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Research Paper

Biogeochemical study of the periglacial slopes of the Nevado del Ruíz volcano (Colombia) as a terrestrial analog of Mars

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ABSTRACT

The Refugio sector of the Nevado del Ruiz Volcano, located in Colombia, has been the focus of multidisciplinary exploration aimed at understanding its geology, geochemistry, mineralogy, and microbiology, with the objective of evaluating its relevance as a potential terrestrial analog of astrobiological interest for Mars. Through detailed physicochemical analyses of soil samples, variability in properties such as pH, electrical conductivity, and nutrient content has been characterized, revealing the diversity of microenvironments within this volcanic region. Comparison with analog samples from Mars, taken from the ISAR collection, has highlighted significant differences in geochemical composition, particularly in MnO, Fe_2O_3, and SiO_2 content, underscoring the importance of Nevado del Ruiz as a key site for studying Martian geological processes. Furthermore, CRISM spectroscopy analyses have confirmed the presence of minerals similar to those found in Mawrth Vallis on Mars, further supporting the site's validity as a terrestrial analog for planetary studies. Finally, the isolation of bacterial species identified as *Klebsiella spallanzanii* and *Bacillus cereus*, two oligotrophic and psychrophilic microorganisms, capable of phosphate solubilization and nitrogen fixation, found at Nevado del Ruiz support the development of biological models of astrobiological relevance. Together with the other findings, this evidence reinforces the importance of Nevado del Ruiz as a natural laboratory for astrobiology and space exploration, with the potential to provide information for future planetary exploration missions.

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1. Introduction

1.1. Relevance of planetary analogs for Latin America

Planetary exploration faces inherent limitations due to the spatial resolution constraints of astronomical observatories based on Earth. Additionally, space missions involve high costs, which restrict access to the space sector for developing countries (Hettrich et al., 2015). However, these countries often possess significant geological, geographical, and ecological diversity, offering a wide range of environments, some of which may be considered extreme or evaluated as analog sites for various rocky bodies in the Solar System. From this perspective, they present a real opportunity for engaging in international research within the space sciences field (Baker, 2014).

Analog sites are widely used in various applications depending on their characteristics and nature (Foucher et al., 2021; Martins et al., 2017). Some are employed for instrument calibration due to their mineralogical and petrological similarities, for the understanding of large-scale geological processes, local mechanical dynamics, astronaut training, and as sites of astrobiological interest (Foucher et al., 2021; Gómez, 2015; Martins et al., 2017; Mouginis-Mark, 2021). An additional factor that may be relevant when selecting a site as a planetary analog is its accessibility and the logistical opportunities it offers for the development of simulated missions (Pantazidis et al., 2019). For this reason, analog sites that support a wide range of perspectives and types of analysis are highly valued for their multifunctionality (Leal et al., 2025b).

In Latin America, several analog sites have been classified: the Atacama Desert (Chile) (Irwin III et al., 2014; Veneranda et al., 2021), the Puna de Atacama (Argentina-Chile) (Kereszturi et al., 2022; Lorenz and Radebaugh, 2016), Pampas de la Joya (Peru) (Valdivia-Silva et al., 2011), and Laguna Negra (Argentina) (Boidi et al., 2020; Gomez et al., 2014).

Among the various approaches to planetary analogs, the study of sites selected for their petrological and mineralogical analogies represents a valuable opportunity for Colombia. Silicates are the most common minerals on rocky bodies in the Solar System and are also abundant in regions with active or extinct volcanism. Some globally recognized analog sites with these characteristics include the islands of Hawaii (Hughes et al., 2019; Ten Kate et al., 2013), Tenerife (Lalla et al., 2015), and Santorini (Pantazidis et al., 2019), and the Mt. Etna on the island of Sicily (Tangari et al., 2020). In the case of Colombia, which hosts 21 volcanoes located in the Central Andes, there is significant potential for conducting analog studies, given the petrological and mineralogical characteristics, and due to the high altitudes of these volcanic edifices, they exhibit glacial and periglacial conditions of great interest for comparison with the prevailing conditions on Mars.

1.2. Geological, periglacial, and climatological conditions of the Nevado del Ruíz volcano

Among the various volcanoes present in Colombia, one of the most extensively studied and most accessible is the Nevado del Ruíz Volcano, a stratovolcano covered by a thick glacier, with its highest zone reaching 5000 m above sea level (Toro Toro et al., 2010). Compositionally, the volcanic lavas consist of porphyritic basalts and andesites (Rayo Rocha, 2012). The products of the Nevado del Ruíz Volcanic Complex are classified as amphibole andesites, with SiO_2 contents ranging from 56.37 % to 69.94 % (basaltic andesite to dacite), medium-to-high K_2O contents (1.16 %–3.12 %), and are typical of a calc-alkaline series formed in subduction zones along active continental margins (Toro Toro et al., 2010).

The Nevado del Ruíz Volcanic Complex (CVNR) consists of three main and superimposed volcanic edifices, named PRE-RUIZ, CVNR-PER, and CVNR-SER; three minor volcanoes (La Olleta, Nereidas, and Piraña); eight volcanic domes (Alfombrales, Arenales, La Laguna, Santana, San Luis, Plazuelas, Recio, and El Plato); and a fissural source (La Esperanza fissural lava flow) (Navarro-Alarcón et al., 2014). The evolutionary history of the Nevado del Ruíz Volcanic Complex is divided into four eruptive periods: (1) PRE-RUIZ, 1.8–0.97 Ma; (2) First Ruíz eruptive period, 0.76–0.2 Ma; (3) Intermediate eruptive period, <0.15 Ma; and (4) Second Ruíz eruptive period, 0.042–0.035 Ma (Rayo Rocha, 2012).

From an environmental perspective, the Nevado del Ruíz Volcano can experience nighttime temperatures as low as −6 °C and midday temperatures around 0 °C in its highest areas, which correspond to the snowline zone (Giraldo Gómez et al., 2018). Some records suggest that the glacier may have formed approximately 2.5 million years ago and extended from elevations starting at 3000 m above sea level (Flórez, 1992). This elevation is defined by the snowline, defined by the treeline, which is thought to have been shaped by diffuse surface runoff under periglacial conditions (Flórez, 1986). These conditions are believed to have persisted at that elevation until around 21,000 years ago, when fluctuations in temperature and humidity caused the snowline—again defined by the absence of vegetation—to rise in altitude, reaching the conditions observed today (Flórez, 2000).

In the periglacial region, surface water and even small streams are observed to freeze during the early morning hours, when temperatures can drop to −2 °C. When this water freezes within fine sediments, it forms pipkrakes, needle-like or columnar ice structures that form on frozen ground when groundwater rises by capillarity and freezes upon contact with cold air, which in some cases lift soil particles by several millimeters. Upon thawing, these formations generate patterned or striated ground (Flórez, 1989). This type of patterned ground is clearly observed in areas such as the Refugio sector of the Nevado del Ruíz Volcano, a region that remained glacier-covered until approximately four decades ago.

This vegetation-free periglacial sector (Bakker and Salomons, 1989), given the conditions and characteristics previously described, could potentially harbor other forms of life, such as bacteria. However, to date, no microbiological records exist for the area, data that would greatly enhance our understanding of the conditions under which life can develop, including in environments analogous to those found on other planets, such as Mars. Furthermore, the possibility of classifying the Nevado del Ruíz Volcano as a Martian analog has not yet been proposed, thus overlooking one of Colombia's greatest potentials for the advancement of space research. For this reason, the present study aims to identify the petrological, physicochemical, and mineralogical characteristics of the Refugio sector of the Nevado del Ruíz Volcano, in order to assess its potential to be classified as an analog for the study of Mars, as well as to evaluate the geomicrobiological perspective of these cold environments.

2. Materials and methods

2.1. Sampling, physicochemical and geochemical analyses, and comparison with previously classified analogs

For the development of this study, a sampling campaign was conducted in the Refugio sector of the Nevado del Ruíz Volcano in October 2017. A *Z*-pattern sampling strategy was employed (Xu and Buchanan, 2019), which is a technique specific to soil microbiology and is used to ensure variability in microbiological terms, collecting 500 g of pyroclastic material at five sampling points located in areas with minimal human intervention, and one additional control point in an area frequently transited by scientists as shown in Fig. 1. Sampling was performed using a previously sterilized shovel, and the samples were collected in sterile Ziplock® bags. As a result of the geological sampling campaign, six samples were collected from the Nevado del Ruíz Volcano. These were labeled R1, R2, R3, R4, and R5 for the samples taken from areas with minimal human intervention, and RC for the sample collected at the arrival point used by the research team, close to the old mountain Hat (Refugio). The different sampling point locations are shown in

Fig. 1, and the context of the sampling sectors is presented in Fig. 2.

Petrographic analysis was carried out using polished thin sections impregnated with resin, observed under Leyca DM50 polarized light petrographic microscope, to identify phenocrysts and matrix minerals. Mineral descriptions followed the abbreviations proposed by Whitney and Evans (2010). For the analysis of major and trace elements, a portable X-ray fluorescence (XRF) analyzer, Niton XL5®, was used.

For the physicochemical analysis, pH and elemental content were examined to determine the conditions relevant to microbial ecology. Phosphorus determination followed the Bray II method, which involves extraction using an ammonium fluoride solution in diluted hydrochloric acid (García and Ballesteros, 2006). Organic carbon content was measured using the Walkley-Black method, which consists of wet oxidation of the soil sample with a potassium dichromate solution. From this, the easily oxidizable carbon was calculated, and the total organic carbon percentage was obtained using a variable correction factor (Eyherabide et al., 2014). Nitrogen content was estimated based on the percentage of organic carbon, using a standardized formula with a nitrogen mineralization constant adjusted to the climate, and a nitrogen availability factor based on soil texture. Calcium, potassium, and magnesium were analyzed using the atomic absorption method, with extraction performed using 1 N ammonium acetate at pH 7. Sulfur was quantified using the turbidimetric method. pH was determined using a saturation paste and a potentiometer.

To enable comparison with previously established analogs, prior analyses conducted by the Colombian Geological Survey at the Nevado del Ruíz Volcano were considered, which indicated the presence of andesites and basaltic andesites. Based on this, a search was carried out in the International Space Analogue Rockstore (ISAR) for rocks of similar composition (Bost, 2012). Samples identified in the database as Trachyandesite and Basaltic Trachyandesite, classified as Martian analogs, were selected, given the absence of data classified as andesite. Once the samples were chosen, the composition of the various oxides present in the samples was examined. Given that the XRF analyses conducted at Nevado del Ruíz were performed using a portable instrument, which limits the ability to measure Na_2O, it was necessary to normalize the data

Fig. 2. General overview of the Refugio sector in the Nevado del Ruíz Volcanic Complex (CVNR) during the sampling at point R2. (Image source: Picture taken by M.A. Leal,during field campaign in 2017).

available from the ISAR collection by excluding this element. After normalization, a univariate analysis was conducted for each oxide, comparing the Nevado del Ruíz samples with the ISAR sample set. A *t*-test was applied to the data using the RStudio software environment.

2.2. Assessment of Martian analogs

Regarding the evaluation of Martian analog sites, and considering that some previous reports suggest the possible detection of signatures consistent with altered andesites or basalts in the Mawrth Vallis region (Hausrath et al., 2014), data from the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM), aboard the Mars Reconnaissance Orbiter, were obtained from the Planetary Data System for the region in question. The data were analyzed based on the spectral signatures, using the BD1900_2 and BD2290 summary products developed by Viviano

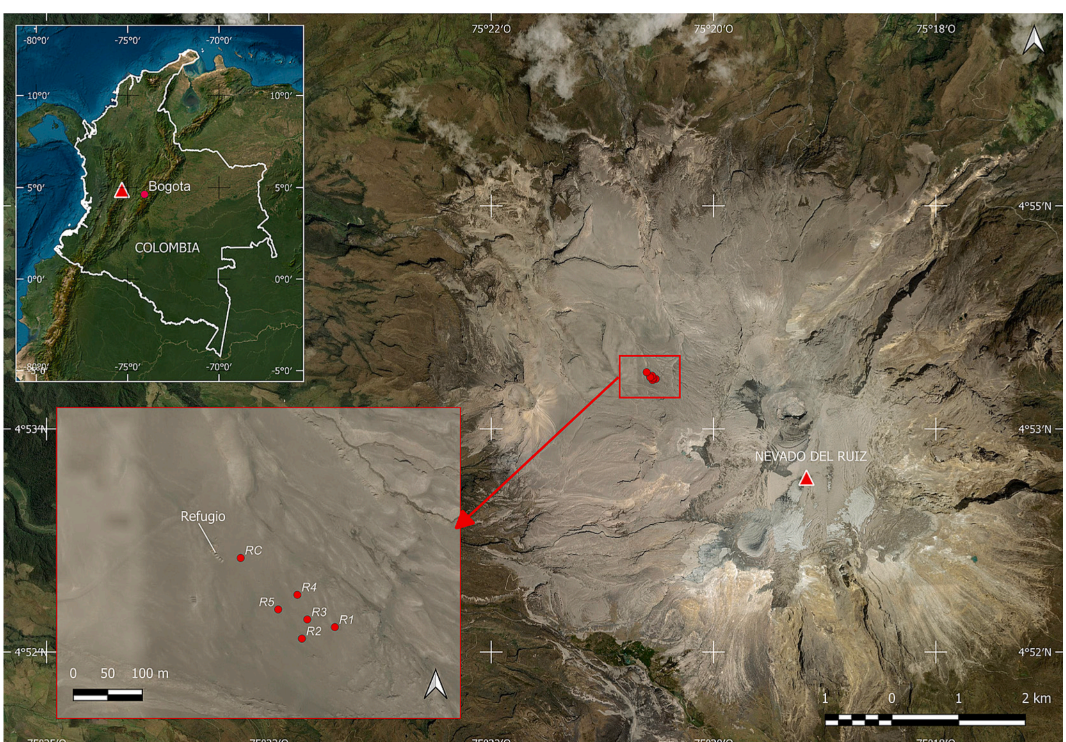

Fig. 1. Sampling points within the Nevado del Ruíz Volcanic Complex (CVNR), in the Refugio sector. (Image source: ESRI satellite world imaginery).

et al. (2014), focusing on the absorption regions associated with the spectral signatures of Hornblende (1400, 2250, 2380 y 2400 nm), according to the sample RK-CMP-055 from RELAB Spectral Database from the Planetary Data System sample analysis (Pieters et al., 2004) and the studies by Ehlmann et al. (2011) and Carter et al. (2013). The goal was to identify spectra with potential similarity to those recorded at the Nevado del Ruíz Volcano and to the selected samples from the International Space Analogue Rockstore (ISAR) collection. For this analysis, three sectors from Mawrth Vallis were selected within the region of interest, and five spectral signatures were extracted from each sector, with each signature corresponding to one pixel. Given that some of the relevant absorption features are less pronounced, the spectra were not normalized in order to facilitate their visual detection.

2.3. Geomicrobiological assays

To isolate bacteria of astrobiological interest, a stock solution was prepared using 10 g of sediment from each of the sampling points at the Nevado del Ruíz Volcano, suspended in 90 mL of sterile 0.85 % saline solution. The suspensions were incubated at 15 °C, considering that the upper limit of the optimal temperature range for psychrophilic microorganisms is 15 °C (Bowman et al., 1997), and for psychrotolerant microorganisms is 20 °C (Moyer and Morita, 2007), in quintuplicate until visible growth was observed, followed by mass inoculations on R2A selective medium (Yadav et al., 2016) to isolate oligotrophic bacteria and verify culture purity. To assess the potential activity (qualitative analysis) for inorganic phosphate solubilization, isolates were inoculated on SRS medium (Gupta et al., 2003) and incubated at 15 °C for 24 to 48 h, following the protocol of the Institute Geographic Agustin Codazzi (Instituto geográfico Agustín Codazzi IGAC, 2006). All assays were performed in triplicate. Positive results were determined based on bacterial growth accompanied by acidification of the culture medium or enzyme production, indicated by the presence of clear halos around the colonies or by a colour change in the medium from purple to yellow.

To evaluate nitrogen fixation activity (qualitative analysis), the NFB (Nitrogen Free Broth) selective medium was used to isolate biological nitrogen-fixing bacteria (Baldani et al., 2014). Cultures were incubated at 15 °C for seven days in triplicate, and positive results were recorded based on observable growth. Bacteria capable of phosphate solubilization, nitrogen fixation, and growth at 15 °C were further evaluated at temperatures of 4 °C, 10 °C, 25 °C, and 37 °C. Growth curves were generated for isolates capable of growing at 4 °C over a 24-h period. Growth was measured as changes in optical density (OD) at 660 nm using a spectrophotometer (Azzota SV-1100, Digital 4NM Visible Spectrophotometer), with aliquots of the culture broth taken at regular time intervals for OD measurements. To estimate the total viable cell count, plate counts were performed after each OD measurement on R2A medium, determining the number of colony-forming units (CFU) per milliliter (ranging from 30 to 300 CFU/g). For the analysis of low-temperature growth, the strain HMI Antart 14, previously isolated from Half Moon Island (Antarctica) and deposited in the Biological Collection “Banco de Cepas y Genes” of the Institute of Biotechnology under the Alexander von Humboldt ID number IBUN-090-04313, was used as a positive control for growth at low temperature. Growth curve analyses were conducted using RStudio software.

Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, USA). The 16S rRNA gene was amplified by PCR under the following conditions (50 μL reaction): 1× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM forward primer 27F (5’-AGAGTTTGATCCTGGCTCAG-3′), 0.4 μM reverse primer 1492R (5’-GGTTACCTTGTTACGACTT-3′), 1.25 U of GoTaq® Flexi DNA Polymerase (Promega), and 10–100 ng of genomic DNA. PCR was performed in a thermal cycler (Mastercycler Pro, Eppendorf AG) with the following program: 2 min at 95 °C; 30 cycles of 20 s at 94 °C, 20 s at 50 °C, and 90 s at 72 °C; followed by 10 min at 72 °C. PCR products were sequenced using Sanger sequencing on an ABI 3500 capillary electrophoresis system at the Molecular Genetics Laboratory of the Colombian Corporation for Agricultural Research. The resulting 16S rRNA sequences were submitted to GenBank with accession numbers PP555749, PP555842, and PP555843. Multiple sequence alignments of 16S rRNA sequences from bacteria associated with cold environments were performed using Highly Similar Sequences (megablast) from the GenBank database. Phylogenetic analysis was conducted using MEGA 11 software. Unrooted trees were constructed using the Maximum Parsimony Tree method, and bootstrap analysis was performed with 1000 replicates of the dataset.

Additionally, considering that the analyzed isolates originated from the same sampling point, a univariate analysis of the various physicochemical parameters from the sampled locations at the Nevado del Ruíz Volcano was performed. A *t*-test was conducted to compare the mean values of the parameters across the sampling sites with those from the specific point where the isolates were obtained. This analysis aimed to identify parameters that were significantly different at the mentioned sampling site. All statistical analyses were performed using RStudio software.

3. Results

3.1. Physicochemical and geochemical analyses, and comparison with previously classified analogs

The volcaniclastic fragments analyzed through petrography correspond, in the case of sample R1 (Fig. S1 supplementary material), to basaltic fragments containing plagioclase, augite, olivine, and enstatite crystals, which are the main phenocrysts typical of this lithology. Hornblende is also present, suggesting a possible compositional change in the volcanic activity. Additionally, volcanic glass lithics associated with basaltic volcanism were observed.

In composition, 47.8 % of the sample consists of subangular plagioclase crystals ranging from 200 to 1000 μm in size, followed by 12.6 % angular augite crystals (100–800 μm), and 12.1 % subangular olivine crystals (50–600 μm). Hornblende, enstatite, and quartz were observed in smaller proportions, with 1.7 %, 1.4 %, and 0.3 %, respectively. Basalt lithics accounted for 18.3 %, volcanic glass lithics for 4.5 %, and opaque minerals for 0.8 %. Finally, alteration minerals such as clays were also identified, representing 0.5 % of the composition.

In sample R2, fragments of volcanic glass and basalt are observed. The crystal fragments consist of plagioclase, olivine, and enstatite, which are the main components of basalts and volcanic glass. Volcanic glass fragments predominate and are characterized by their trachytic, vesicular texture. In composition, 54.3 % of the sample consists of subangular plagioclase crystals ranging from 200 to 1500 μm, followed by 29.7 % volcanic glass lithics (40–800 μm), 3.1 % augite, 2.9 % basalt lithics, 2.3 % olivine, and 2.0 % enstatite. Minor proportions of quartz, hornblende, opaque minerals, and clays are also present (Fig. 3).

In sample R3 (Fig. S2 supplementary material), basaltic fragments are observed, with abundant plagioclase, augite, enstatite, and olivine crystals. These basalt fragments are accompanied by volcanic glass, which, upon alteration to clay minerals, forms palagonite fragments. Notably, this sample shows the presence of quartz, likely associated with hydrothermal processes, and lamprobolite, related to metasomatic processes. In composition, 61.5 % of the sample consists of subangular plagioclase crystals ranging from 80 to 1000 μm in size, 10 % subangular basaltic lithics (500–5500 μm), 8 % augite, 7.3 % enstatite, 4.7 % olivine, 4.3 % volcanic glass lithics, 1.7 % fine ash lithics, 1 % opaque minerals, 0.7 % palagonite lithics, 0.3 % quartz, 0.3 % lamprobolite, and 0.3 % clay minerals.

Sample R4 (Fig. S3 supplementary material) consists of volcanic glass and basalt fragments, containing plagioclase, enstatite, and olivine crystals, which are primary components of basalt. Numerous vesicles are observed within the glass, indicative of subaerial cooling processes. Additionally, whitish ash fragments are present. In composition, the

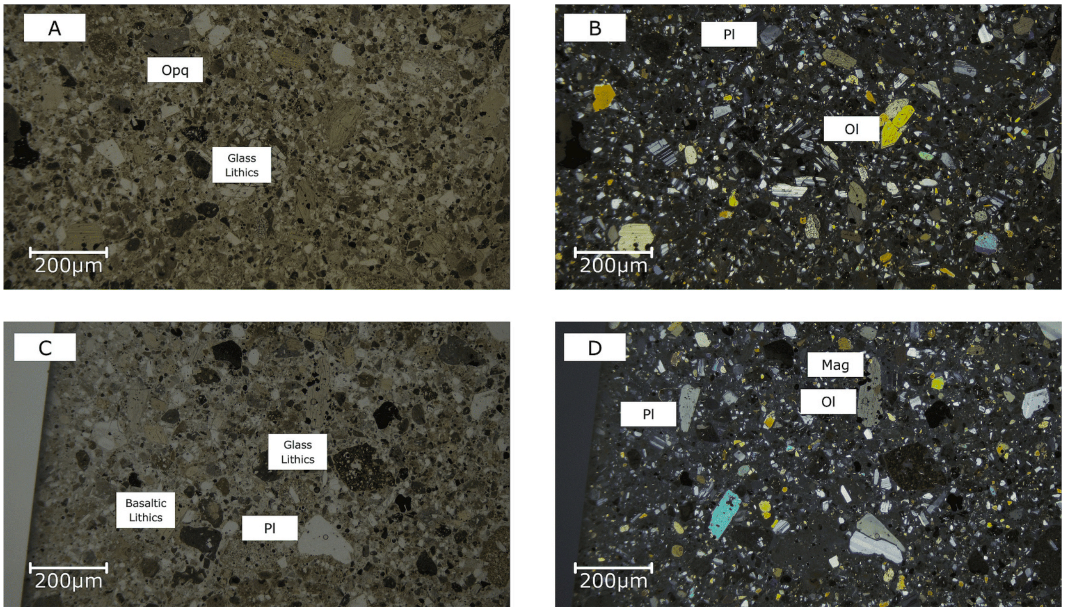

Fig. 3. Thin sections of volcanoclastic fragments from sample R2. A) Photomicrograph under plain light showing opaque minerals (Opq) in grayish tones and glass lithics in dark brown. B) Photomicrograph under cross-polarized light, where plagioclase (Pl) appears gray-blue and olivine (Ol) appears yellow. C) Photomicrograph under plain light showing glass lithics in dark brown, basaltic lithics in black, and plagioclase (Pl) in white. D) Photomicrograph under cross-polarized light, where plagioclase (Pl) appears gray-blue, olivine (Ol) appears yellow, and magnetite (Mag) occurs as black inclusions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

predominant mineral is plagioclase, comprising 47.1 % of the sample, represented by subangular crystals ranging from 100 to 2000 μm. Enstatite accounts for 15.4 %, volcanic glass lithics for 13.8 %, olivine for 8.6 %, basaltic lithics for 6.2 %, and fine ash lithics for 3.1 %. Smaller proportions include 2.2 % augite, 0.9 % opaque minerals, 0.6 % palagonite lithics, 0.6 % hornblende, 0.3 % lamprobolite, and 0.3 % clay minerals.

Sample R5 (Fig. S4 supplementary material) consists of basaltic fragments, with a predominant presence of enstatite over plagioclase, both of which constitute a significant portion of the phenocrysts in basalts and volcanic glass. Hornblende is also observed, possibly associated with variations in magma composition or metasomatic processes. In composition, angular enstatite crystals make up 34.3 % of the sample and range in size from 100 to 800 μm. Subangular plagioclase crystals account for 27.1 %, with sizes between 20 and 2500 μm. Basaltic lithics represent 14.7 %, volcanic glass lithics 11.8 %, while augite and olivine are both present in equal proportions at 4 %. Fine ash lithics constitute 2.4 % of the sample, clays 0.8 %, opaque minerals 0.5 %, and hornblende 0.3 %.

Sample RC (Fig. S5 supplementary material), composed of volcanic glass and basaltic fragments, contains abundant plagioclase, augite, enstatite, and olivine crystals, minerals commonly found as phenocrysts. Volcanic glass predominates over basalt, although both likely share a common effusive origin. Ash fragments, also resulting from the same origin, are present. The sample also displays hornblende crystals, suggesting the presence of a slightly more acidic magma. In composition, 58.6 % of the sample consists of subangular plagioclase crystals ranging from 50 to 350 μm, 18.9 % of volcanic glass lithics, 8 % of basaltic lithics, 6.3 % of augite, 3.4 % of enstatite, and 2.6 % of hornblende. Fine ash, olivine, and opaque minerals are each present at 0.6 %. Finally, quartz and clay minerals are each present at 0.3 %.

Based on the petrographic analysis infers minerals were identified, a search was conducted in the ISAR collection for andesites, resulting in the identification of one Martian analog sample classified as basaltic trachyandesite: sample 14FR03. In addition, two other samples consistent with Martian trachyandesites were identified: samples 14FR02 and 14FR21. All three samples were collected in the Massif Central (France), within the volcanic region of Auvergne.

As a result of the XRF analysis conducted on each of the samples from the Nevado del Ruíz Volcano, along with the normalization of the samples from the ISAR collection, the percentage of each oxide is presented in Table 1. Additionally, based on the TiO_2/Zr ratio, the material was identified as originating from an intermediate-composition rock, which is consistent with the minerals previously identified as shown in Fig. 4.

Regarding the physicochemical parameters analyzed in the different samples from the Nevado del Ruíz Volcano, the recorded values are presented in Table 2.

3.2. Comparison with previously established analogs

Following the comparative analysis between the CVNR samples and those from the ISAR collection to determine whether significant differences existed among the oxides present, it was found, as shown in Table 3 and Fig. 5, that only manganese oxide (MnO), ferric oxide (Fe_2O_3), and silicon dioxide (SiO_2) exhibited statistically significant differences between the two sample sets. In the case of silicon dioxide, the mean value for the Nevado del Ruíz Volcano is higher than that of the samples from France. However, the remaining differences were minor. No statistically significant differences were found for the other oxides.

3.3. Assessment of Martian analogy

Based on the analysis of available CRISM data, it was found that in record frt0003bfb, when band composition was applied using the BD1900_2 and BD2290 products, white regions appeared in the resulting RGB composite image. This visual combination suggests the possible presence of plagioclase and amphibole minerals, the characterization of this region can be observed in detail in Fig. 6. Considering the minerals observed in the samples from the Nevado del Ruíz Volcano, hornblende was selected as the representative amphibole, based on the likely parental material (andesite), This considers the reference spectrum of hornblende obtained from the RELAB database, which is shown in Fig. 7. Additionally, spectral analysis of sectors S1, S2, and S3 in Mawrth Vallis

Table 1
Content of the main oxides present in each of the analyzed samples from the Nevado del Ruíz Volcano and the ISAR collection.

Sample	Fe2O3%	CaO %	MgO %	SiO2%	Al2O3%	K2O %	TiO2%	MnO %	P2O5%
R1	4.537	3.577	1.955	67.443	18.640	2.350	0.610	0.047	0.840
R2	5.013	4.057	3.045	65.791	18.603	1.935	0.623	0.075	0.859
R3	4.801	3.631	2.484	66.232	19.247	2.026	0.641	0.065	0.873
R4	4.565	3.937	2.054	66.014	19.886	1.978	0.665	0.065	0.838
R5	4.383	3.947	2.783	65.561	20.078	1.881	0.605	0.058	0.703
RC	4.596	3.519	2.243	67.045	18.823	2.156	0.690	0.055	0.872
14FR02	7.352	4.816	2.069	60.927	19.001	3.660	1.241	0.233	0.700
14FR03	10.051	6.617	3.235	56.150	18.254	2.657	1.849	0.210	0.977
14FR21	8.572	7.019	3.737	56.849	17.238	3.675	2.122	0.166	0.621

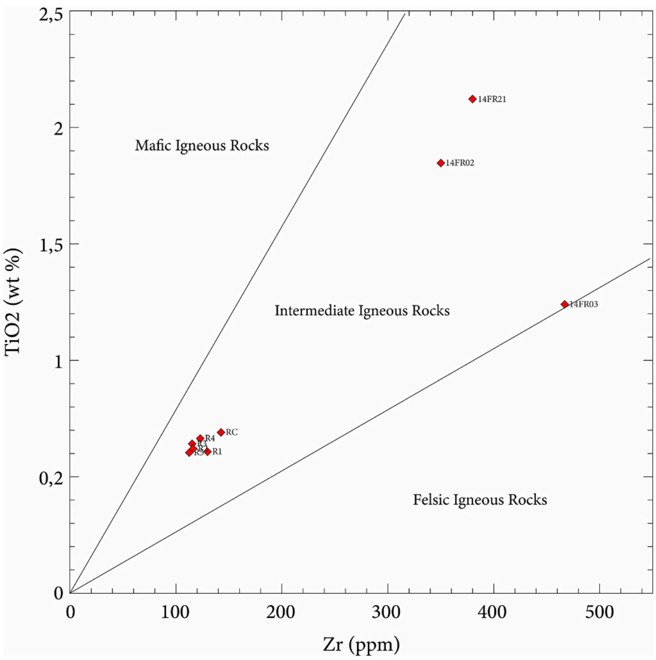

Fig. 4. Type of composition of the materials identified in the Refugio sector of the Nevado del Ruíz Volcano, based on the TiO_2/Zr ratio, according to the XRF analyses, the figure shows that both the samples from Nevado del Ruíz and those from previously established analogs correspond to intermediate rocks, which is useful for their comparison.

Table 2
Physicochemical properties of the samples from the Nevado del Ruíz Volcano.

Sample	R1	R2	R3	R4	R5	RC
pH	3.970	4.110	3.890	4.140	4.310	4.030
CE (dS/m)	0.800	1.120	0.930	0.980	1.110	0.770
Average moisture saturation (%)	15.000	13.800	13.200	13.900	13.600	10.700
Oxidizable Organic Carbon %	0.272	0.236	0.340	0.312	0.348	0.356
Organic Matter %	0.469	0.407	0.586	0.538	0.600	0.614
N-Nh4+ (mg/kg)	1.240	1.090	1.140	0.801	0.919	1.030
N-No3- (mg/kg)	0.924	0.756	0.689	0.678	0.656	0.505
P (mg/kg)	0.035	0.041	0.036	0.041	0.040	0.026
K (mg/kg)	5.070	3.420	3.230	3.030	2.980	2.230
Ca (mg/kg)	21.400	39.200	29.700	35.000	43.600	17.500
Mg (mg/kg)	6.030	6.430	3.760	4.860	5.610	3.530
Na (mg/kg)	13.700	10.300	11.700	9.120	10.200	6.610
Al (mg/kg)	2.790	1.850	1.700	1.140	1.440	0.980
S (mg/kg)	30.300	45.000	35.500	39.200	44.400	22.300
Fe (mg/kg)	0.022	0.025	0.015	0.012	0.015	0.014
Mn (mg/kg)	0.017	0.019	0.015	0.016	0.019	0.009
Cu (mg/kg)	0.017	0.018	0.014	0.014	0.011	0.006
Zn (mg/kg)	0.038	0.023	0.022	0.023	0.021	0.007
B (mg/kg)	0.115	0.136	0.114	0.129	0.144	0.086

Table 3
t-test comparing the oxide content between the sample set from the CVNR and the ISAR collection.

Oxide	CVNR	ISAR collection	LB[1]	Diff[2]	UB[3]	p-value[4]
Al_2O_3	19.21	18.16	−0.74	1.05	2.84	0.1621
CaO	3.78	6.15	−5.21	−2.37	0.47	0.0701
Fe_2O_3	4.65	8.66	−7.31	−4.01	−0.71	**0.0343**
K2O	2.05	3.33	−2.65	−1.28	0.09	0.0575
MgO	2.43	3.01	−2.45	−0.59	1.28	0.3586
MnO	0.06	0.20	−0.22	−0.14	−0.06	**0.0160**
P_2O_5	0.83	0.77	−0.37	0.06	0.50	0.6138
SiO_2	66.35	57.98	2.29	8.37	14.45	**0.0262**
TiO_2	0.64	1.74	−2.21	−1.10	0.02	0.0515

[1] Lower bound of confidence interval.
[2] Difference of means.
[3] Upper bound of confidence interval.
[4] p-value of t-test for independent samples.

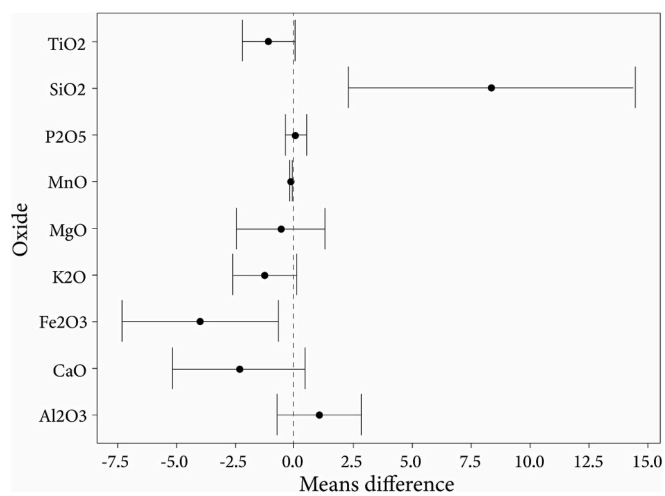

Fig. 5. Confidence intervals for the mean differences in oxide content between the CVNR sample set and the ISAR collection.

yielded the results presented in Figs. 8, 9, and 10, respectively. In Figs. 8 through 10, vertical black dashed lines were added to highlight the characteristic wavelengths of hornblende absorption peaks, which, according to Carter et al. (2013) and Ehlmann et al. (2011), correspond to 1400, 2250, 2380, and 2400 nm.

3.4. Geomicrobiological assays

Stock solutions were prepared from each of the CVNR sampling points. From these solutions, a total of 56 bacterial isolates capable of growing at 15 °C were obtained using R2A medium for oligotrophic organisms. All 56 isolates were evaluated on SRS medium using $AlPO_4$, $FePO_4$, and $Ca_3(PO_4)_2$ as phosphate sources. Qualitative analysis

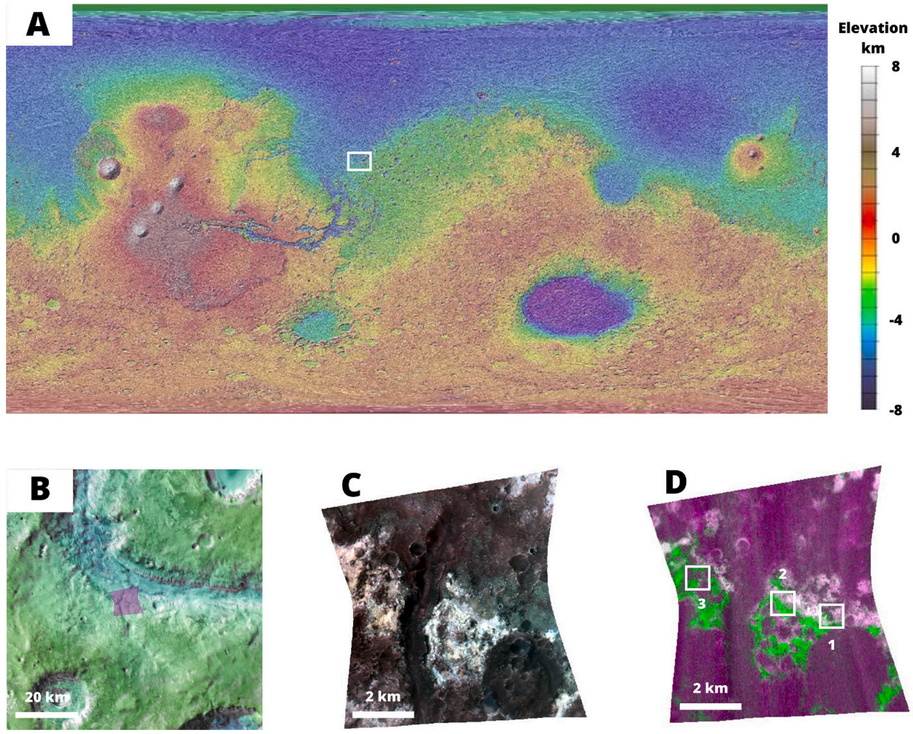

Fig. 6. Location of the site with andesite-like composition-related spectral signatures on Mars, at the Mawrth Vallis region. A) Location of the Mawrth Vallis region on Mars. B) Topographic features of the Mawrth Vallis region, with the location of image C. C) CRISM image frt0003bfb of the analyzed sector in Mawrth Vallis. D) Analysis of the selected region based on the summary products. Purple indicates features highlighted by the BD2290 product, while green indicates features highlighted by the BD1900_2 product (Viviano et al., 2014). The white-outlined areas correspond to the analyzed sectors from right to left: S1, S2, and S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

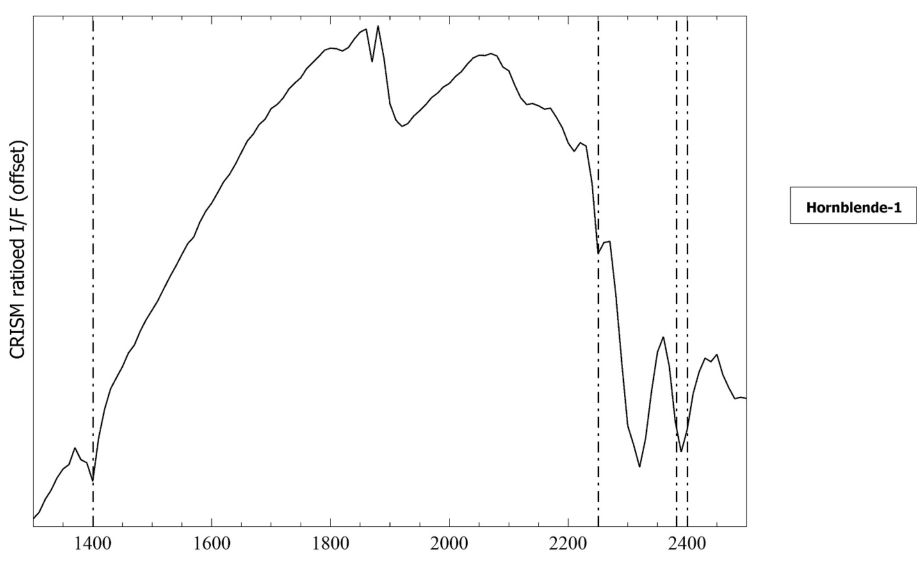

Fig. 7. Reference spectral signature of the mineral hornblende. Three reference spectra from the RELAB database are shown. Vertical lines indicate the most characteristic absorption bands.

determined that 38 of these isolates had the ability to solubilize phosphates. Similarly, the initial isolates were evaluated qualitatively on Nitrogen-Free Bacteria (NFB) medium, resulting in 39 isolates with nitrogen-fixing capability.

Subsequently, isolates exhibiting both phosphate-solubilizing and nitrogen-fixing activity were selected, totaling 21 isolates. These were cultured at different temperatures (4 °C, 15 °C, 25 °C, and 37 °C) for 24 h. Only two isolates were capable of growing at 4 °C during this period: 43–1 (registered in the IBUN "Banco de Cepas y Genes" collection under Alexander von Humboldt ID IBUN-090-04682) and 2–3 (registered under Alexander von Humboldt ID IBUN-090-04673). These strains, along with strain HMI Antart 14 from Antarctica, used as a positive control, were subjected to growth curve analysis to determine optimal growth times, as shown in Figs. 11, 12, and 13.

In the growth curves of the control strain and isolate 2–3 (Figs. 12 and 13), a growth trend is observed in which the stationary phase begins

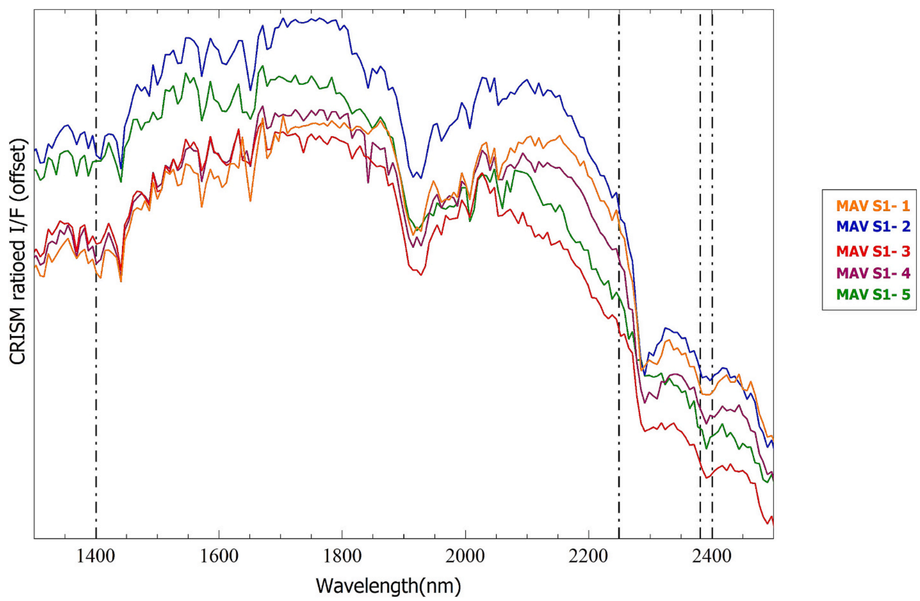

Fig. 8. Spectral signature analysis of Sector 1 in Mawrth Vallis (MAV).

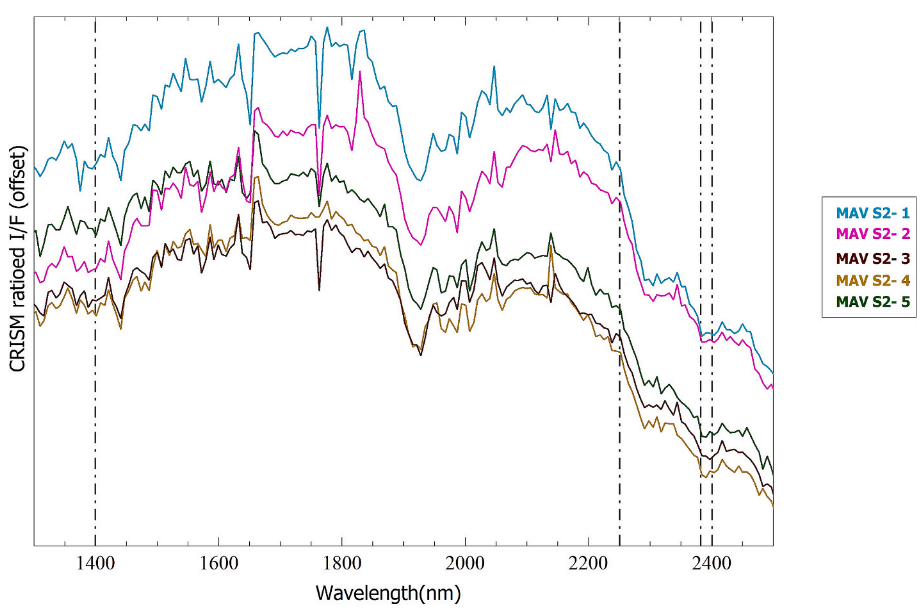

Fig. 9. Spectral signature analysis of Sector 2 in Mawrth Vallis (MAV).

approximately eight hours after inoculation, with an average Optical Density (OD) of 0.230, regardless of the incubation temperature during the experiment. In contrast, the growth curves of isolate 43–1 clearly show a positive effect of temperature on growth. At 4 °C, the stationary phase was reached after 16 h, with an OD of approximately 0.230, making it the least efficient treatment. At the other tested temperatures, the stationary phase was reached more quickly, with the 37 °C treatment showing the fastest onset of the stationary phase, occurring at approximately 8 h with an OD of around 0.240.

Viability assays were performed once the cultures exceeded an optical density of 0.200, with measurements taken two days after inoculation. The results of these assays exceeded the standard maximum detection range (300 CFU/mL) in all tested treatments.

Considering that both isolates 43–1 and 2–3 from the CVNR originated from sampling point R2, a *t*-test was conducted to compare the mean values of the physicochemical parameters with those at point R2. The results revealed statistically significant differences in the content of copper, iron, magnesium, organic matter, oxidizable organic carbon, and sulfur, as shown in Table 4 and Fig. 14.

The 16S rRNA gene analysis identified isolate CVNR 43–1 as *Klebsiella spallanzanii*, isolate CVNR 2–3 as *Bacillus cereus*, and the control HMI Antart 14 as *Bacillus velezensis*, as shown in Fig. 15.

4. Discussion

4.1. Geochemical variability in volcanic environments

This study provides a first overview of the geochemistry and microbiology of the Nevado del Ruíz Volcano (CVNR), highlighting its relevance for understanding geological evolution and biology in volcanic environments at high elevations and relatively cold climatic conditions. Particularly, volcanic material in periglacial environments where

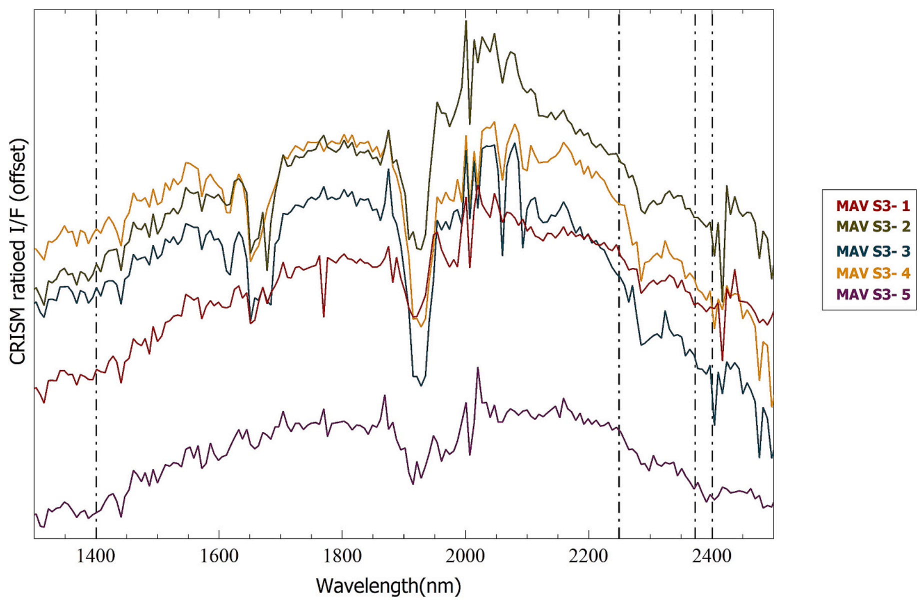

Fig. 10. Spectral signature analysis of Sector 3 in Mawrth Vallis (MAV).

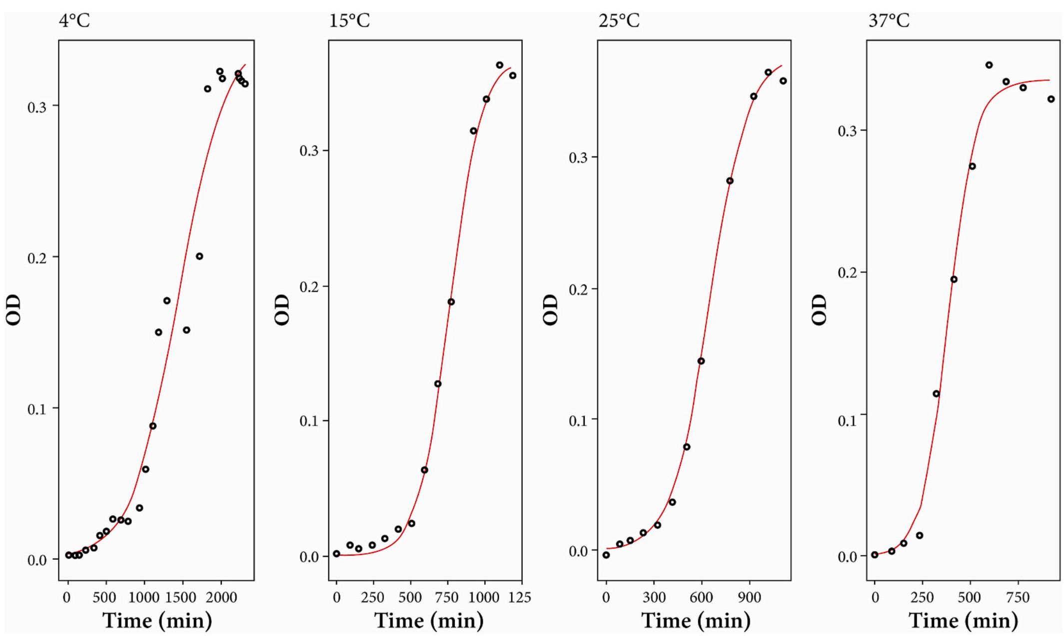

Fig. 11. Average growth curves at different temperatures for the isolate 43–1 from the CVNR, showing the growth duration until the end of the bacterial exponential phase at four different temperatures.

the presence of ice played a significant role and low temperatures (below 0 °C) are ubiquitous. The results obtained from the mineralogical characterization are consistent with the typical features of andesitic materials, showing a predominant presence of minerals such as plagioclase, augite, olivine, and amphiboles (Smith and Jones, 2023). These findings align with previous research on the petrology of the volcanic region, supporting the consistency of the mineralogical composition in the CVNR (García et al., 2023).

The significant differences observed in certain mineralogical and physicochemical parameters between the CVNR samples and those from the ISAR collection in France underscore the importance of understanding geological and environmental variability in volcanic settings, while some statistically significant differences were observed in Fe_2O_3, MnO, and SiO_2 content, these are not unexpected. These differences may be attributed to several factors, including variability in magma composition, alteration processes, and the influence of both past and present volcanic activity. Moreover, the differences in physicochemical parameters may reflect variations in environmental conditions and soil dynamics within the CVNR, highlighting the complexity of volcanic systems and their influence on the biogeochemistry of surrounding areas (Pérez and Gómez, 2023). Such differences must be also considered when analyzing environments with potential andesitic materials on the Martian surface.

4.2. Possible presence of intermediate rocks in Mawrth Vallis

Multiple studies have proposed the presence of andesitic materials on Mars. Bandfield et al. (2000), based on data from the Thermal

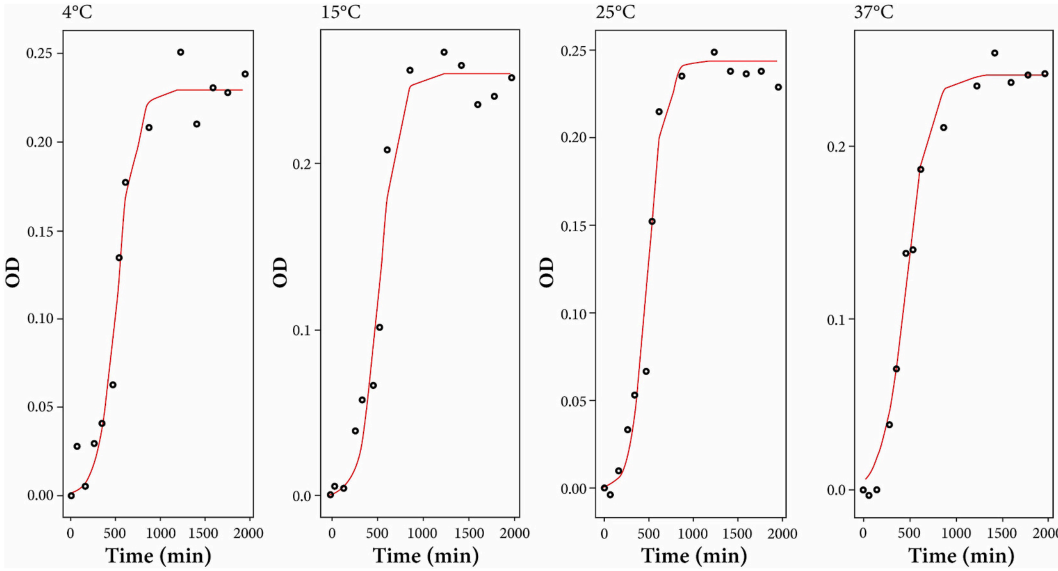

Fig. 12. Average growth curves at different temperatures for the isolate 2–3 from the CVNR, showing the growth duration until the end of the bacterial exponential phase at four different temperatures.

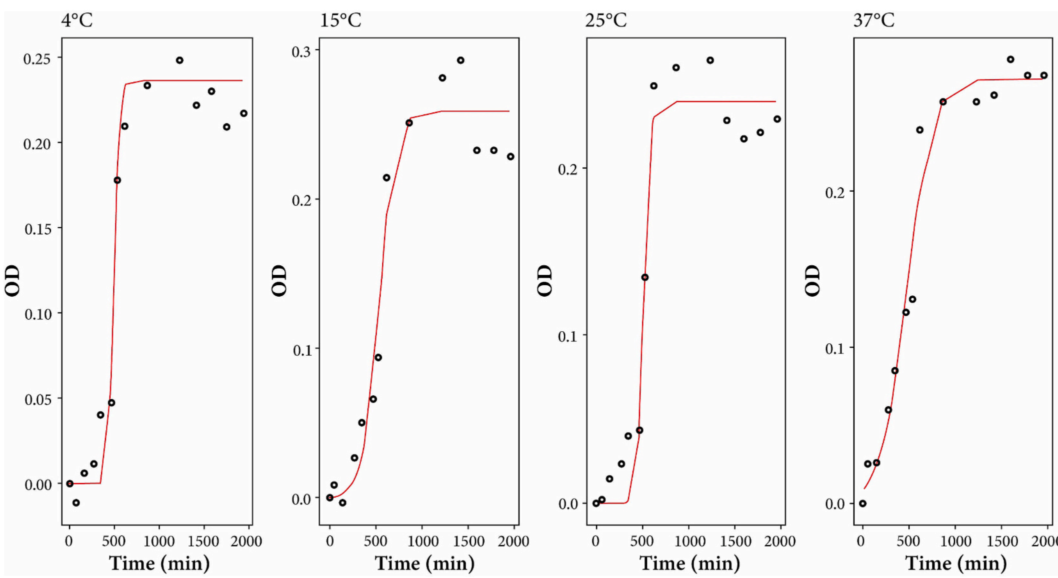

Fig. 13. Average growth curves at different temperatures for the isolate HMI Antart 14 from Half Moon Island, Antarctica, used as a positive control, showing the growth duration until the end of the bacterial exponential phase at four different temperatures.

Emission Spectrometer (TES), suggested their occurrence in regions such as Acidalia Planitia and Ares Vallis. Zuber et al. (2000) proposed that in the Arabia Terra region, basalts might be covered by a layer of andesite, which could have been transported from other regions due to the finer grain size of andesitic material compared to basalts. However, the TES data had spatial resolutions ranging from 3.5 to 3600 km^2/pixel, which made it difficult to accurately characterize specific regions where andesites were detected and to develop hypotheses about their origin (Rogers and Christensen, 2003). The present analyses contribute to a more detailed characterization of regions with andesitic presence by utilizing CRISM data, which offer a much finer resolution of 18 m^2/pixel. This enables the confirmation of andesitic materials and supports the analysis of geological, environmental, and temporal factors that may have contributed to their formation and evolution.

In the Mawrth Vallis region in particular, abundant clay deposits and other substrates have been identified, which are long-standing indicators of water–rock interaction and are highly relevant from an astrobiological perspective (Poulet et al., 2020). Spectral data from CRISM and observations from the Thermal Emission Spectrometer (TES) have enabled the detection of various classes of phyllosilicates, such as smectites, kaolinites, and in some cases, allophane/imogolite (Wills et al., 2009). These clays are formed through hydrothermal alteration and weathering processes in the presence of water, implying that the Martian environment in Mawrth Vallis was, at some point, sufficiently persistent to support complex chemical reactions (Poulet et al., 2020).

The layered and porous structure of these clays not only preserves the mineral alteration history but also provides stable microenvironments capable of adsorbing and preserving organic matter (Shevchenko, 2025; Hazen and Sverjensky, 2010). This is crucial, as clays may act as natural capsules, protecting potential biosignatures from radiation and other surface-degrading agents on Mars. Furthermore, the mineral diversity of these clays, including aluminum-rich varieties and those with

Table 4
Results of the t-test of the physicochemical attributes at sampling point R2 compared to the mean of the sampling points in the CVNR.

Attribute	Estimate	LB[1]	UB[2]	R2 Value	p-value[3]
Al	1.61	0.72	2.50	1.85	0.4949
Average humidity saturation	13.28	11.31	15.25	13.80	0.5050
B	0.12	0.09	0.14	0.14	0.1279
Ca	29.44	16.44	42.44	39.20	0.1056
CE (dS/m)	0.92	0.75	1.09	1.12	**0.0310**
Cu	0.01	0.01	0.02	0.02	**0.0395**
Fe	0.02	0.01	0.02	0.03	**0.0051**
K	3.31	2.00	4.62	3.42	0.8241
Mg	4.76	3.39	6.13	6.43	**0.0275**
Mn	0.02	0.01	0.02	0.02	0.0872
$N\text{-}Nh^{4+}$	1.03	0.81	1.24	1.09	0.4566
$N\text{-}No^{3-}$	0.69	0.50	0.88	0.76	0.3842
Na	10.27	6.95	13.58	10.30	0.9787
Organic Matter	0.56	0.49	0.63	0.41	**0.0043**
Oxidizable organic carbon	0.33	0.28	0.37	0.24	**0.0043**
P	0.04	0.03	0.04	0.04	0.1119
pH	4.07	3.87	4.27	4.11	0.5957
S	34.34	23.81	44.87	45.00	**0.0482**
Zn	0.02	0.01	0.04	0.02	0.8786

[1] Lower bound of confidence interval.
[2] Upper bound of confidence interval.
[3] p-value of t-test.

high magnesium and iron content, suggests the existence of chemical gradients that could have served as sources of energy and nutrients for possible microbial communities (Poulet et al., 2020).

4.3. Nevado del Ruíz volcano as a functional analog to Mawrth Vallis

Mawrth Vallis is known for its complex geological history, including hydrothermal activity, fluvial transport, chemical weathering, and possibly the presence of intermediate volcanic deposits (Poulet et al., 2020; Bishop and Rampe, 2016). This complexity has led to the proposal of diverse lithologies: altered basalts, andesites, and even felsic rocks, to explain the spectral signatures observed. Our study aims to contribute to this ongoing debate by providing evidence from a terrestrial analog that integrates intermediate lithology, hydrothermal alteration, and periglacial conditions. Although no analog site can reproduce Martian conditions perfectly, the CVNR shows a significant mineralogical match, particularly in the presence of Fe/Mg smectites, iron oxides, silica, and amphiboles, that is consistent with CRISM spectral features. This supports its classification as a functional analog, focused not on absolute compositional identity, but on the study of geological, geochemical, and biogeochemical processes relevant to habitability, phosphorus redistribution, and biosignature preservation (Foucher et al., 2021; Bandfield et al., 2000; Zuber et al., 2000; Rogers and Christensen, 2003).

This study contributes to the identification of spectral data associated with andesites on the surface of Mawrth Vallis. Of the 15 spectra analyzed across the three studied sectors, three (MAV S1–2, MAV S2–5, MAV S3–5) showed all four diagnostic absorption bands used in the characterization (Figs. 8, 9, and 10). These findings add crucial evidence to the historical evolution of local aqueous environments on Mars. The presence of these rocks, with fine-grained transport characteristics (Zuber et al., 2000) and associations with alteration minerals, suggests sedimentary processes of material redistribution, potentially linked to fluvial or hydrothermal activity. Previous studies have shown that regions with spectral signatures compatible with andesites also exhibit mineralogical diversity that reflects changes in water chemistry (Zuber et al., 2000). This reinforces the classification of Mawrth Vallis as a site

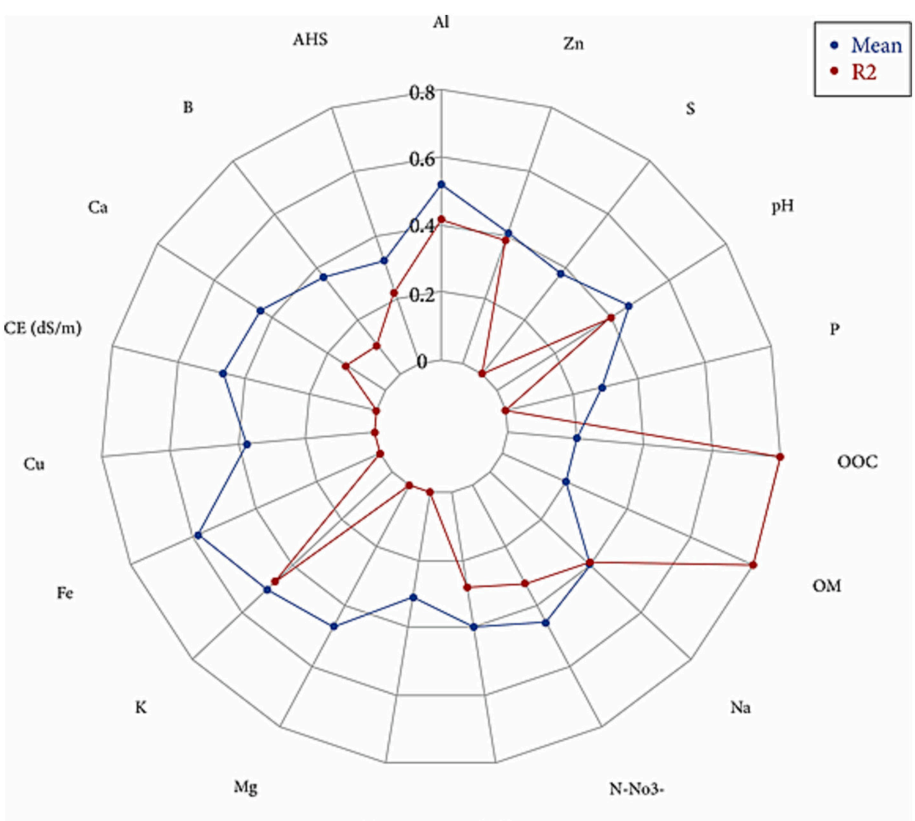

Fig. 14. Profile of the physicochemical attributes at sampling point R2 compared to the mean of the sampling points in the CVNR. (AHS: Average Humidity Saturation, OOC: Oxidizable organic carbon; OM: Organic Matter).

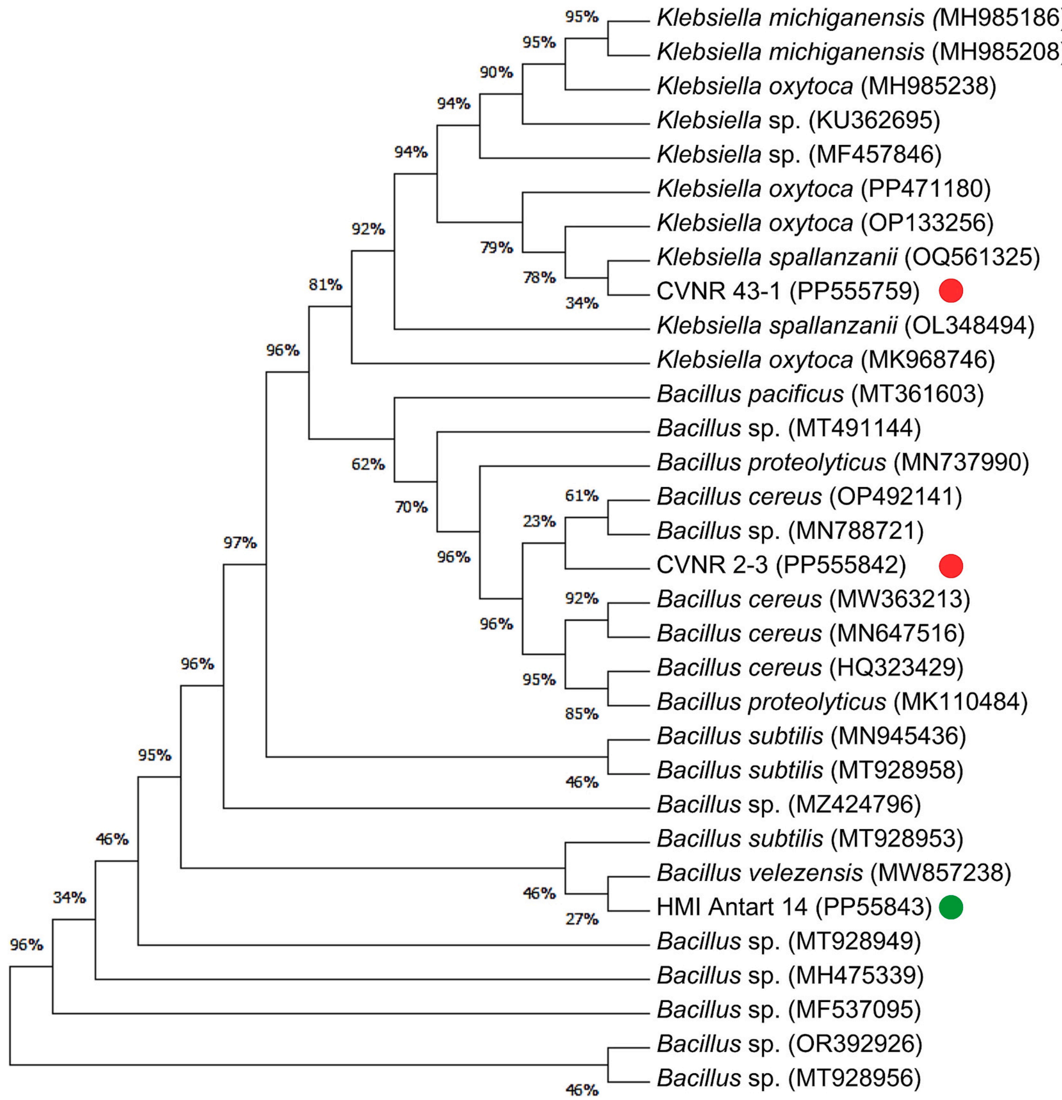

Fig. 15. Maximum Parsimony Tree showing the positions of isolates CVNR 2–3, CVNR 43–1, and the control HMI Antart 14.

of high interest for the search for biosignatures, such as amino acids with L-enantiomeric excess or certain fatty acids, offering a stratigraphic record that documents the transition from wet environments to more arid conditions (Bishop et al., 2013; Bishop and Rampe, 2016), similar to the evolutionary patterns observed in the periglacial conditions of the Refugio sector at the Nevado del Ruíz Volcano.

Taken together, the coexistence of andesites, clays, and other altered substrates in Mawrth Vallis not only documents past episodes of aqueous activity, but also establishes a scenario in which habitable conditions may have favored the emergence and preservation of life. These findings reinforce the site's potential to host biosignatures and highlight the importance of continuing integrated studies that combine spectral, geochemical, and mineral alteration analyses to unravel the complex history of this Martian environment.

The study of the Nevado del Ruíz Volcano (CVNR), with its intricate interaction of volcanic, periglacial, and chemical alteration processes, provides a valuable analog framework for understanding the dynamics that may have operated in Mawrth Vallis. Geochemical and petrographic analyses conducted at the CVNR have revealed the presence of andesitic materials, altered basalts, and clays, very similar to those identified in Mawrth Vallis, and probably existing in other locations of Mars. Furthermore, the periglacial conditions at Nevado del Ruíz (Flórez, 2000), where daily and seasonal thermal fluctuations, combined with the presence of ice, drive freeze-thaw cycles, create an environment in which both physical and chemical weathering act intensively. This scenario favors the formation of clays, the redistribution of essential elements such as phosphorus, and the development of microenvironments that, taken together, mirror the alteration and biosignature preservation processes expected under Mars' extreme climates.

At the CVNR, repeated freeze-thaw cycles not only contribute to the fragmentation and reconfiguration of volcanic materials, but also promote the release and reprecipitation of chemical compounds that may be utilized by life. These mechanisms are comparable to those hypothesized for Mawrth Vallis, where the interaction between water, radiation, and soil has produced complex and diverse stratigraphic records. The similarity in mineral composition and alteration processes, including clay formation and phosphorus mobilization (Hausrath et al., 2014), supports the extrapolation of mechanisms observed at CVNR to the geological and biogeochemical evolution of Mawrth Vallis.

Thus, the study of the CVNR not only contributes to our understanding of volcanic and periglacial environmental evolution on Earth but also offers key insights for interpreting the aqueous history and biosignature preservation potential on Mars. When evaluated as an analog, the detection of psychrophilic bacteria through microbiological techniques at the CVNR strengthens the plausibility of life in Mawrth Vallis and informs strategies for its future exploration.

The current Martian environment, with an average surface pressure of approximately 6 mbar, precludes the stable existence of liquid water at the surface. Consequently, any potential hydrothermal habitat must be located beneath the surface, where lithostatic pressure may increase sufficiently to maintain water in a liquid state, particularly when associated with salts or other compounds that lower its freezing point. Kurokawa et al. (2018) estimated that atmospheric pressure on early Mars, around 4.1 Ga, may have exceeded 0.5 bar, which could have supported transient episodes of surface or near-surface liquid water. However, subsequent atmospheric collapse likely restricted habitable aqueous environments to the subsurface. Supporting this hypothesis, experimental studies have demonstrated that certain methanogenic archaea can metabolize and produce methane at pressures as low as 50 mbar, comparable to those expected in the Martian shallow subsurface, although their metabolic activity is significantly reduced under such stress conditions (Kral et al., 2011).

Beyond pressure constraints, the absence of a substantial magnetic field and the consequent exposure to intense ionizing radiation render the Martian surface particularly hostile to microbial life. Ultraviolet and galactic cosmic radiation can damage cellular structures and genetic material, posing a significant barrier to surface habitability (Nicholson et al., 2013). In contrast, the subsurface offers natural shielding and a more stable chemical environment. Nicholson et al. (2013) reported that several strains of Carnobacterium isolated from Siberian permafrost were capable of growth under simulated Martian conditions, including low temperature (0 °C), anoxic CO_2-dominated atmospheres, and pressures as low as 7 mbar. These findings suggest that microorganisms adapted to permafrost or deep subsurface environments on Earth may serve as viable analogs for putative life on Mars, supporting the hypothesis that if microbial life exists or once existed on Mars, it is more likely to persist below the surface, where pressure, temperature, and radiation conditions are more conducive to habitability.

4.4. Biogeochemical and Astrobiological implications

The bacterial isolates identified at the Nevado del Ruíz Volcano, particularly the two organisms that exhibited both phosphate-solubilizing and nitrogen-fixing activity and were able to grow at 4 °C, *Klebsiella spallanzanii* (Gram-negative bacillus with capsule) (Merla et al., 2019) and *Bacillus cereus* (Gram-positive bacillus with endospore, facultative anaerobe) (Abbas et al., 2014), represent a substantial model for understanding the capacity of microorganisms to thrive in cold, volcanic conditions. These bacteria, with demonstrated abilities to solubilize phosphate and fix nitrogen, hold dual significance. On one hand, their presence supports the hypothesis that extreme environments can host active microbial communities, reinforcing the rationale for seeking life in Mawrth Vallis, where conditions have been similarly harsh and variable. The ability to survive and grow under low-temperature and high-volcanic-activity conditions suggests that even on Mars, where temperature fluctuations and radiation are intense microbial niches might exist that are adapted to such challenges.

Additionally, phosphate solubilization is critical for the release of phosphorus into the soil, an essential nutrient for the biochemistry of life, as it forms part of key molecules such as ATP, nucleic acids, and cellular membranes. Given that phosphorus measurements on Mars have shown limited concentrations, and that in many cases, phosphorus exists in less reactive mineral forms (Hausrath et al., 2024), microorganisms similar to those isolated from Nevado del Ruíz could have contributed to increasing phosphorus availability in Martian environments. This mobilization of phosphorus could not only have facilitated potential prebiotic processes but also played a crucial role in sustaining microbial communities, had they developed, enabling life to emerge and persist under initially unfavorable conditions.

The phosphate-solubilizing capacity of these bacteria is closely linked to the amount of bioavailable phosphorus in the soil. In Martian environments, where water–rock interactions may have limited phosphorus reactivity by trapping it in low-solubility minerals, microbial activity could have facilitated its release and subsequent assimilation (Hausrath et al., 2024). In this context, the mechanisms observed at Nevado del Ruíz, such as the production of organic acids and enzymatic actions that break mineral bonds, could be extrapolated to Mawrth Vallis. The presence of phosphate-bearing minerals and chemical alteration induced by aqueous processes suggest an environment in which biology, if ever developed, could have interacted to enhance phosphorus bioavailability.

The integrated set of findings presented in this study—from the mineralogical and geochemical characterization of andesite and other materials in Mawrth Vallis to the identification of microorganisms with biogeochemical capabilities at the Nevado del Ruíz Volcano—reinforces the importance of terrestrial analogs in astrobiological research. Not only is Mawrth Vallis validated as a site of high interest for future in situ exploration missions (Gross et al., 2024), but Nevado del Ruíz is also positioned as a potential terrestrial analog of this Martian region. These results demonstrate that extreme environments on Earth can effectively model processes that may have facilitated the emergence or preservation of life on Mars. The combination of spectral, geochemical, and geomicrobiological data enables the construction of a holistic model that integrates both inorganic and organic processes, an essential approach for understanding the evolution of habitability on the Red Planet.

Moreover, the presence of bacteria such as *Klebsiella spallanzanii* and *Bacillus cereus* at Nevado del Ruíz highlights the adaptability of life to extreme conditions, providing concrete examples of biochemical strategies that may have been employed in Martian environments. These microorganisms not only contribute to the mobilization of essential nutrients, but also influence mineral formation and alteration, creating microenvironments conducive to biosignature preservation. The similarity in alteration mechanisms and biological responses observed in this terrestrial analog supports the idea that, even on a planet as inhospitable as Mars, biogeochemical processes compatible with life may exist or may have once existed.

Notably, both microorganisms were isolated from sampling point R2, where, as shown in Fig. 14 and Table 4, the parameter with the most significant difference is organic matter content. As detailed in Table 2, this point exhibited the lowest level of organic matter, reinforcing the oligotrophic nature of the identified microorganisms, a trait highly relevant to Martian environments. Studies such as those by Pérez and Martínez (2023) and Rodríguez and López (2023) further support the existence of a wide variety of microorganisms at Nevado del Ruíz.

Therefore, future astrobiology research at Nevado del Ruíz should focus on conducting controlled experimental studies that simulate Martian conditions, such as oxygen depletion and radiation exposure. These studies should investigate the interaction between phosphate-solubilizing microorganisms and phosphate minerals to assess their role in nutrient bioavailability in Martian soils (Kumari and Prakash, 2024), as well as their potential in biotechnological applications (González and Sánchez, 2023) or in the production of plant growth-promoting biocompounds for future crewed space missions (Handy et al., 2021). Additionally, further exploration of the geological and geochemical characteristics of other sectors within the volcanic complex could help refine models for analyzing and interpreting the history of Mawrth Vallis, or other sites of Mars with geochemical similarities. In addition, comparative studies could be conducted with other Martian analogs that feature volcanoes and glaciers, such as those found in Antarctica (Leal et al., 2025a; Leal et al., 2025b; Bendia et al., 2018).

5. Conclusions

This multidisciplinary study has integrated geochemical, mineralogical, and microbiological data obtained from the Nevado del Ruíz Volcano (CVNR) and compared them with information collected from Mawrth Vallis, establishing the Refugio sector of the CVNR as a consolidated astrobiological analog for Mars.

5.1. Relevance of mineral and geochemical composition

The identification of potential andesites in Mawrth Vallis, based on high-resolution spectral data (CRISM), provides essential evidence of past aqueous activity and alteration processes. The presence of these materials, characterized by their fine transportability and association with alteration minerals (such as various clays and phyllosilicates), suggests the existence of fluvial and hydrothermal environments in the past, enhancing the site's potential for biosignature preservation.

5.2. Value of the terrestrial analog at Nevado del Ruíz

The study of the CVNR, with its complex interaction of volcanic, periglacial, and chemical alteration processes, provides an experimental framework for extrapolating mechanisms of mineral alteration and transport to Martian environments. The similarity in mineralogical composition and the processes of material formation and phosphorus redistribution reinforce the utility of Nevado del Ruíz as an analog for understanding the evolution of Mawrth Vallis and, by extension, the aqueous and climatic history of Mars.

5.3. Biogeochemical implications of microbial activity

The bacterial isolates identified at Nevado del Ruíz, particularly *Klebsiella spallanzanii* and *Bacillus cereus*, demonstrate the ability of life to adapt to extreme conditions. These bacteria, capable of phosphate solubilization and nitrogen fixation, may have promoted the mobilization and bioavailability of phosphorus in the soil. Given that phosphorus is an essential nutrient and has been reported in limited concentrations on Mars, these microbial mechanisms suggest the possibility that biological activity could have contributed to improving local habitability in a Martian environment.

5.4. Integration of inorganic and organic processes

The synergy between volcanic activity, periglacial processes, and microbial action highlights the complex interaction between inorganic and organic processes in extreme environments. This interaction is key to understanding how biosignatures can form and be preserved under adverse conditions, with profound implications for the search for past or present life on Mars.

5.5. Future perspectives and astrobiological relevance

The results of this study emphasize the importance of using terrestrial analogs to develop interpretive models of Martian environmental evolution. Future research at Nevado del Ruíz should focus on conducting laboratory experiments that simulate additional Martian conditions, expanding sampling efforts to other sectors of the volcano, and deepening the integration of orbital spectral data with geochemical and microbiological field analyses, as well as comparisons with other cold environments such as Antarctica. These approaches will help refine site selection criteria for in situ missions and improve our understanding of the processes that may have facilitated the emergence and preservation of life on the Red Planet.

Permits

For the development of the project, a sampling campaign was conducted in 2017. At that time, the samples could not be accredited with a collection permit. However, under Article 6 of Law 1955 of 2019 (Colombia), the Biological Collection "Banco de Cepas y Genes" of the Institute of Biotechnology at the National University of Colombia adhered to the amnesty process. As a result, the strains were legalized under the IDs authorized by the Alexander von Humboldt Institute (HMI Antart 14: IBUN-090-04313, 43–1: IBUN-090-04682, and 2–3: IBUN-090-04673). The project currently holds Collection Authorization No. 005 of 2023 for Los Nevados and Gorgona National Natural Parks, within the framework of the General Permit for the Collection of Wild Specimens of Biological Diversity for Non-Commercial Scientific Research, granted to the National University of Colombia through Resolution No. 255 of 2014 by the National Environmental Licensing Authority (ANLA).

Author contribution

Leal, Tovar and de Pablo, were responsible for conceptualization; investigation; visualization; writing original draft, writing, review and editing. Bolaños, Ruíz, Buitrago and Velez contributed to the research and the execution of the analyses. Bonilla, Leone, Tchegliakova, Molina, San Martín, Chacón, Abrevaya, Torres, Reyes, Cancino-Escalante and Acevedo-Barrios, were in charge of, review original draft, writing, review and editing. Sánchez was in charge of conceptualization, resources, writing, review and supervision of the research. E. Ruíz, Writing – review & editing; Methodology

CRediT authorship contribution statement

M.A. Leal: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **D. Tovar:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M.A. de Pablo:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **M.A. Bonilla:** Writing – review & editing, Supervision, Conceptualization. **J. Bolaños:** Writing – review & editing, Methodology, Formal analysis. **J. Sánchez:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **J. Buitrago:** Writing – review & editing, Methodology. **G. Leone:** Writing – review & editing, Formal analysis. **N. Tchegliakova:** Writing – review & editing. **A. Molina:** Writing – review & editing, Conceptualization. **J. San Martín:** Writing – review & editing, Formal analysis. **Z. Chacón:** Writing – review & editing, Methodology, Conceptualization. **X. Abrevaya:** Writing – review & editing, Formal analysis. **F. Vélez:** Writing – review & editing, Formal analysis, Data curation. **A. Torres:** Writing – review & editing, Investigation, Funding acquisition. **R. Reyes:** Writing – review & editing, Formal analysis. **G. Cancino-Escalante:** Writing – review & editing, Funding acquisition. **R. Acevedo-Barrios:** Writing – review & editing, Investigation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.icarus.2025.116783.

Data availability

All the data generated or analyzed during this study are included in this published article.

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