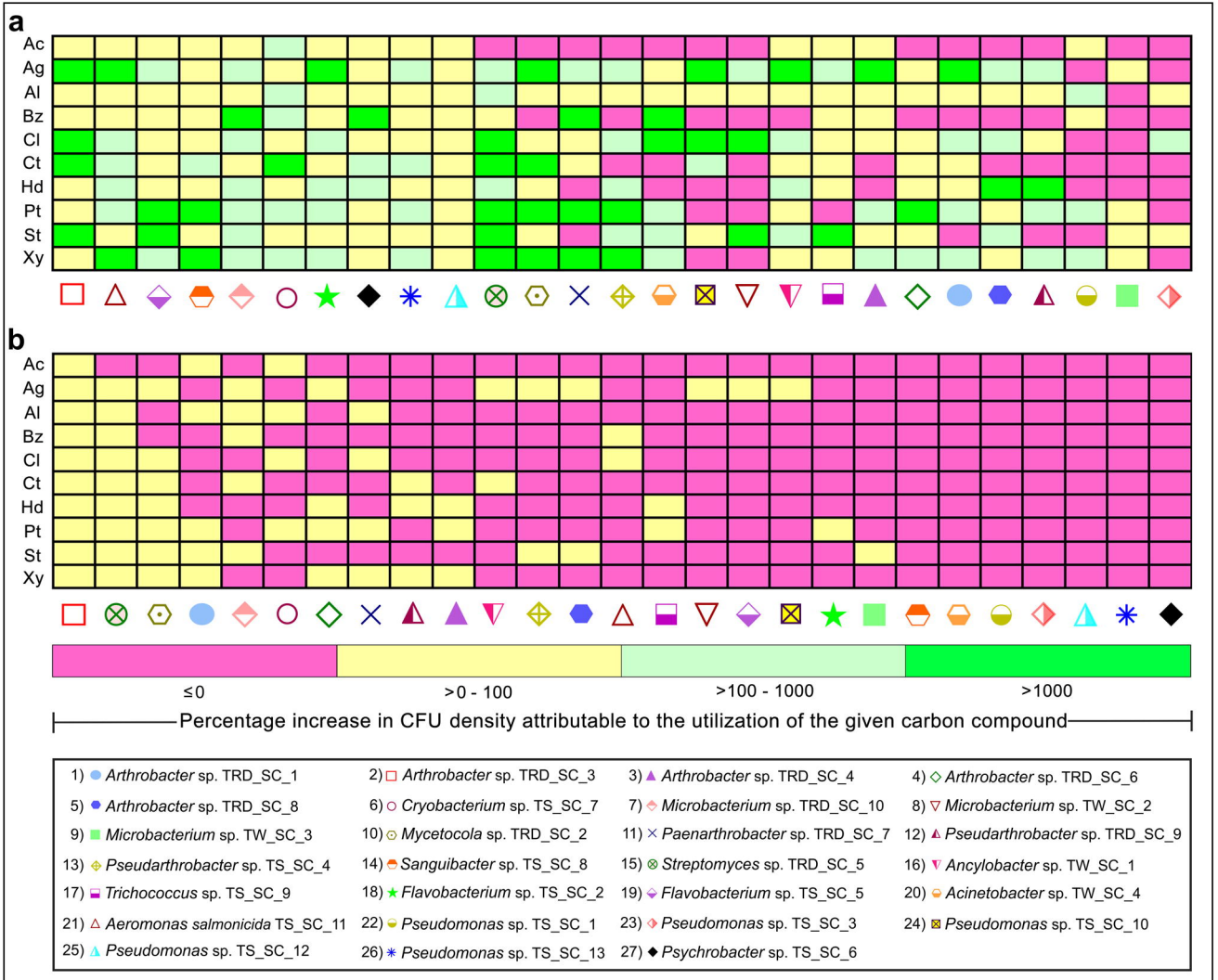
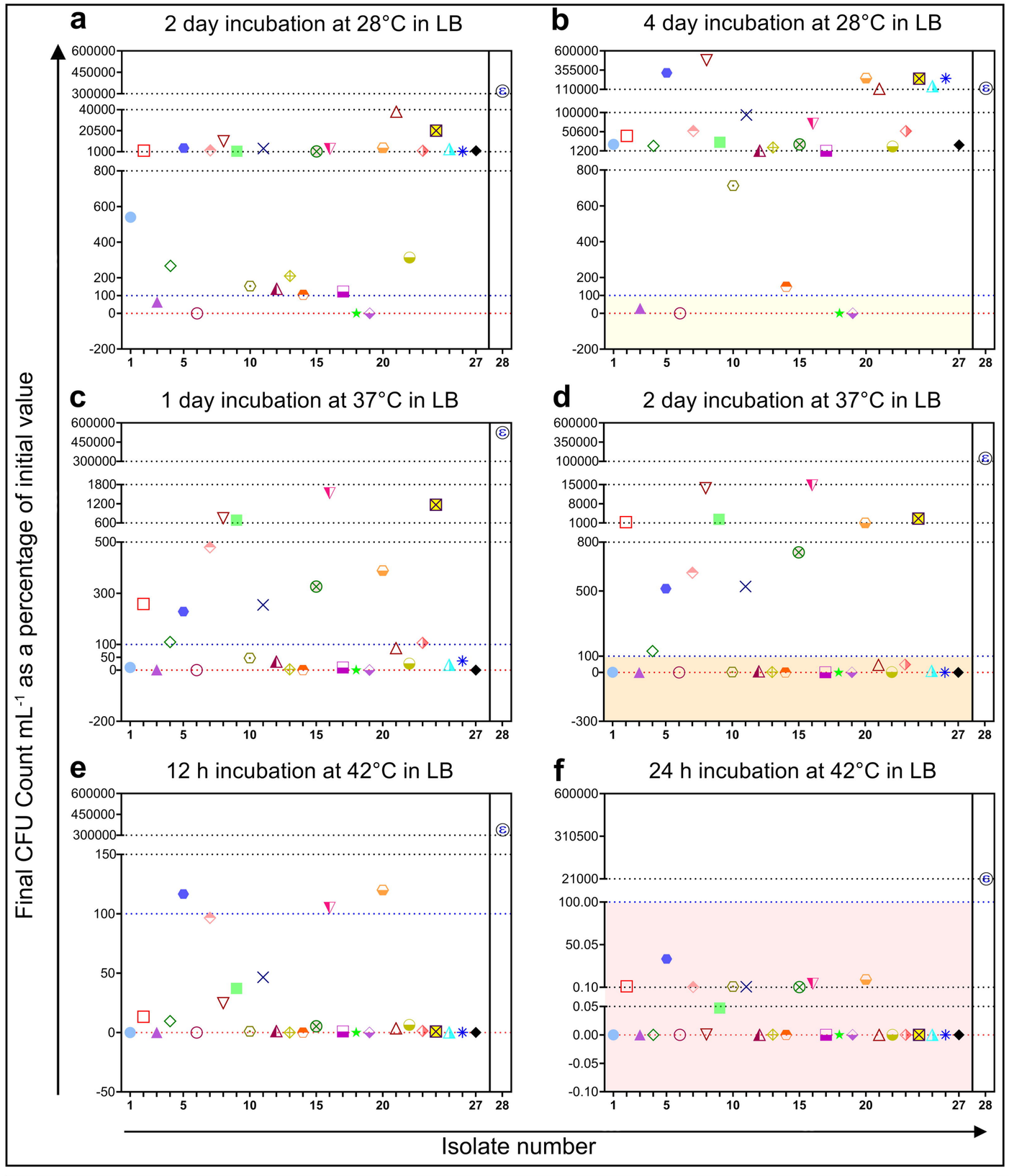
UR 0.02
LL 53.18
LR 0.00
FITC-A
FSC-A


Pseudomonas sp. TS_SC_12
UL 67.00
UR 0.03
LL 32.94
LR 0.03
FITC-A
FSC-A


Pseudomonas sp. TS_SC_13
UL 81.06
UR 0.60
LL 18.34
LR 0.00
FITC-A
FSC-A


Psychrobacter sp. TS_SC_6
UL 88.87
UR 1.10
LL 10.03
LR 0.00
FITC-A
FSC-A


a
Ac
Ag
Al
Bz
Cl
Ct
Hd
Pt
St
Xy
b
Ac
Ag
Al
Bz
Cl
Ct
Hd
Pt
St
Xy
≤0
>0 - 100
>100 - 1000
>1000
Percentage increase in CFU density attributable to the utilization of the given carbon compound
1) Arthrobacter sp. TRD_SC_1
2) Arthrobacter sp. TRD_SC_3
3) Arthrobacter sp. TRD_SC_4
4) Arthrobacter sp. TRD_SC_6
5) Arthrobacter sp. TRD_SC_8
6) Cryobacterium sp. TS_SC_7
7) Microbacterium sp. TRD_SC_10
8) Microbacterium sp. TW_SC_2
9) Microbacterium sp. TW_SC_3
10) Mycetocola sp. TRD_SC_2
11) Paenarthrobacter sp. TRD_SC_7
12) Pseudarthrobacter sp. TRD_SC_9
13) Pseudarthrobacter sp. TS_SC_4
14) Sanguibacter sp. TS_SC_8
15) Streptomyces sp. TRD_SC_5
16) Ancylobacter sp. TW_SC_1
17) Trichococcus sp. TS_SC_9
18) Flavobacterium sp. TS_SC_2
19) Flavobacterium sp. TS_SC_5
20) Acinetobacter sp. TW_SC_4
21) Aeromonas salmonicida TS_SC_11
22) Pseudomonas sp. TS_SC_1
23) Pseudomonas sp. TS_SC_3
24) Pseudomonas sp. TS_SC_10
25) Pseudomonas sp. TS_SC_12
26) Pseudomonas sp. TS_SC_13
27) Psychrobacter sp. TS_SC_6



Phenotypes of the strains lacking growth at 28°C, 37°C and/or 42°C

g

Strain name	Percentage of CFUs retained after four days at 28°C	Percentage of cells remaining metabolically active after four days at 28°C
Arthrobacter sp. TRD_SC_4	27.58621	58.43
Cryobacterium sp. TS_SC_7	0	0.24
Flavobacterium sp. TS_SC_2	0	18.23
Flavobacterium sp. TS_SC_5	0	21.56

h

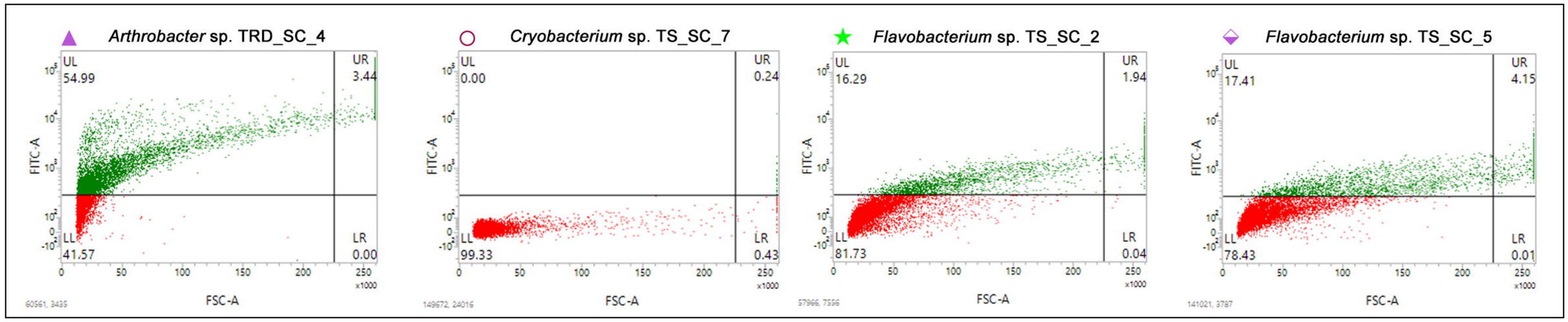
Strain name	Percentage of CFUs retained after two days at 37°C	Percentage of cells remaining metabolically active after two days at 37°C
Arthrobacter sp. TRD_SC_1	0.83810	20.13
Arthrobacter sp. TRD_SC_4	0.04000	10.45
Cryobacterium sp. TS_SC_7	0	0.08
Mycetocola sp. TRD_SC_2	2.40000	44.98
Pseudarthrobacter sp. TRD_SC_9	6.14286	47.65
Pseudarthrobacter sp. TS_SC_4	2.21875	4.47
Sanguibacter sp. TS_SC_8	0.04100	4.16
Trichococcus sp. TS_SC_9	0.39326	7.50
Flavobacterium sp. TS_SC_2	0	3.96
Flavobacterium sp. TS_SC_5	0	10.36
Aeromonas salmonicida TS_SC_11	46.42857	65.06
Pseudomonas sp. TS_SC_1	1.69014	39.67
Pseudomonas sp. TS_SC_3	48.18182	58.75
Pseudomonas sp. TS_SC_12	8.94444	19.73
Pseudomonas sp. TS_SC_13	0.38462	75.93
Psychrobacter sp. TS_SC_6	0.01095	39.26

i

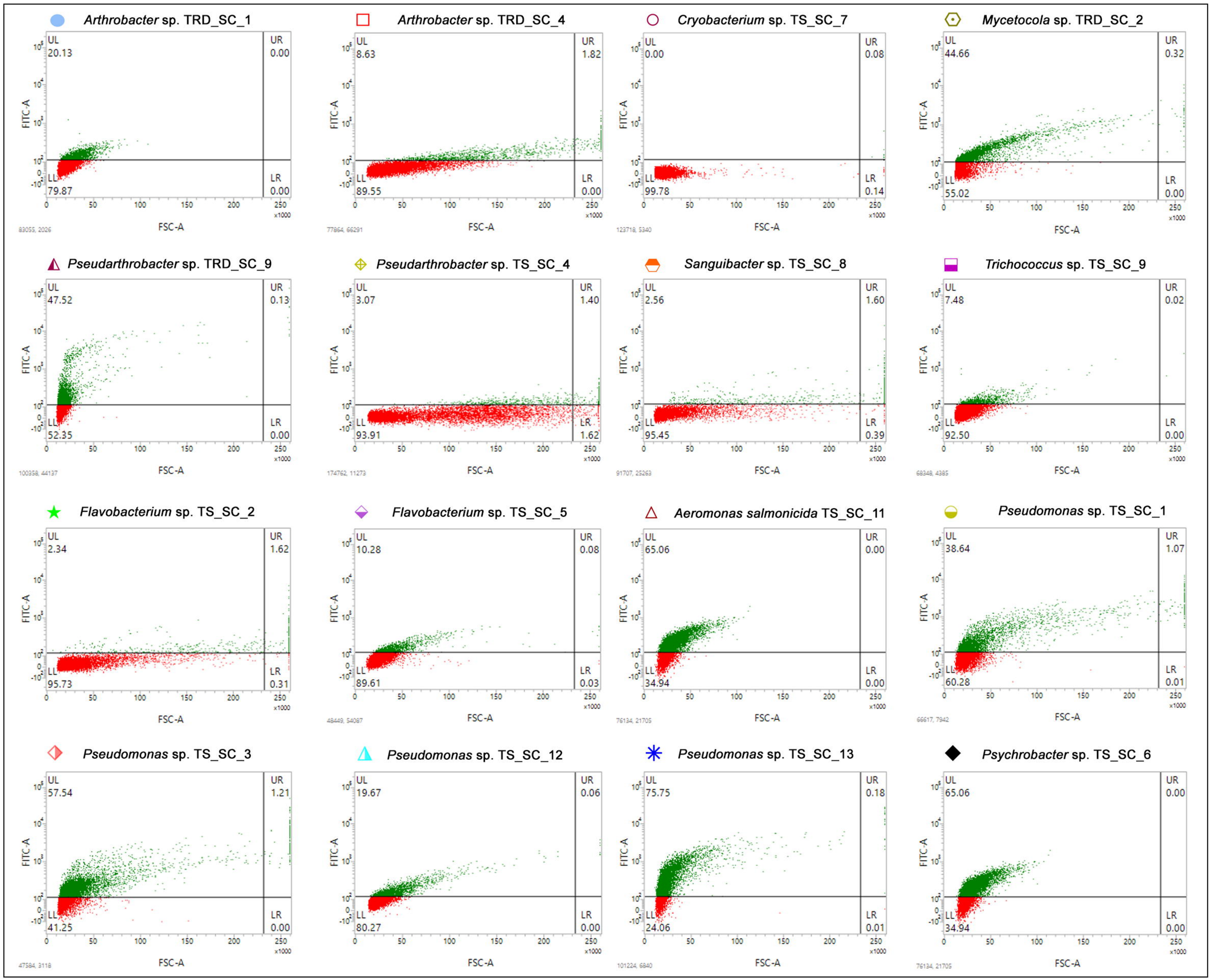
Strain name	Percentage of CFUs retained after one day at 42°C	Percentage of cells remaining metabolically active after one day at 42°C
Arthrobacter sp. TRD_SC_1	0	14.98
Arthrobacter sp. TRD_SC_3	1.24138	7.18
Arthrobacter sp. TRD_SC_4	0	0.07
Arthrobacter sp. TRD_SC_6	0	0.06
Arthrobacter sp. TRD_SC_8	33.33333	39.89
Cryobacterium sp. TS_SC_7	0	0
Microbacterium sp. TRD_SC_10	0.43750	14.67
Microbacterium sp. TW_SC_2	0.00073	57.92
Microbacterium sp. TW_SC_3	0.04737	57.13
Mycetocola sp. TRD_SC_2	1.06145	43.36
Paenarthrobacter sp. TRD_SC_7	0.82759	18.37
Pseudarthrobacter sp. TRD_SC_9	0	45.5
Pseudarthrobacter sp. TS_SC_4	0.00036	0.93
Sanguibacter sp. TS_SC_8	0	1.09
Streptomyces sp. TRD_SC_5	0.35294	10.92
Ancylobacter sp. TW_SC_1	4.32584	49.41
Trichococcus sp. TS_SC_9	0	0.03
Flavobacterium sp. TS_SC_2	0	0.23
Flavobacterium sp. TS_SC_5	0	0.08
Acinetobacter sp. TW_SC__4	9.28571	87.34
Aeromonas salmonicida TS_SC_11	0	57.43
Pseudomonas sp. TS_SC_1	0	31.38
Pseudomonas sp. TS_SC_3	0	0.1
Pseudomonas sp. TS_SC_10	0	5.9
Pseudomonas sp. TS_SC_12	0	14.98
Pseudomonas sp. TS_SC_13	0	41.18
Psychrobacter sp. TS_SC_6	0	12.68

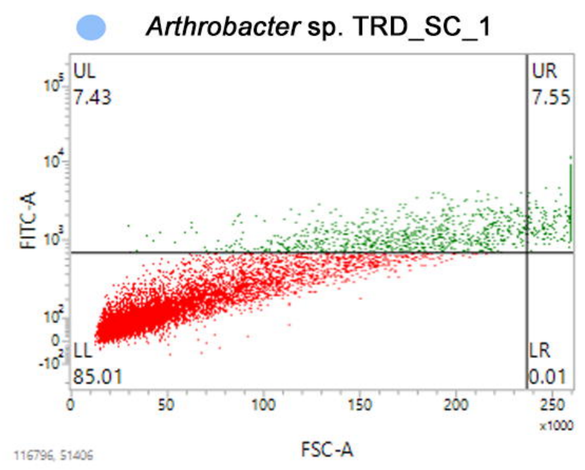
1) *Arthrobacter* sp. TRD_SC_1
2) *Arthrobacter* sp. TRD_SC_3
3) *Arthrobacter* sp. TRD_SC_4
4) *Arthrobacter* sp. TRD_SC_6
5) *Arthrobacter* sp. TRD_SC_8
6) *Cryobacterium* sp. TS_SC_7
7) *Microbacterium* sp. TRD_SC_10
8) *Microbacterium* sp. TW_SC_2
9) *Microbacterium* sp. TW_SC_3
10) *Mycetocola* sp. TRD_SC_2
11) *Paenarthrobacter* sp. TRD_SC_7
12) *Pseudarthrobacter* sp. TRD_SC_9
13) *Pseudarthrobacter* sp. TS_SC_4
14) *Sanguibacter* sp. TS_SC_8
15) *Streptomyces* sp. TRD_SC_5
16) *Ancylobacter* sp. TW_SC_1
17) *Trichococcus* sp. TS_SC_9
18) *Flavobacterium* sp. TS_SC_2
19) *Flavobacterium* sp. TS_SC_5
20) *Acinetobacter* sp. TW_SC_4
21) *Aeromonas salmonicida* TS_SC_11
22) *Pseudomonas* sp. TS_SC_1
23) *Pseudomonas* sp. TS_SC_3
24) *Pseudomonas* sp. TS_SC_10
25) *Pseudomonas* sp. TS_SC_12
26) *Pseudomonas* sp. TS_SC_13
27) *Psychrobacter* sp. TS_SC_6
28) *Escherichia coli* K-12

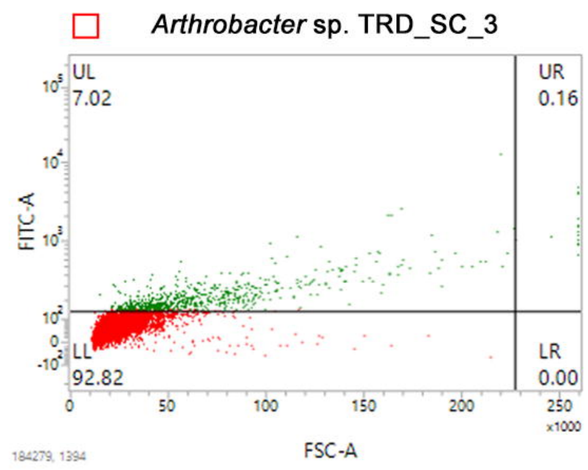
a

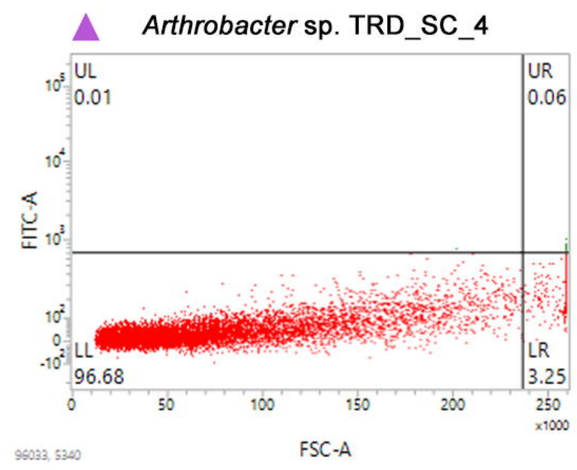

Arthrobacter sp. TRD_SC_4
UL 54.99
UR 3.44
LL 41.57
LR 0.00
Cryobacterium sp. TS_SC_7
UL 0.00
UR 0.24
LL 99.33
LR 0.43
Flavobacterium sp. TS_SC_2
UL 16.29
UR 1.94
LL 81.73
LR 0.04
Flavobacterium sp. TS_SC_5
UL 17.41
UR 4.15
LL 78.43
LR 0.01
FITC-A
FSC-A
x1000

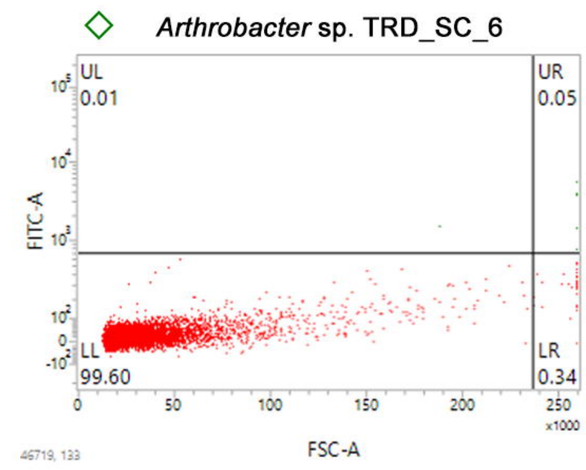
b

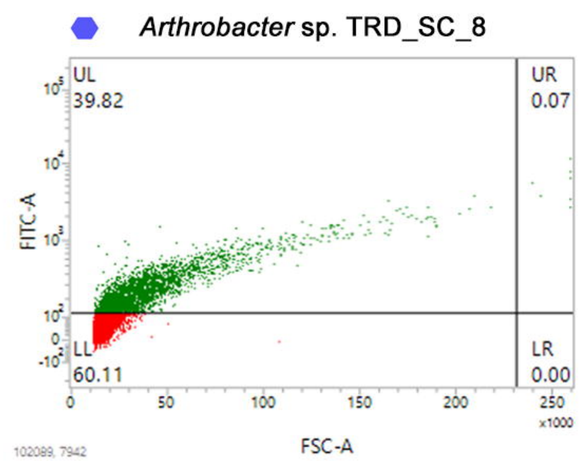

Arthrobacter sp. TRD_SC_1
UL 20.13
UR 0.00
LL 79.87
LR 0.00
Arthrobacter sp. TRD_SC_4
UL 8.63
UR 1.82
LL 89.55
LR 0.00
Cryobacterium sp. TS_SC_7
UL 0.00
UR 0.08
LL 99.78
LR 0.14
Mycetocola sp. TRD_SC_2
UL 44.66
UR 0.32
LL 55.02
LR 0.00
Pseudarthrobacter sp. TRD_SC_9
UL 47.52
UR 0.13
LL 52.35
LR 0.00
Pseudarthrobacter sp. TS_SC_4
UL 3.07
UR 1.40
LL 93.91
LR 1.62
Sanguibacter sp. TS_SC_8
UL 2.56
UR 1.60
LL 95.45
LR 0.39
Trichococcus sp. TS_SC_9
UL 7.48
UR 0.02
LL 92.50
LR 0.00
Flavobacterium sp. TS_SC_2
UL 2.34
UR 1.62
LL 95.73
LR 0.31
Flavobacterium sp. TS_SC_5
UL 10.28
UR 0.08
LL 89.61
LR 0.03
Aeromonas salmonicida TS_SC_11
UL 65.06
UR 0.00
LL 34.94
LR 0.00
Pseudomonas sp. TS_SC_1
UL 38.64
UR 1.07
LL 60.28
LR 0.01
Pseudomonas sp. TS_SC_3
UL 57.54
UR 1.21
LL 41.25
LR 0.00
Pseudomonas sp. TS_SC_12
UL 19.67
UR 0.06
LL 80.27
LR 0.00
Pseudomonas sp. TS_SC_13
UL 75.75
UR 0.18
LL 24.06
LR 0.01
Psychrobacter sp. TS_SC_6
UL 65.06
UR 0.00
LL 34.94
LR 0.00
FITC-A
FSC-A
x1000

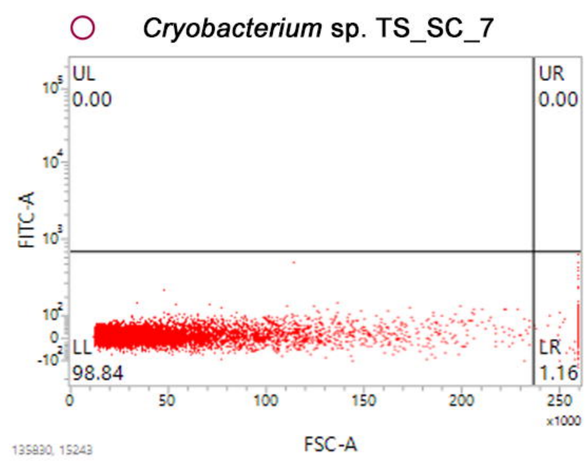

Arthrobacter sp. TRD_SC_1
UL 7.43
UR 7.55
LL 85.01
LR 0.01
FITC-A
FSC-A

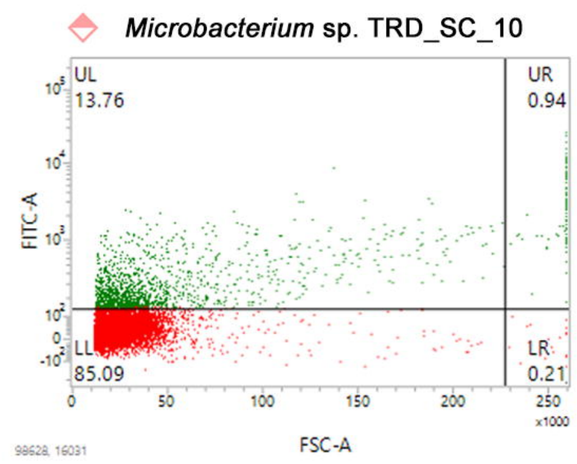

Arthrobacter sp. TRD_SC_3
UL 7.02
UR 0.16
LL 92.82
LR 0.00
FITC-A
FSC-A

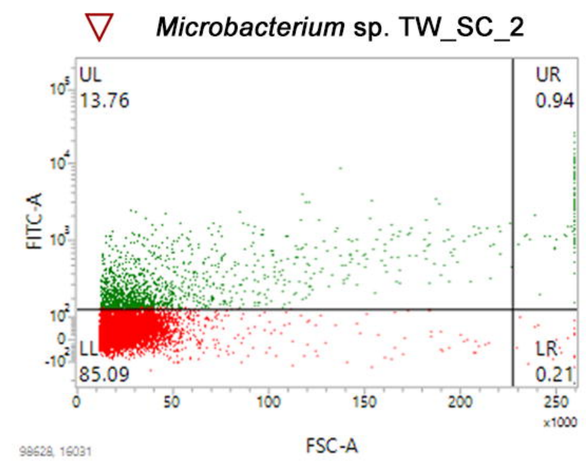

Arthrobacter sp. TRD_SC_4
UL 0.01
UR 0.06
LL 96.68
LR 3.25
FITC-A
FSC-A

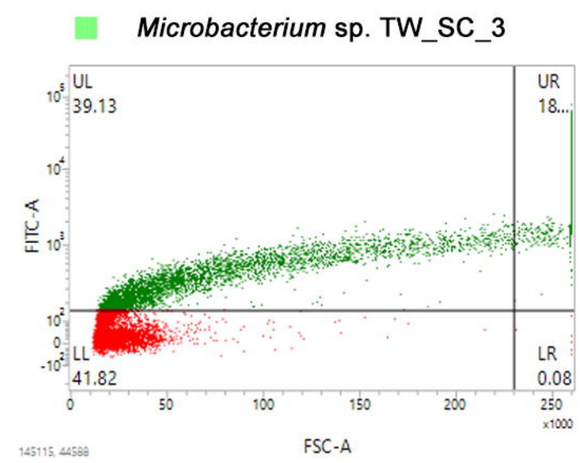

Arthrobacter sp. TRD_SC_6
UL 0.01
UR 0.05
LL 99.60
LR 0.34
FITC-A
FSC-A

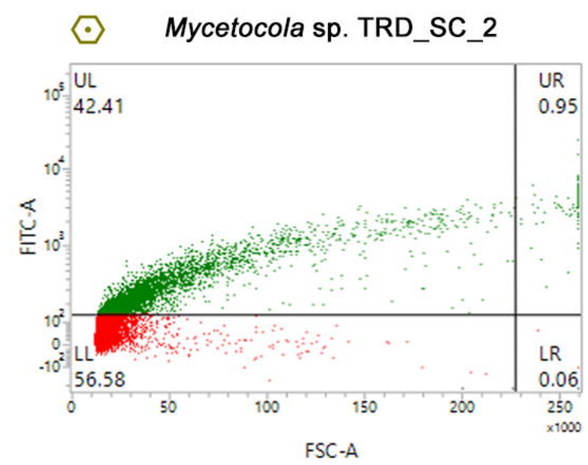

Arthrobacter sp. TRD_SC_8
UL 39.82
UR 0.07
LL 60.11
LR 0.00
FITC-A
FSC-A

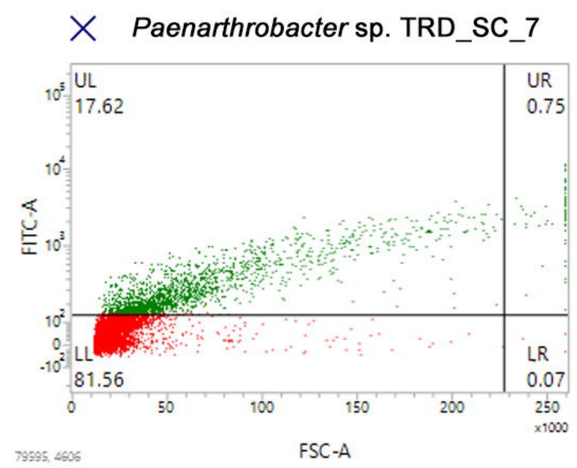

Cryobacterium sp. TS_SC_7
UL 0.00
UR 0.00
LL 98.84
LR 1.16
FITC-A
FSC-A

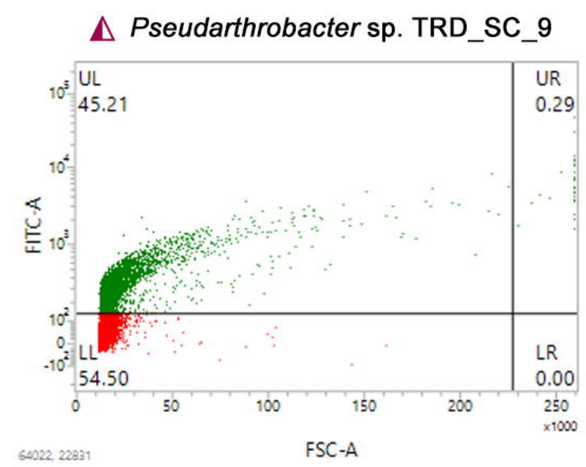

Microbacterium sp. TRD_SC_10
UL 13.76
UR 0.94
LL 85.09
LR 0.21
FITC-A
FSC-A

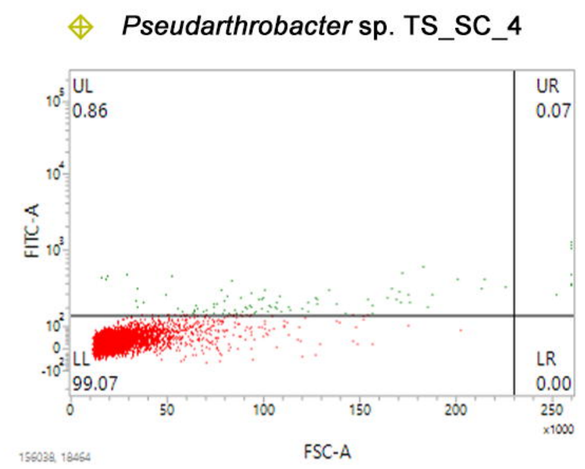

Microbacterium sp. TW_SC_2
UL 13.76
UR 0.94
LL 85.09
LR 0.21
FITC-A
FSC-A

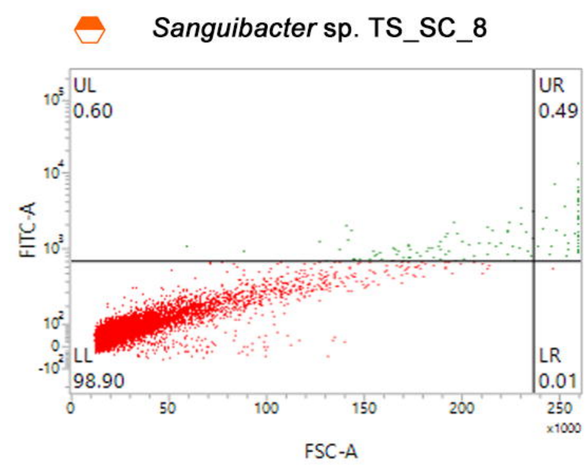

Microbacterium sp. TW_SC_3
UL 39.13
UR 18...
LL 41.82
LR 0.08
FITC-A
FSC-A

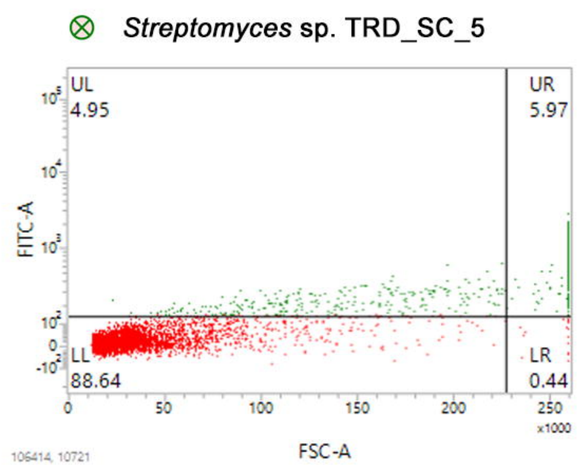

Mycetocola sp. TRD_SC_2
UL 42.41
UR 0.95
LL 56.58
LR 0.06
FITC-A
FSC-A

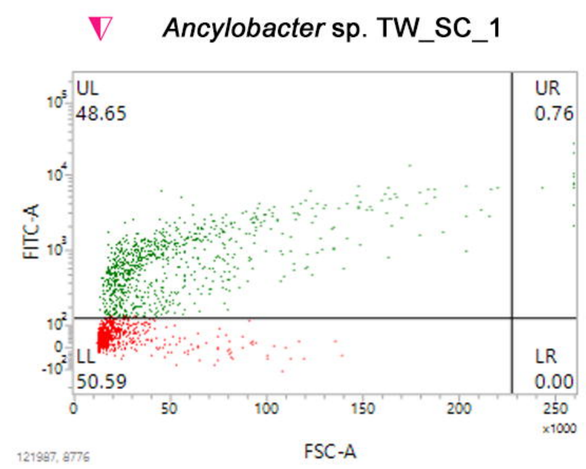

Paenarthrobacter sp. TRD_SC_7
UL 17.62
UR 0.75
LL 81.56
LR 0.07
FITC-A
FSC-A

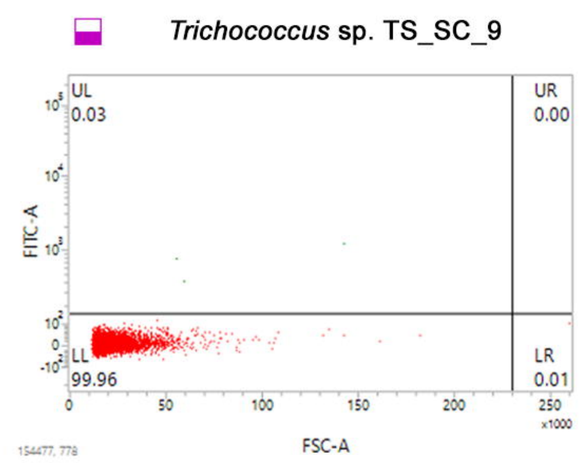

Pseudarthrobacter sp. TRD_SC_9
UL 45.21
UR 0.29
LL 54.50
LR 0.00
FITC-A
FSC-A

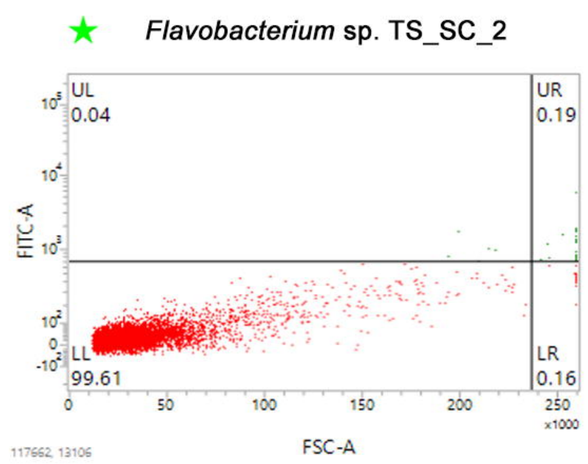

Pseudarthrobacter sp. TS_SC_4
UL 0.86
UR 0.07
LL 99.07
LR 0.00
FITC-A
FSC-A

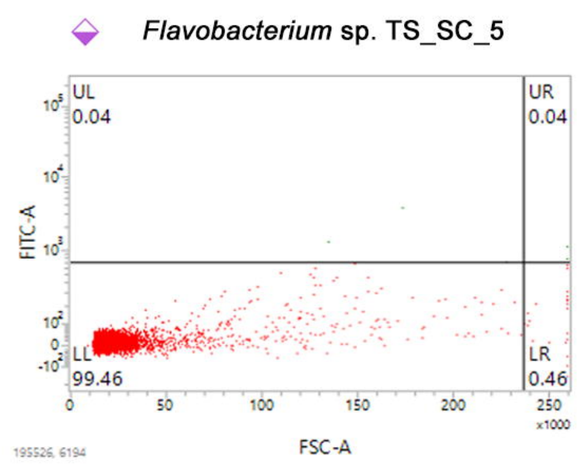

Sanguibacter sp. TS_SC_8
UL 0.60
UR 0.49
LL 98.90
LR 0.01
FITC-A
FSC-A

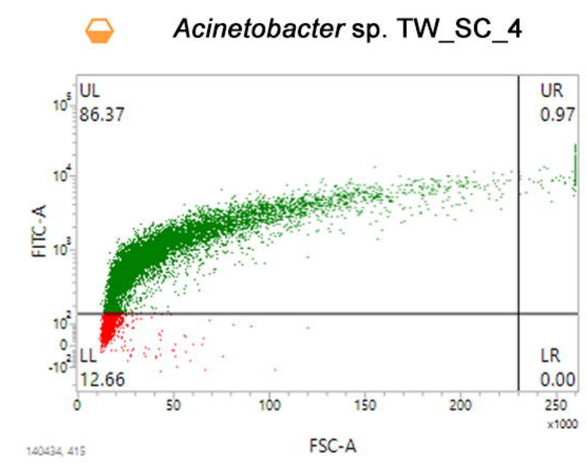

Streptomyces sp. TRD_SC_5
UL 4.95
UR 5.97
LL 88.64
LR 0.44
FITC-A
FSC-A

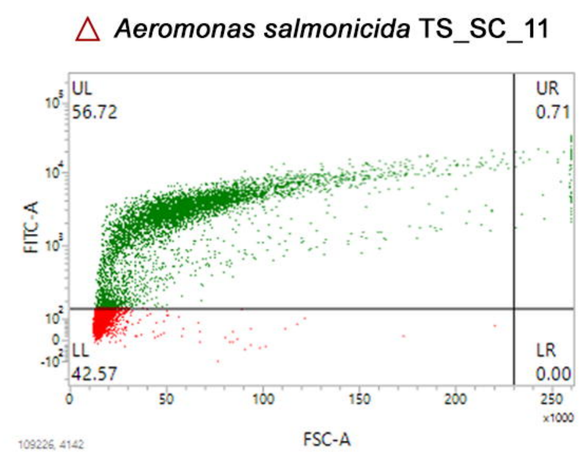

Ancylobacter sp. TW_SC_1
UL 48.65
UR 0.76
LL 50.59
LR 0.00
FITC-A
FSC-A

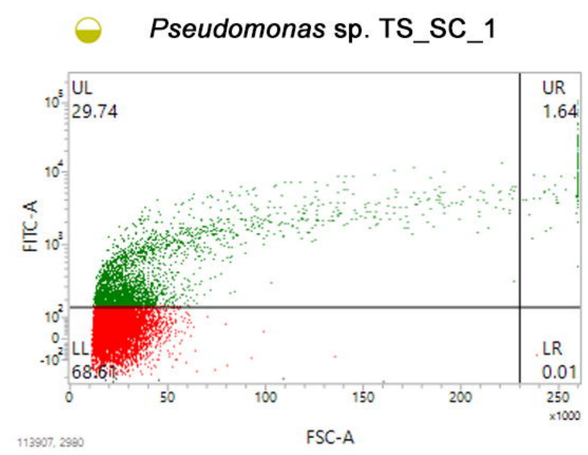

Trichococcus sp. TS_SC_9
UL 0.03
UR 0.00
LL 99.96
LR 0.01
FITC-A
FSC-A

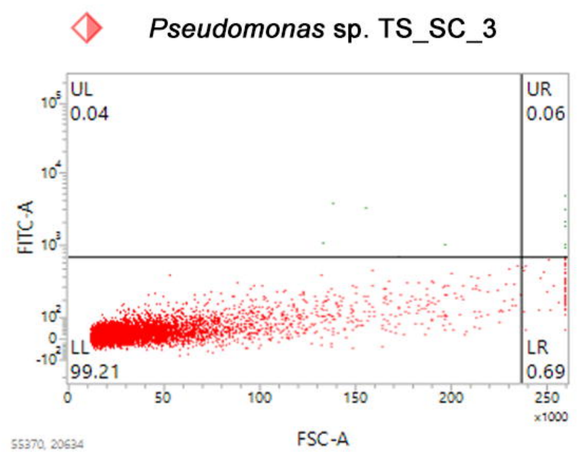

Flavobacterium sp. TS_SC_2
UL 0.04
UR 0.19
LL 99.61
LR 0.16
FITC-A
FSC-A

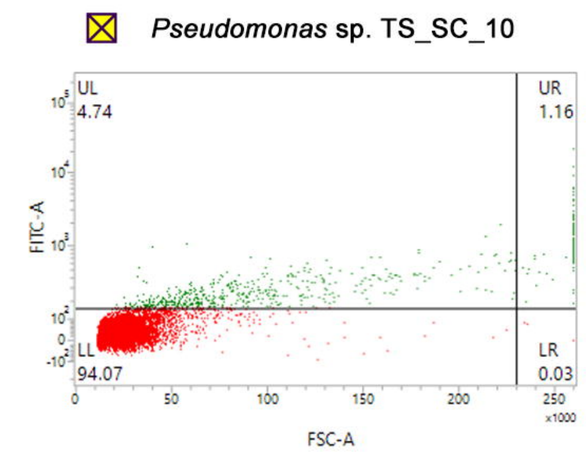

Flavobacterium sp. TS_SC_5
UL 0.04
UR 0.04
LL 99.46
LR 0.46
FITC-A
FSC-A

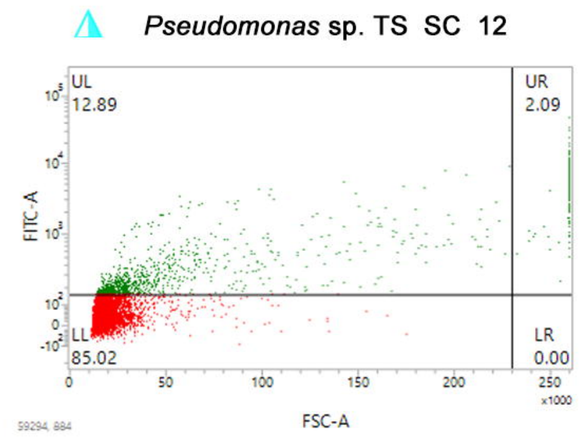

Acinetobacter sp. TW_SC_4
UL 86.37
UR 0.97
LL 12.66
LR 0.00
FITC-A
FSC-A

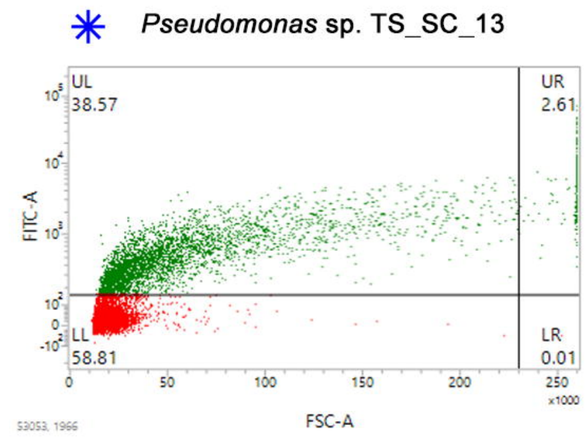

Aeromonas salmonicida TS_SC_11
UL 56.72
UR 0.71
LL 42.57
LR 0.00
FITC-A
FSC-A

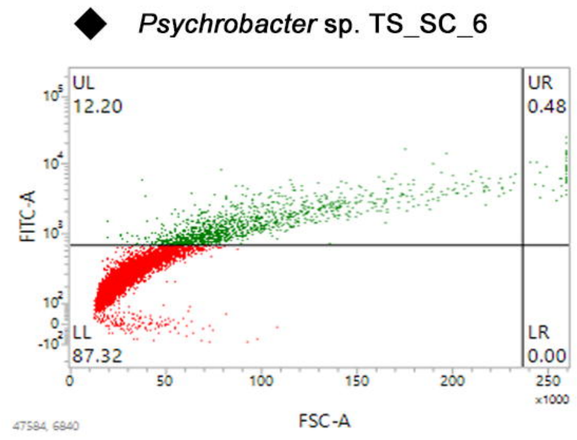

Pseudomonas sp. TS_SC_1
UL 29.74
UR 1.64
LL 68.61
LR 0.01
FITC-A
FSC-A

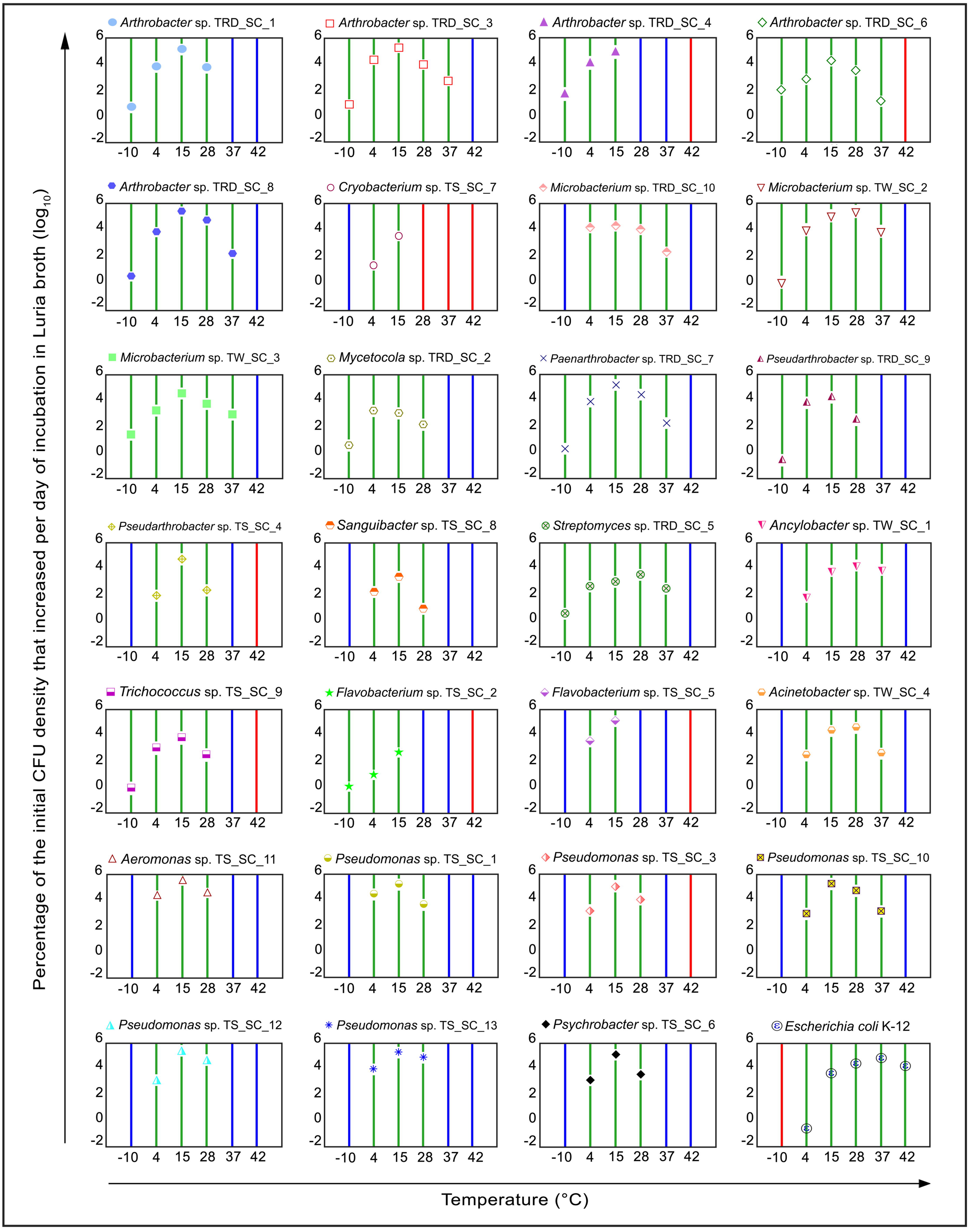

Pseudomonas sp. TS_SC_3
UL 0.04
UR 0.06
LL 99.21
LR 0.69
FITC-A
FSC-A


Pseudomonas sp. TS_SC_10
UL 4.74
UR 1.16
LL 94.07
LR 0.03
FITC-A
FSC-A

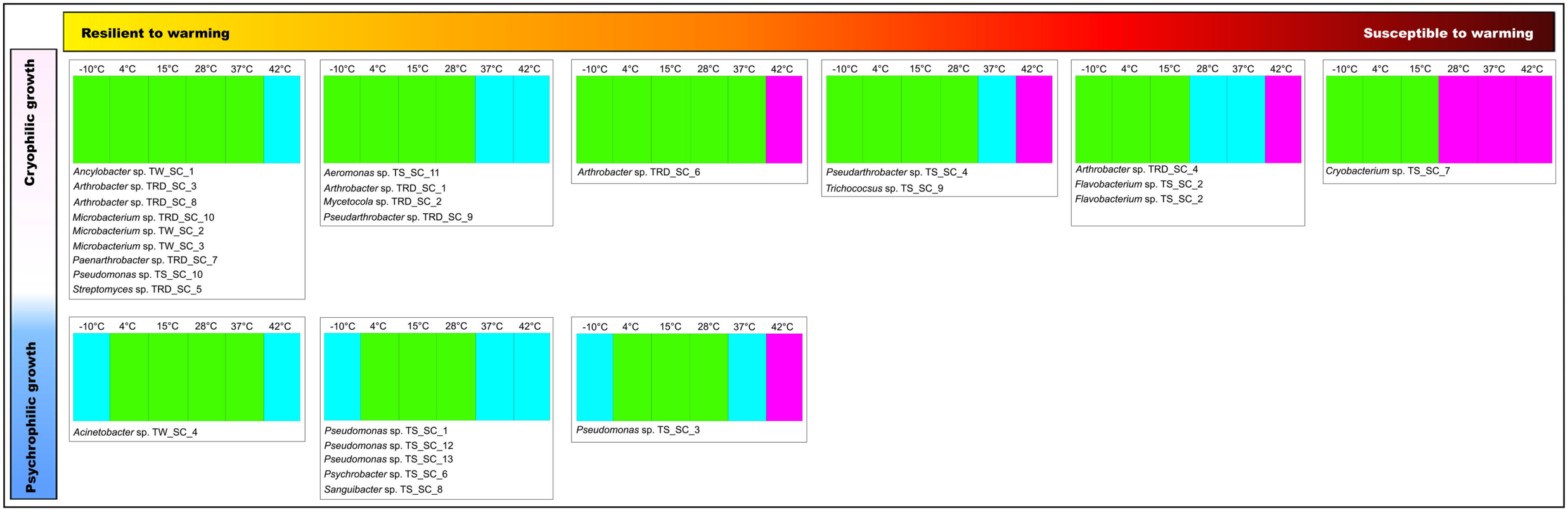

Pseudomonas sp. TS_SC_12
UL 12.89
UR 2.09
LL 85.02
LR 0.00
FITC-A
FSC-A


Pseudomonas sp. TS_SC_13
UL 38.57
UR 2.61
LL 58.81
LR 0.01
FITC-A
FSC-A


Psychrobacter sp. TS_SC_6
UL 12.20
UR 0.48
LL 87.32
LR 0.00
FITC-A
FSC-A


Arthrobacter sp. TRD_SC_1
Arthrobacter sp. TRD_SC_3
Arthrobacter sp. TRD_SC_4
Arthrobacter sp. TRD_SC_6
Arthrobacter sp. TRD_SC_8
Cryobacterium sp. TS_SC_7
Microbacterium sp. TRD_SC_10
Microbacterium sp. TW_SC_2
Microbacterium sp. TW_SC_3
Mycetocola sp. TRD_SC_2
Paenarthrobacter sp. TRD_SC_7
Pseudarthrobacter sp. TRD_SC_9
Pseudarthrobacter sp. TS_SC_4
Sanguibacter sp. TS_SC_8
Streptomyces sp. TRD_SC_5
Ancylobacter sp. TW_SC_1
Trichococcus sp. TS_SC_9
Flavobacterium sp. TS_SC_2
Flavobacterium sp. TS_SC_5
Acinetobacter sp. TW_SC_4
Aeromonas sp. TS_SC_11
Pseudomonas sp. TS_SC_1
Pseudomonas sp. TS_SC_3
Pseudomonas sp. TS_SC_10
Pseudomonas sp. TS_SC_12
Pseudomonas sp. TS_SC_13
Psychrobacter sp. TS_SC_6
Escherichia coli K-12
6
4
2
0
-2
-10
4
15
28
37
42
Percentage of the initial CFU density that increased per day of incubation in Luria broth (log10)
Temperature (°C)

a



b

Weathered rock dust of a lake-side hill						
Strain	Temperature (°C)					
	-10	4	15	28	37	42
Arthrobacter sp. TRD_SC_3						
Arthrobacter sp. TRD_SC_8						
Paenarthrobacter sp. TRD_SC_7						
Streptomyces sp. TRD_SC_5						
Arthrobacter sp. TRD_SC_1						
Mycetocola sp. TRD_SC_2						
Pseudarthrobacter sp. TRD_SC_9						
Microbacterium sp. TRD_SC_10						
Arthrobacter sp. TRD_SC_6						
Arthrobacter sp. TRD_SC_4						

Lake water						
Strain	Temperature (°C)					
	-10	4	15	28	37	42
Microbacterium sp. TW_SC_2						
Microbacterium sp. TW_SC_3						
Acinetobacter sp. TW_SC_4						
Ancylobacter sp. TW_SC_1						

Lake sediment						
Strain	Temperature (°C)					
	-10	4	15	28	37	42
Trichococsus sp. TS_SC_9						
Flavobacterium sp. TS_SC_2						
Pseudomonas sp. TS_SC_10						
Aeromonas sp. TS_SC_11						
Pseudomonas sp. TS_SC_1						
Pseudomonas sp. TS_SC_12						
Pseudomonas sp. TS_SC_13						
Psychrobacter sp. TS_SC_6						
Sanguibacter sp. TS_SC_8						
Pseudarthrobacter sp. TS_SC_4						
Pseudomonas sp. TS_SC_3						
Flavobacterium sp. TS_SC_5						
Cryobacterium sp. TS_SC_7						

Increase recorded in the CFU densities of LB, and/or single C-compound, grown cultures

No increase in CFU density in any type of medium, but >1% cells of the LB culture stained with FDA

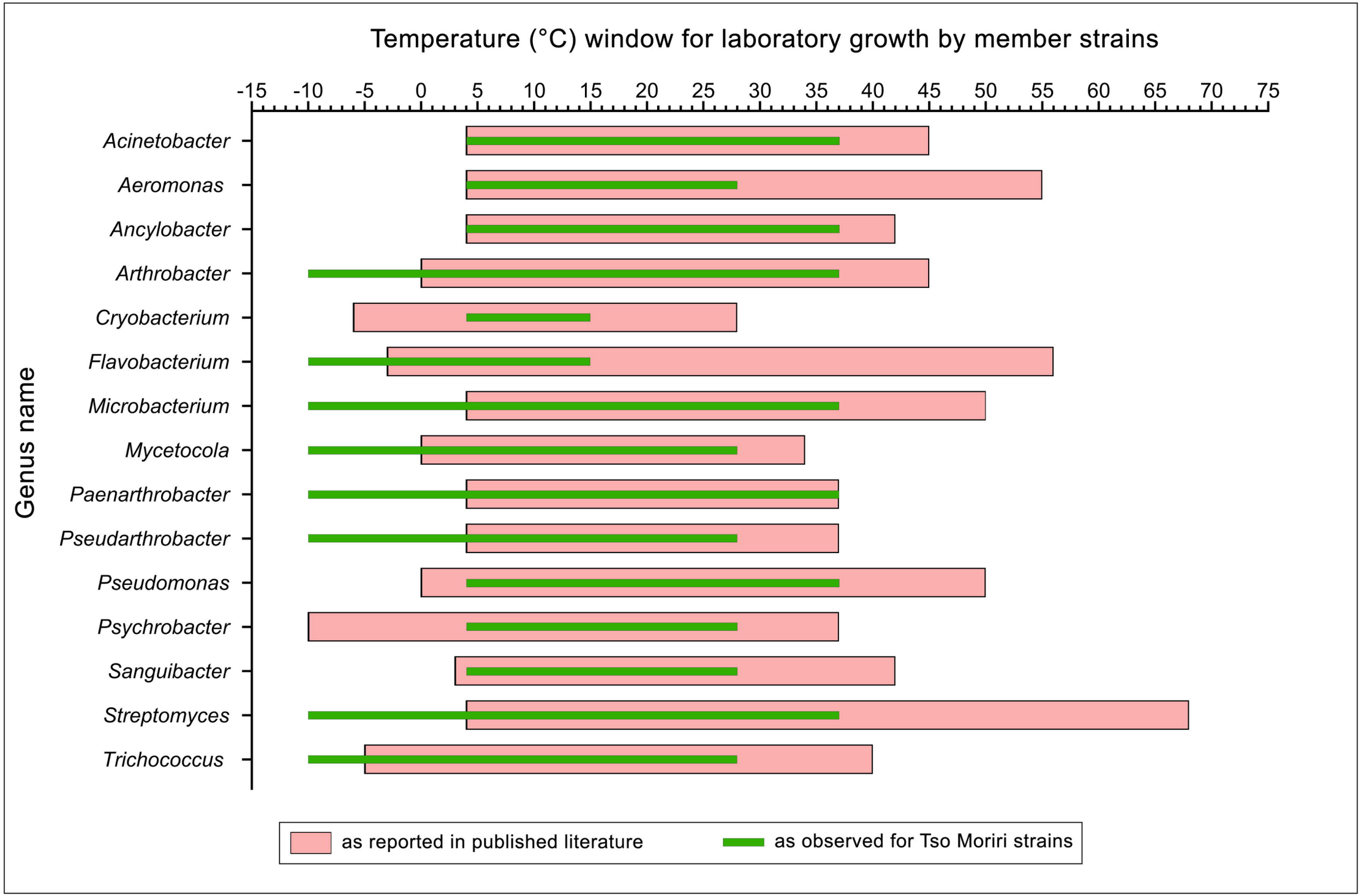
No increase in CFU density in any type of medium, and <1% cells of the LB culture stained with FDA

Potential antagonists (Tso Moriri actinobacteria)

Targets of potential antibiosis (foreign as well as Tso Moriri bacteria)

Origin and identity of the organisms tested			Isolates from the weathered rock dust of a lake-side hill										Isolates from lake-water		Isolates from lake-sediment		
			Arthrobacter TRD_SC_1	*Arthrobacter* TRD_SC_3	*Arthrobacter* TRD_SC_4	*Arthrobacter* TRD_SC_6	*Arthrobacter* TRD_SC_8	*Microbacterium* TRD_SC_10	*Mycetocola* TRD_SC_2	*Paenarthrobacter* TRD_SC_7	*Pseudarthrobacter* TRD_SC_9	*Streptomyces* TRD_SC_5	*Microbacterium* TW_SC_2	*Microbacterium* TW_SC_3	*Cryobacterium* TS_SC_7	*Pseudarthrobacter* TS_SC_4	*Sanguibacter* TS_SC_8
Mesophiles from other habitats	Gram positive bacteria	*Lysinibacillus fusiformis* LAPE1_SD1															
		Bacillus licheniformis PAMA2_SD1															
		Bacillus subtilis SC_1															
	Gram negative bacteria	*Advenella kashmirensis* WT001															
		Paracoccus SMMA_5															
		Escherichia coli K-12															
Tso Moriri isolates outside Actinomycetota	Isolates from lake-sediment	*Aeromonas salmonicida* TS_SC_11															
		Flavobacterium TS_SC_2															
		Flavobacterium TS_SC_5															
		Pseudomonas TS_SC_1															
		Pseudomonas TS_SC_3															
		Pseudomonas TS_SC_10															
		Pseudomonas TS_SC_12															
		Pseudomonas TS_SC_13															
		Psychrobacter TS_SC_6															
		Trichococcus TS_SC_9															
	Isolates from lake-water	*Acinetobacter* TW_SC_4															
		Ancylobacter TW_SC_1															
Tso Moriri isolates belonging to Actinomycetota	Isolates from lake-sediment	*Sanguibacter* TS_SC_8															
		Pseudarthrobacter TS_SC_4															
		Cryobacterium TS_SC_7															
	Isolates from lake-water	*Microbacterium* TW_SC_3															
		Microbacterium TW_SC_2															
	Isolates from the weathered rock dust of a lake-side hill	*Streptomyces* TRD_SC_5															
		Pseudarthrobacter TRD_SC_9															
		Paenarthrobacter TRD_SC_7															
		Mycetocola TRD_SC_2															
		Microbacterium TRD_SC_10															
		Arthrobacter TRD_SC_8															
		Arthrobacter TRD_SC_6															
		Arthrobacter TRD_SC_4															
		Arthrobacter TRD_SC_3															
		Arthrobacter TRD_SC_1															

	Uninhibited lawn of growth ...	... for the incoming organism, around the pre-established colony of the incumbent actinobacterium (potential antagonist).
	Cleared zone of eradication ...	


Temperature (°C) window for laboratory growth by member strains
-15
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
Genus name
Acinetobacter
Aeromonas
Ancylobacter
Arthrobacter
Cryobacterium
Flavobacterium
Microbacterium
Mycetocola
Paenarthrobacter
Pseudarthrobacter
Pseudomonas
Psychrobacter
Sanguibacter
Streptomyces
Trichococcus
as reported in published literature
as observed for Tso Moriri strains