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Bioprospecting Antiproliferative Marine Microbiota From Submarine Volcano Tagoro

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Marine ecosystems are unique and rich reservoirs of biodiversity with high potential toward improving the quality of human life. The extreme physical–chemical conditions of the oceans have favored marine organisms to produce a great variety of new molecules as a mechanism to ensure their survival, and such compounds possess great biopharmaceutical interest. In particular, marine microbiota represent a promising and inexhaustible source for the development of new drugs. This work presents the taxonomic study of the samples obtained from the underwater volcano Tagoro, which has allowed us to develop a collection of 182 marine bacterial strains. On October 10th, 2011, at La Restinga–El Mar de Las Calmas Marine Reserve, an underwater eruption gave rise to a novel shallow submarine volcano at 1.8 km south of the island of El Hierro, Canary Islands, Spain. During the first 6 months, extreme physical–chemical perturbations, comprising thermal changes, water acidification, deoxygenation, and metal enrichment, resulted in significant alterations of the marine ecosystem. After March 2012, the submarine volcano Tagoro entered an active hydrothermal phase that involved a release of heat, gases, metals, and micronutrients that continues till our present. During 2016, our research team had the opportunity to participate in one of the monitoring oceanographic cruises carried out in the area in order to isolate microorganisms associated with both rock samples and deep-sea invertebrates over Tagoro submarine volcano. In this study, Proteobacteria revealed as the most abundant Phylum with 70.2% among all isolated strains, followed by Firmicutes 19%, Actinobacteria 9.5%, and Bacteroidetes 1.2%. Furthermore, we present the results of the antiproliferative assays of the extracts obtained from small-scale cultures of selected bacterial strains. An analysis of the effects of culture conditions in the antiproliferative activity showed that strains grown in Marine Broth (MB) presented lower GI_{50} values than those cultured in a modified medium (MM1). This effect is improved when the strains are incubated under agitation conditions. The antiproliferative

potential of genera such as *Halobacillus*, *Kangiella*, *Photobacterium*, and *Halomonas* is revealed. Their biotechnological development provides an excellent starting point to access novel secondary metabolites and enzymes with potential for pharmaceutical and industrial applications.

Keywords: bioprospection, microbial biodiversity, marine bacteria, OSMAC, antiproliferative activity, Tagoro, singular ecosystem, submarine volcanoes and hydrothermal vents

INTRODUCTION

Marine environments represent more than 70% of the earth's surface with the deep sea comprising the largest part of the oceans, which is characterized by extreme conditions of pressure, temperature, light, salinity, and nutrient availability. Deep-sea benthic environments are predominantly occupied by microbial organisms (Hoshino et al., 2020), which are strongly influenced by the singular physical–chemical characteristics in the marine habitats (Poli et al., 2017). As a mechanism to ensure their survival, marine microbiota, such as bacteria, cyanobacteria, yeasts, fungi, and microalgae, respond by biosynthesizing a great variety of molecules. These compounds represent a unique reservoir of new bioactive substances of great biopharmaceutical potential for the development of new drugs (Carroll et al., 2021).

Microbial richness of marine environmental microbiomes is estimated to range between 10^4 and 10^6 prokaryotes/mL (Whitman et al., 1998; Hoshino et al., 2020). Despite this huge biodiversity, most of microorganisms that inhabit the marine environment remain uncultured under laboratory conditions (Kaeberlein et al., 2002; Mu et al., 2021), an essential requisite for biodiscovery and the access to new chemical entities. Nonetheless, innovative approaches for isolation and culture marine microbes represent a growing research field (Joint et al., 2010; Mu et al., 2021). For these reasons, the study of singular marine ecosystems is considered a very interesting source for biodiscovery (Rotter et al., 2021) that allow to access novel microorganisms with unique metabolic and physiological capabilities to colonize extreme habitats and biosynthesize compounds as an adaptive mechanism to the environmental conditions (Nikapitiya, 2012; Silva et al., 2020). This particularity has led to a constant description of bioactive metabolites from marine bacteria (Buijs et al., 2019; Puglisi et al., 2019). Marine compounds display high cytotoxicity; since the 1990s it was suggested that over 1% of the marine samples analyzed possesses antitumoral activity while the same is 0.2% of terrestrial samples (Wang et al., 2020).

Among singular marine ecosystems, deep-sea hydrothermal vents and submarine volcanoes constitute unique environments for bioprospection studies with significant emission of gases and solutes that react with seawater leading to physical–chemical changes that impact consequently the marine ecosystem (Fraile-Nuez et al., 2012). Thus, hydrothermal vents have been recognized as "natural hot spots" of bacterial strains that have developed multiple resistances as a result of the adaptive phenotypic and genotypic responses to environmental conditions (Farias et al., 2015).

On October 10th, 2011, at La Restinga–El Mar de Las Calmas Marine Reserve, an underwater eruption gave rise to a novel shallow submarine volcano at 1.8 km south of the island of El Hierro, Canary Island, Spain (27°37.116′N, 017°59.466′W). During the first 6 months (October 2011–March 2012), extreme physical–chemical perturbations caused by this event, comprising thermal changes, water acidification, deoxygenation, and metal enrichment, resulted in significant alterations of the marine ecosystem. After March 2012, the submarine volcano Tagoro entered an active hydrothermal phase that involved a release of heat, gases, metals, and micronutrients that continues till our present. In this way, volcano Tagoro became one of the newest and shallowest active submarine volcanoes in Europe, representing an excellent natural laboratory to study how the extreme physical–chemical and biological anomalies caused by this eruptive process have transformed one of the richest marine ecosystems of the Spanish coast (Fraile-Nuez et al., 2012, 2018).

Although Tagoro submarine volcano dataset has turned into one of the longest and more complete multidisciplinary time-series for the study of how physical–chemical anomalies impacted over the marine ecosystem (Fraile-Nuez et al., 2012, 2018; Rivera et al., 2013; Santana-Casiano et al., 2013, 2016, 2018; Ariza et al., 2014; Coca et al., 2014; Eugenio et al., 2014; Blanco et al., 2015; Danovaro et al., 2017; Santana-González et al., 2017; Álvarez-Valero et al., 2018; Sotomayor-García et al., 2019, 2020; González-Vega et al., 2020), just a few studies have shed light in the characterization of the microbial communities with geologically active marine environments, partly due to the difficulty in collecting samples as well as the eruption prediction and monitoring, usually in remote locations (Ferrera et al., 2015). These previous studies have exhibited the effects of the eruption on the survival and colonization by benthic and demersal species (Sotomayor-García et al., 2019) as well as microorganism communities (Ferrera et al., 2015).

In October 2016, our research team participated in one of the monitoring oceanographic cruises carried out in the area with the aim to explore the microbial biodiversity associated with both rock samples and deep-sea invertebrates over Tagoro submarine volcano. This work reports on the results of the taxonomic study of the samples obtained from this singular marine ecosystem which has allowed us to obtain a collection of 182 strains of marine bacteria. Furthermore, the results of the antiproliferative assays of the extracts from small-scale cultures of selected bacterial strains are showed. This collection represents an excellent starting point to access novel secondary metabolites and enzymes with potential for pharmaceutical and industrial applications.

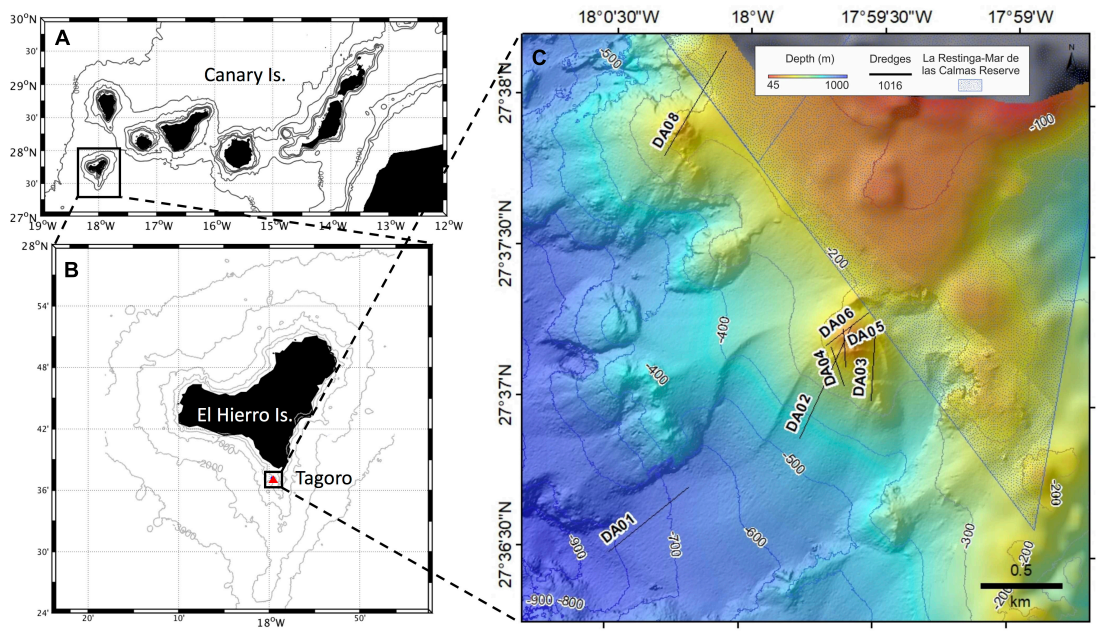

FIGURE 1 | Location map. **(A)** Location of the island of El Hierro in the Canary Archipelago. **(B)** Location of Tagoro submarine volcano south of El Hierro island. **(C)** Rock dredge sampling over the lava flow of Tagoro submarine volcano and control area northwest of the submarine volcano. Shaded triangle represents the domain of La Restinga - Mar de Las Calmas Marine Reserve.

MATERIALS AND METHODS

In October 2016, 5 years after the completion of the magmatic activity, a multidisciplinary oceanographic cruise, on board R/V Ángeles Alvariño of the Spanish Institute of Oceanography, was carried out in the context of the VULCANO-II project in order to characterize the physical–chemical and biological anomalies of the underwater volcano in a degassing stage (**Figure 1**).

Bathymetry Data

Multibeam echosounder datasets were acquired using a Kongsberg-Simrad EM710 during VULCANO-II-1016, which operates at sonar frequencies in the 70–100 kHz range and were processed using CARIS HIPS and SIPS yielding bathymetric grid resolution of 5 m with 100% coverage.

Sample Collection

Benthic organisms were sampled using a benthic dredge with a horizontal opening of 1 m and a net mesh size of 1 cm. Although samplings were mainly performed to carry out a geological characterization of substrate types, samples of benthic organisms were also collected: in the base of Tagoro volcanic complex (DA01, **Figure 1**), around the main edifice and secondary and primary craters of Tagoro submarine volcano (DA02–06), and in the unaffected area in the interior of La Restinga-Mar de Las Calmas Marine Reserve (DA08). In that sense, a total of 30 samples were obtained from seven different benthic dredges (DA01–06, DA08) in a ranging depth of 160–800 m. The samples (**Supplementary Table 1**), which included deep-sea algae, invertebrates, and sediments occluded into pores of volcanic rocks, were washed with sterile sea water, stored in sterile tubes and kept at 4°C on board until processed in the laboratory.

Physical–Chemical Data at the Collection Site

A SeaBird 911-plus CTD instrument, equipped with dual temperature and conductivity sensors, with accuracies of 0.001°C and 0.0003 S/m, respectively, was used to collect temperature, conductivity, salinity, pressure, dissolved oxygen, fluorescence, and pH data over the study area. The CTD continuously recording data with a sampling interval of 24 Hz and the sensors were calibrated at the SeaBird laboratory before and after the cruises. Discrete water samples for inorganic nutrient analysis were obtained using a rosette of 24–12-L Niskin bottles.

During the cruise, 16 vertical profiles of CTD were carried out around the main volcano edifice together with 48 tow-yo transect, which involve continuously lowering and raising the rosette between 1 and 40 m above the seafloor, with the ship moving at 0.2–0.4 kn in Dynamic Positioning, obtaining a sawtooth-shaped dataset with high spatial resolution and closer to the source of the emissions. Physical–chemical parameters at sampling stations, including pH (ΔpH) and temperature ($\Delta\theta$) anomalies around the main and secondary crater (DA04–06) are summarized in **Supplementary Table 1**.

Isolation of Bacterial Strains

The macroscopic samples (**Figure 2**) were surface sterilized with sterile seawater, followed by immersion in ethanol three times and processed according to their nature through two different methods: (a) by chopping, and (b) by homogenization with sterile

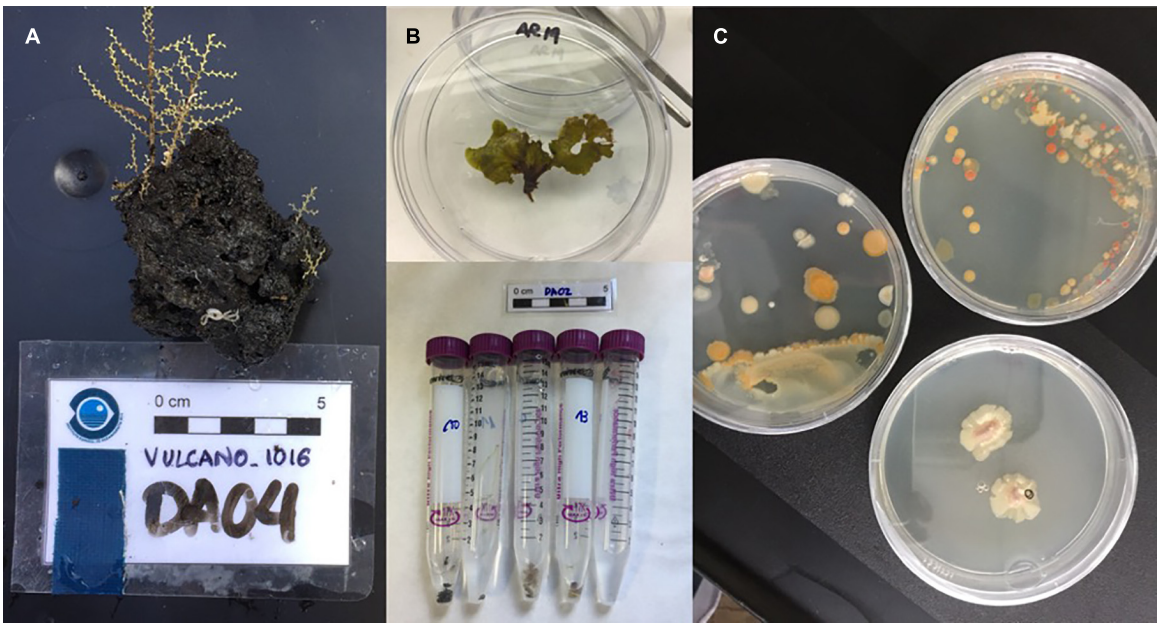

FIGURE 2 | (A) Hydrozoan *Sertularella* sp. (Depht: −170 to 270 m) from DA04. **(B)** Environmental samples AR19 and AR10–AR14 in sterile containers. **(C)** Microbial diversity observed in processed samples.

seawater and vortexed for 5 min. Sediments were diluted in sterile seawater (50 mg/mL) and vortexed for 5 min. After sample settling, sequential dilutions of the supernatant were performed, and transferred in duplicate into Petri dishes containing two solid media: MA (Marine Agar 2216, Panreac) and MM1 [Yeast extract (4 g/L); starch (10 g/L); bacteriological peptone (2 g/L); agar (15 g/L); and filtered seawater (90%)]. Inoculated Petri dishes were incubated at 20°C and microbial growth was monitored periodically over 6 months. The microbial colonies observed were isolated in pure culture by repeated transfer from a single colony, until strains were established. All isolates were grown in liquid media and cryopreserved at −80°C in 20% glycerol and belong to the Marine Microbial Collection of IUBO-ULL.

DNA Extraction and 16S rRNA Gene Sequencing

DNA extraction, 16S rRNA gene amplification and sequencing was carried out at the Genomic Service of the General Research Support Services of Universidad de La Laguna. DNA was extracted from liquid cultures after centrifugation (5 min at 18,000 × *g*) and the cell pellet was recovered by re-suspending in 200 μL of lysis buffer [40 mM Tris–HCl pH 7.8, 20 mM sodium acetate, 1 mM EDTA, 1% SDS, 0.04 μg/μL RNase-A (Promega), 0.04 μg/μL lysozyme (Thermo Fisher Scientific)]. Samples were incubated for 5 min at room, and 5 M NaCl and chloroform sequentially added. After centrifugation (18 000 × *g*, 2 min), the aqueous phase was recovered, DNA was precipitated with cold absolute ethanol and washed twice with 70% ethanol. DNA pellets were dried at room temperature and then suspended in 50 μL of H_2O. 16S rRNA gene was amplified using universal primers E8F (5′-TAGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Weisburg et al., 1990). Commercial PCR kit AmpONE Taq DNA polymerase (GeneAll Biotech) was performed using a PTC200 MJ Research Thermal Cycler with the following conditions: initial denaturation at 95°C for 2 min, 35 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 1.5 min, followed by a final extension at 72°C for 7 and 5 min at 16°C. The PCR-amplified products were checked for the presence of a single band of the expected size by standard agarose electrophoresis. PCR products were purified using the EXO-SAP-IT kit (Affymetrix-USB) following manufacturer's instructions in a total volume of 7 μL and incubated at 35°C for 15 min, followed by heating inactivation to 80°C for 15 min. Sanger Sequencing was carried out using the BigDye^{TM} Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and 3500 Series Genetic Analyzer automatic sequencer (Thermo Fisher Scientific). The SeqScape (Thermo Fisher Scientific) software was used for the analysis of sequencing data and filtering out low quality reads.

Taxonomic Identification and Phylogenetic Analysis

A total of 84 selected sequences were analyzed using Chromas (Chromas software 2.6.6, Technelysium, Pty. Ltd.[1]) and Seaview 5 (Gouy et al., 2010) programs. Consensus sequences were created and compared to the GenBank database by the Blast(n) algorithm to establish their closest relatives. Using Mothur program (Schloss et al., 2009), sequences were grouped by operational taxonomic units (OTUS) at 97% identity using the command classify.otu (Chappidi et al., 2019). Once identified

[1]http://technelysium.com.au/wp/chromas/

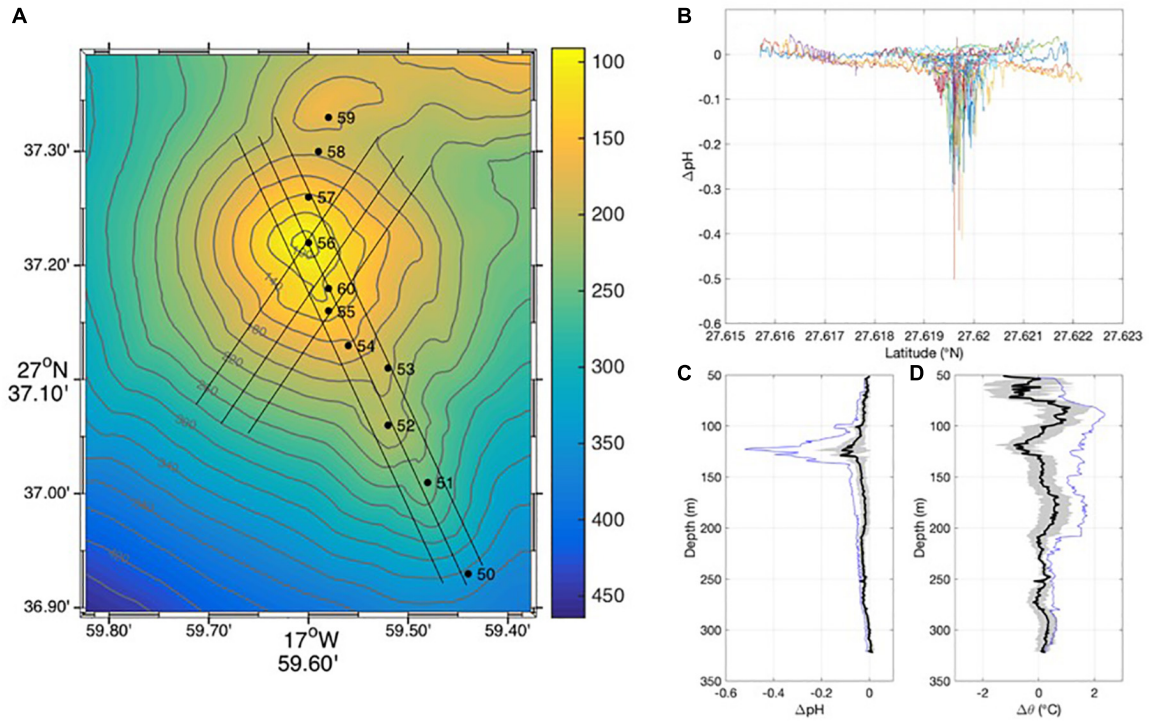

FIGURE 3 | Physical–chemical anomalies over Tagoro submarine volcano. **(A)** High resolution bathymetry of Tagoro submarine volcano. CTD stations are marked with dots from 50 to 60. Blank lines represent the CTD tow-yo transects over the main volcanic edifice. **(B)** pH anomaly for every tow-yo transect with latitude. **(C)** Vertical mean pH anomaly (bold line) with its standard deviation (gray) and the minimum values (blue line). **(D)** Vertical mean potential temperature anomaly (bold line) with its standard deviation (gray) and the maximum values (blue line).

the hits with high maximum identity, reference sequences were used to be aligned using MAFFT v7 (Katoh and Standley, 2013) with G-INS-i strategy (recommended for <200 sequences with global homology). The alignment obtained was used to construct the phylogenetic tree using PhyML v3.1 (Guindon and Gascuel, 2003; Anisimova and Gascuel, 2006; Dereeper et al., 2008, 2010) under the GTR model with bootstrap 1000 replicates value. *Thermogymnomonas acidicola* was used as outgroup. Tree manipulation and editing were done using FigTree v14.0 program.[2] The 16S rRNA gene sequences were submitted to the GenBank database under the following accession numbers: MW826671–MW826754.

Culture and Preparation of Crude Extracts

Bacterial isolates were cultured in four low-nutrient experimental conditions in 500 mL Erlenmeyer flasks containing 300 mL of 1:5 dilution ratio of marine broth (MB/5, Marine Broth 2216, Panreac) and MM1 medium (MM1/5). The flasks were incubated both under static conditions and in an orbital shaker (IKA KS501, Labortechnik) at 100 rpm, at 20°C in the dark for 20–30 days. After the incubation process, cultures were extracted three times with ethyl acetate (3 × 100 mL EtOAc). The organic layer was dried in a rotary evaporator and the resulting extracts stored at −20°C until required for biological tests.

Antiproliferative Assays

Weighted dried organic extracts were dissolved in dimethyl sulfoxide to prepare a stock solution of 50 mg/mL for bioactivity assays. To evaluate the antiproliferative activity of the samples we selected the cancer cell lines A549 and SW1573 (non-small cell lung), HBL-100 and T-47D (breast), HeLa (cervix), and WiDr (colon). The tests were performed in 96-well plates using the SRB assay (Orellana and Kasinski, 2016) with the following specifications. Cell seeding densities were 2500 cells/well for A549, HBL-100, HeLa and SW1573, and 5000 cells/well for T-47D and WiDr. Drug incubation times were 48 h. The optical density of each well was measured at 530 (primary) and 620 (secondary) nm. The antiproliferative activity, expressed as 50% growth inhibition (GI_{50}), was calculated according to NCI formulas (Monks et al., 1991).

RESULTS

Physical–Chemical Anomalies Over Tagoro Submarine Volcano

Figure 3 shows the physical–chemical anomalies over the submarine volcano Tagoro with the use of a CTD and tow-yos

[2]http://tree.bio.ed.ac.uk/software/figtree/

close to the seabed. Although the pH anomalies are quite visible in a wide area around the volcano edifice (400–500 m radius around sta56), close to the main crater (sta60), and secondary crater (sta56) the pH anomalies reach a maximum value of −0.5. The anomaly plume is centered around 127 meters depth that correspond with the depth of the main crater (sta60) and could transport with the local currents for hundreds of meters (Santana-Casiano et al., 2016).

Moreover, in the interior of the main crater, not only a pH anomaly is found but also a thermal increase of up to +2.37°C, a salinity decrease of −1.02, and a density decrease of −1.43 (kg m^{-3}). The combined effect of these anomalies is generating a buoyant plume over the volcano that returns downward waters by diffusion to close the circulation cells generating a horizontal flow by this vertical. Theoretically, this vortex is of sufficient magnitude to trap water in a local recirculation that could have a crucial effect on the expected dispersal of properties, including minerals, tracers, and organisms (Speer, 1989).

Isolation and Identification of Marine Bacteria From Volcano Tagoro

From the analysis and bioprospection of the microbiota of this unique and extreme habitat, a total of 182 bacterial strains were isolated from volcanic sediments clogged into rocks and as endophytes from the inner tissues of algae and deep-sea invertebrates sampled in the range of depths of −160 to −800 m (**Figure 1** and **Supplementary Table 1**) for over 6 months. Marine samples were incubated in duplicates into two different solid media, MA and modified marine agar (MM1) as described in section "Sample Collection." No fungal growing was observed. Among all isolated bacterial strains, 95 and 87 grew on MA and MM1, respectively. Those strains obtained from the same environmental sample which showed similar morphological characteristics were grouped and a representative strain was selected to continue the taxonomic and phylogenetic studies.

Thus, the 16S rRNA gene of 84 selected strains were sequenced and identified by its similarity with 16S rRNA gene sequences deposited in the GenBank database from the National Center for Biotechnology Information, NCBI (**Table 1**). Sequences were grouped into 80 OTUs comprising 28 genera classified on 4 Phyla. Proteobacteria is the most abundant Phylum and represents 70.2% of all isolated strains (18 genera, 59 strains), followed by Firmicutes 19% (3 genera, 16 strains), Actinobacteria 9.5% (6 genera, 8 strains), and Bacteroidetes 1.2% (1 genus, 1 strain), as represented in **Figure 4**.

The evolutionary relationships between the isolated bacterial strains are shown in the 16S rRNA phylogenetic tree (**Figure 5**). From the 84 isolates, 66 out strains exhibited more than 99% similarity compared to 16S rRNA gene sequences deposited in GenBank and 6 isolates exhibited <97% similarity; lower sequence similarities were observed mainly for three strains of *Vibrio* (AR30-01, AR30-05, and AR32-02) isolated from invertebrates collected at depths ranging 200–300 m (DA06 and DA08), **Supplementary Table 2**.

There are diverse studies focused on the diversity of culturable bacteria from marine sources such as sediment, invertebrates, and water samples, among other marine samples, within Proteobacteria representing an important proportion of the isolates (Buijs et al., 2019). In the present study, Proteobacteria were isolated from all the seven depths analyzed and, interestingly, strains isolated of samples from the crater (DA06), especially of inner sediment volcanic rocks, comprises the four phyla identified in this work. Although it is noticeable that some groups of samples are represented by a certain class, such as sponges from DA08, since type and number of samples are different for each dredge, no tendencies were observed in relation to the sample and depth.

Antiproliferative Potential of the Extracts of Marine Bacteria From Volcano Tagoro

Previous studies that show the pharmacological relevance of marine microorganisms prompted us to evaluate the antiproliferative properties of bacterial strains isolated from volcano Tagoro against A549, HBL-100, HeLa, SW1573, T-47D, and WiDr cell lines.

A selection of 33 bacterial isolates representing 21 different phylogenetic classes were cultured in 300 mL liquid media under low-nutrient conditions, using two different media, under static and agitation conditions, for a period of 30 days for each strain. The use of oligotrophic media and large fermentation time was intended to induce stress conditions that stimulate the activation of secondary-metabolism biogenetic gene clusters following an one strain many compounds (OSMAC)-based strategy (Bode et al., 2002). This strategy has revealed as a simple and effective approach to induce the activation of the biosynthetic mechanisms of microorganisms to produce new secondary metabolites by changing culture conditions (Reen et al., 2015). Successful examples include the addition of enzyme inhibitors, addition of signaling molecules, co-cultivation with other microbial strain, modification of medium composition, or changes of physical parameters, among others (Romano et al., 2018; Pan et al., 2019).

Microbial cultures were extracted with EtOAc and the solvent dried. The antiproliferative analysis of the obtained extracts (**Table 2** and **Supplementary Table 3**) revealed that one actinobacteria, two Proteobacteria, and two Firmicutes showed GI_{50} < 20 μg/mL against at least one cell line: *Micromonospora* AR22-02, *Kangiella* AR14-04, *Pseudoalteromonas* AR04-01, and *Halobacillus* AR14-06 and AR01-01. Interestingly, besides the lowest GI_{50} values, the two *Halobacillus* strains were the most active bacteria evaluated, since they exhibited antiproliferative properties for at least five out from the six cell lines assayed. Also, 11 bacterial strains showed GI_{50} values ranging from 20 to 39 μg/mL against at least one cell line, the Bacteroidetes *Salinimicrobium* AR29-06; two Actinobacteria: *Micrococcus* AR07-11A and *Mycobacterium* AR05-15C; three Firmicutes comprising the genus *Bacillus*: AR13-02, AR14-07B, AR19-02; and five Proteobacteria: *Limimaricola* AR29-03, *Aliivibrio* AR26-02, *Kangiella* AR14-04, *Photobacterium* AR27-02 and *Vibrio* AR05-02. Overall, strains comprising the genera *Halobacillus*, *Salinimicrobium*, *Micrococcus*, *Aliivibrio*, *Kangiella*, *Photobacterium*, and *Pseudoalteromonas* showed GI_{50} < 40 μg/mL against at least four out from the six cell lines tested. Additionally, it was observed that 24 strains comprising

TABLE 1 | Summary of the bacterial isolates and phylogenetically closely related species from GenBank according to 16S rRNA gene sequence similarity.

Phylum	Class	Strain ID.	Source	Genus/species	Accession number	Closest neighbor		
						Genus/species	Accession number (NCBI)	Identity (%)
Actinobacteria	Actinobacteria	AR10-02	Iron oxide sediments	*Glutamicibacter* sp.	MW826695	*Glutamicibacter* sp.	LC512313.1	99.16
		AR32-01	White sponge (*Sarcotragus* sp.)	*Microbacterium* sp.	MW826745	*Microbacterium barkeri*	MT214325.1	98.17
		AR07-11A	Hydrozoa	*Micrococcus* sp.	MW826688	*Micrococcus luteus*	MT539734.1	99.48
		AR28-05	Inner sediment volcanic rock	*Micrococcus* sp.	MW826735	*Micrococcus luteus*	MT367834.1	99.23
		AR07-07	Hydrozoa	*Micrococcus yunnanensis*	MW826686	*Micrococcus yunnanensis*	MT225648.1	99.93
		AR22-02	Hydrozoa (*Sertularella* sp.)	*Micromonospora* sp.	MW826725	*Micromonospora viridifaciens*	GQ153845.1	99.64
		AR07-15C	Hydrozoa	*Mycobacterium hippocampi*	MW826692	*Mycobacterium hippocampi*	MW012620.1	99.70
		AR14-11D	Pink filament	*Pseudonocardia* sp.	MW826708	*Pseudonocardia* sp.	MN631183.1	97.82
Bacteroidetes	Flavobacteriia	AR29-06	Inner sediment volcanic rock	*Salinimicrobium* sp.	MW826739	*Salinimicrobium* sp.	MW012343.1	98.07
Firmicutes	Bacilli	AR13-02	Seaweed/green hydrozoa	*Bacillus* sp.	MW826699	*Bacillus algicola*	KT984000.1	98.05
		AR06-02	Hydrozoa	*Alkalihalobacillus berkeleyi*	MW826678	*Bacillus berkeleyi*	NR_109459.1	99.86
		AR29-01	Inner sediment volcanic rock	*Bacillus* sp.	MW826736	*Bacillus idriensis*	MN410537.1	98.95
		AR06-01	Hydrozoa	*Bacillus* sp.	MW826677	*Bacillus* sp.	GQ284497.1	98.57
		AR14-07B	Pink filament	*Bacillus* sp.	MW826705	*Bacillus* sp.	FN179280.1	100.00
		AR07-04	Hydrozoa	*Bacillus vietnamensis*	MW826683	*Bacillus vietnamensis*	MH384991.1	99.78
		AR07-05	Hydrozoa	*Bacillus* sp.	MW826684	*Bacillus* sp.	MG893128.1	99.93
		AR10-01	Iron oxide sediments	*Bacillus vietnamensis*	MW826694	*Bacillus vietnamensis*	MH384991.1	99.93
		AR11-01	Seaweed/green hydrozoa	*Bacillus vietnamensis*	MW826696	*Bacillus vietnamensis*	MH384991.1	99.93
		AR14-02	Pink filament	*Bacillus vietnamensis*	MW826702	*Bacillus vietnamensis*	MH384991.1	99.93
		AR19-02	Brown seaweed	*Bacillus* sp.	MW826713	*Bacillus vietnamensis*	MH384991.1	96.97
		AR23-01	Hydrozoa (*Sertularella* sp.)	*Bacillus vietnamensis*	MW826726	*Bacillus vietnamensis*	MH384991.1	100.00
		AR15_01	Hydrozoa (*Sertularella* sp.)	*Domibacillus* sp.	MW826709	*Domibacillus iocasae*	NR_148625.1	99.64
		AR14-06	Pink filament	*Halobacillus dabanensis*	MW826704	*Halobacillus dabanensis*	MG566184.1	100.00
		AR01_01	Black coral (*Stichopathes* sp.)	*Halobacillus* sp.	MW826671	*Halobacillus* sp.	MK922526.1	99.50
		AR16-01B	Briozoo	*Halobacillus trueperi*	MW826710	*Halobacillus trueperi*	MT125721.1	100.00
Proteobacteria	Alphaproteobacteria	AR29-03	Inner sediment volcanic rock	*Limimaricola* sp.	MW826738	*Limimaricola* sp.	MN435786.1	99.29
		AR21-03	Iron oxide sediments	*Henriciella barbarensis*	MW826721	*Henriciella barbarensis*	NR_157787.1	99.84
		AR21-05	Iron oxide sediments	*Hyphomonas* sp.	MW826723	*Hyphomonas* sp.	KY770375.1	99.85
		AR35-02	Orange sponge (*Geodiidae*)	*Ruegeria atlantica*	MW826749	*Ruegeria atlantica*	MT072134.1	99.87
		AR07-02B	Hydrozoa	*Ruegeria* sp.	MW826680	*Ruegeria* sp.	MN099589.1	99.92
		AR07-03C	Hydrozoa	*Ruegeria* sp.	MW826682	*Ruegeria* sp.	MN704271.1	95.26

(Continued)

TABLE 1 | Continued

Phylum	Class	Strain ID.	Source	Genus/species	Accession number	Closest neighbor		
						Genus/species	Accession number (NCBI)	Identity (%)
		AR07-16	Hydrozoa	*Ruegeria* sp.	MW826693	*Ruegeria* sp.	KY363633.1	97.95
		AR19-09	Brown seaweed	*Ruegeria* sp.	MW826716	*Ruegeria* sp.	MH023307.1	99.92
		AR23-02	Hydrozoa (*Sertularella* sp.)	*Ruegeria* sp.	MW826727	*Ruegeria* sp.	KY363633.1	99.85
		AR31-04	White sponge (*Phakellia* sp.)	*Ruegeria* sp.	MW826744	*Ruegeria* sp.	MK411263.1	99.92
	Gammaproteobacteria	AR19-05	Brown seaweed	*Agarivorans* sp.	MW826714	*Agarivorans* sp.	KF577091.1	99.76
		AR26-02	Black coral (*Stichopathes* sp.)	*Aliivibrio* sp.	MW826732	*Aliivibrio* sp.	MN049669.1	99.25
		AR37-03B	Black sponge (*Geodiidae*)	*Cobetia* sp.	MW826752	*Cobetia* sp.	LC549335.1	99.93
		AR07-08	Hydrozoa	*Halomonas* sp.	MW826687	*Halomonas denitrificans*	MT525289.1	99.64
		AR07-13	Hydrozoa	*Halomonas neptunia*	MW826690	*Halomonas neptunia*	LR722836.1	99.86
		AR21-02	Iron oxide sediments	*Halomonas* sp.	MW826720	*Halomonas* sp.	MG309470.1	100.00
		AR21-06A	Iron oxide sediments	*Halomonas* sp.	MW826724	*Halomonas* sp.	MN784249.1	99.33
		AR29-02	Inner sediment volcanic rock	*Halomonas* sp.	MW826737	*Halomonas* sp.	EU880513.1	96.18
		AR14-04	Pink filament	*Kangiella geojedonensis*	MW826703	*Kangiella geojedonensis*	NR_109098.1	99.71
		AR13-04	Seaweed/green hydrozoa	*Leclercia* sp.	MW826700	*Leclercia adecarboxylata*	MT133354.1	99.18
		AR24-01	Ash	*Marinobacter* sp.	MW826728	*Marinobacter sediminum*	LR218090.1	99.67
		AR07-15A	Hydrozoa	*Marinobacter similis*	MW826691	*Marinobacter similis*	KJ547704.1	99.71
		AR20-02B	Iron oxide sediments	*Marinobacter* sp.	MW826717	*Marinobacter* sp.	MT830217.1	99.71
		AR20-03	Iron oxide sediments	*Marinobacter* sp.	MW826718	*Marinobacter* sp.	MK819193.1	99.92
		AR07-02A	Hydrozoa	*Photobacterium sanguinicancri*	MW826679	*Photobacterium sanguinicancri*	GQ454934.2	99.79
		AR27-02	Black coral (*Stichopathes* sp.)	*Photobacterium* sp.	MW826734	*Photobacterium* sp.	JQ082197.1	99.71
		AR26-03	Black coral (*Stichopathes* sp.)	*Pseudoalteromonas* sp.	MW826733	*Pseudoalteromonas carrageenovora*	MN746256.1	97.05
		AR14-08B	Pink filament	*Pseudoalteromonas* sp.	MW826706	*Pseudoalteromonas lipolytica*	MK439576.1	98.95
		AR25-05D1	Iron oxide	*Pseudoalteromonas* sp.	MW826730	*Pseudoalteromonas lipolytica*	MK439576.1	99.21
		AR04-01	Seaweed/hydrozoa	*Pseudoalteromonas* sp.	MW826672	*Pseudoalteromonas* sp.	MT187764.1	99.64
		AR05-06	Hydrozoa	*Pseudoalteromonas* sp.	MW826676	*Pseudoalteromonas* sp.	MH782067.1	99.78
		AR14-01	Pink filament	*Pseudoalteromonas* sp.	MW826701	*Pseudoalteromonas* sp.	MK577347.1	99.39
		AR17-07	Volcanic sediment	*Pseudoalteromonas* sp.	MW826712	*Pseudoalteromonas* sp.	EU143362.1	99.79
		AR24-02	Ash	*Pseudoalteromonas* sp.	MW826729	*Pseudoalteromonas* sp.	MT645493.1	99.79
		AR31-02	White sponge (*Phakellia* sp.)	*Pseudoalteromonas* sp.	MW826743	*Pseudoalteromonas* sp.	MK377267.1	99.71

(Continued)

TABLE 1 | Continued

Phylum	Class	Strain ID.	Source	Genus/species	Accession number	Closest neighbor		
						Genus/species	Accession number (NCBI)	Identity (%)
		AR11-05	Seaweed/green hydrozoa	*Pseudomonas marincola*	MW826697	*Pseudomonas marincola*	KY940349.1	99.86
		AR13-01	Seaweed/green hydrozoa	*Pseudomonas marincola*	MW826698	*Pseudomonas marincola*	KY940349.1	99.86
		AR25-06	Iron oxide	*Psychrobacter* sp.	MW826731	*Psychrobacter alimentarius*	MN758808.1	98.55
		AR21-04	Iron oxide sediments	*Psychrobacter* sp.	MW826722	*Psychrobacter pocilloporae*	MH725521.1	99.47
		AR05-05B	Hydrozoa	*Shewanella* sp.	MW826675	*Shewanella* sp.	MN049695.1	99.79
		AR07-11B	Hydrozoa	*Shewanella* sp.	MW826689	*Shewanella* sp.	JN618154.1	99.36
		AR26-01	Black coral (*Stichopathes* sp.)	*Shewanella* sp.	MW826754	*Shewanella* sp.	JN618154.1	99.36
		AR14-11C	Pink filament	*Vibrio alginolyticus*	MW826707	*Vibrio alginolyticus*	MT491126.1	99.77
		AR31-03	White sponge (*Phakellia* sp.)	*Vibrio alginolyticus*	MW826743	*Vibrio alginolyticus*	MT325883.1	99.77
		AR32-02	White sponge (*Sarcotragus* sp.)	*Vibrio* sp.	MW826746	*Vibrio alginolyticus*	MT368033.1	96.92
		AR33-03B	Yellow sponge (*Sarcotragus* sp.)	*Vibrio* sp.	MW826748	*Vibrio alginolyticus*	CP054701.1	98.71
		AR36-03A	Brown sponge (*Geodiidae*)	*Vibrio* sp.	MW826750	*Vibrio alginolyticus*	MT368033.1	99.01
		AR37-02	Black sponge (*Geodiidae*)	*Vibrio* sp.	MW826751	*Vibrio alginolyticus*	MT299676.1	98.87
		AR30-01	Hydrozoa	*Vibrio* sp.	MW826740	*Vibrio chagasii*	MK102593.1	95.51
		AR05-02	Hydrozoa	*Vibrio* sp.	MW826674	*Vibrio coralliirubri*	MT269626.1	99.50
		AR07-03B	Hydrozoa	*Vibrio* sp	MW826681	*Vibrio coralliirubri*	MT269626.1	99.64
		AR17-04	Volcanic sediment	*Vibrio coralliirubri*	MW826711	*Vibrio coralliirubri*	MT269626.1	99.93
		AR19-06A	Brown seaweed	*Vibrio coralliirubri*	MW826715	*Vibrio coralliirubri*	MT269626.1	99.93
		AR33-01	Yellow sponge (*Sarcotragus* sp.)	*Vibrio* sp.	MW826747	*Vibrio crassostreae*	MT510175.1	98.43
		AR30-05	Hydrozoa	*Vibrio* sp.	MW826741	*Vibrio harveyi*	MK102617.1	95.69
		AR05-01	Hydrozoa	*Vibrio* sp.	MW826673	*Vibrio* sp.	MF975584.1	99.42
		AR07-06	Hydrozoa	*Vibrio* sp.	MW826685	*Vibrio* sp.	MG388156.1	99.79
		AR21-01	Iron oxide sediments	*Vibrio* sp.	MW826719	*Vibrio* sp.	KU579285.1	99.79
	Epsilonproteobacteria	AR37-04B	Black sponge (*Geodiidae*)	*Malaciobacter canalis*	MW826753	*Arcobacter canalis*	NR_163659.1	100.00

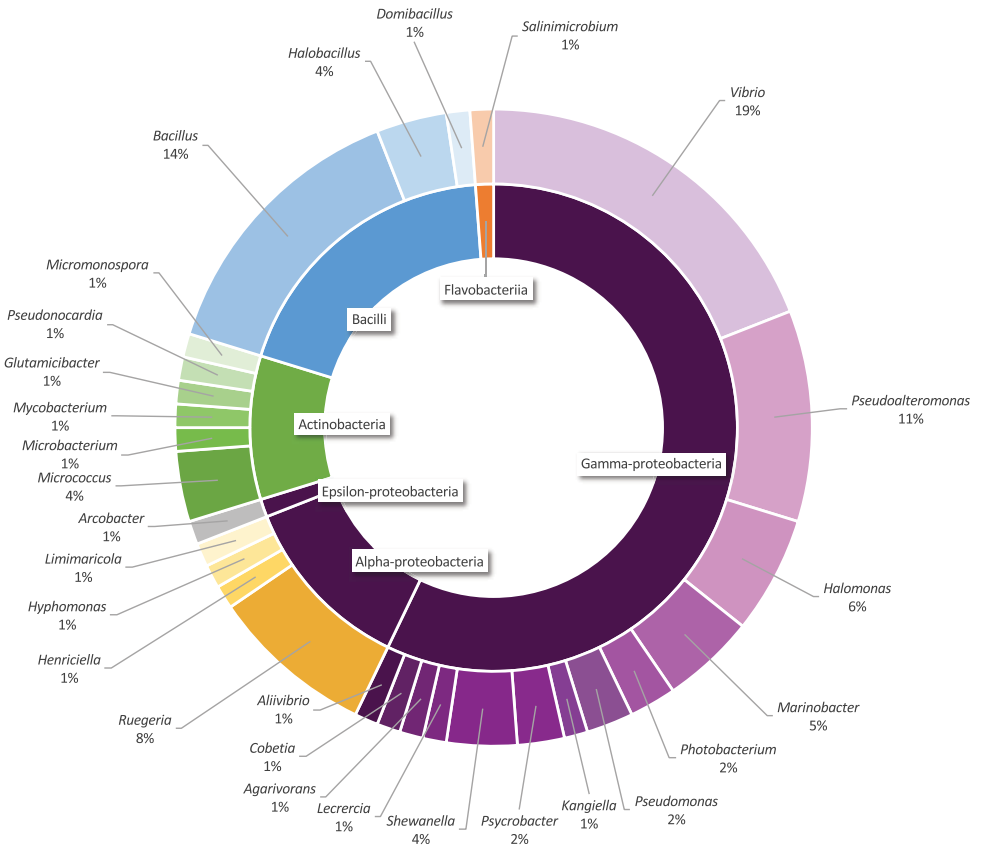

FIGURE 4 | Genera (external ring) and classes (internal ring) distribution of the marine bacterial collection isolated from volcano Tagoro.

5 Actinobacteria, 5 Firmicutes and 14 Proteobacteria exhibited $40 \leq GI_{50} \leq 60$ μg/mL.

Among the most active strains, the genus *Halobacillus* showed the best antiproliferative activities in MB, particularly when grown under agitation conditions. On the other hand, in the case of *Pseudoalteromonas* sp. strain AR04-01 the best results of activity were observed when grown under static culture in MM1 medium. Overall, comparison of the strains that exhibited $GI_{50} < 40$ μg/mL, the most sensible cells were A549, HeLa, and SW1573, whereas HBL-100 was the most resistant.

An analysis of the effects of culture conditions in the antiproliferative activity of the tested strains is showed in **Figure 6**. The radar charts reveal that strains grown in MB medium (1:5 diluted) showed lower GI_{50} values than the same strains cultured in a MM1 medium (1:5 diluted), with higher content in nutrients than the former. This effect is improved when the strains are incubated under agitation conditions compared to those grown in static cultures and is maintained in all cell lines tested (**Supplementary Figure 1**).

DISCUSSION

Tagoro submarine volcano is considered a natural laboratory which has brought the opportunity to carry out a regular multidisciplinary monitoring from the beginning of the unrest. The monitoring has exhibited that the changes produced by the eruption were temporal and all microbial parameters analyzed indicated that the microbial community returned to normal levels (Ferrera et al., 2015). However, there is still an enormous potential for the bioprospection of this unique ecosystem. To date, Tagoro submarine volcano represents the last hydrothermal system reported in the Canary Islands, and a hypothetical modern analog of a seafloor under iron formation in genesis (González et al., 2020).

Some studies on the analysis of microbial communities surrounding submarine volcanoes, such as the Kolumbo volcano and the Solfatara Crater, point out Proteobacteria as the most abundant phylum followed by other taxa represented into Firmicutes, Bacteroidetes, and Acidobacteria (Glamoclija et al., 2004; Christakis et al., 2018).

In this study it is showed that, among Proteobacteria, *Vibrio* was the most abundant genus, with 20% of the isolates among Gammaproteobacteria, whereas *Bacillus* was the most abundant genus representing 75% of the isolated strains within Firmicutes. In this context and taking into consideration the high metabolic flexibility and genome plasticity of *Vibrio* spp. (Wang et al., 2019), and in order to determine its phylogenetic diversity, our results suggest continuing with the investigation of the *Vibrio* community isolated from volcano Tagoro.

FIGURE 5 | Circular maximum-likelihood phylogenetic tree of isolated bacterial strains and their closest NCBI (BLAST) relatives based on 16S rRNA gene sequences. Bootstrap values calculated from 1000 replicates using maximum likelihood are shown the most representatives at their representative node. Colors indicate distribution by Classes: Epsilonproteobacteria (gray), Gammaproteobacteria (purple), Alphaproteobacteria (yellow), Actinobacteria (green), and Bacilli (blue).

All Firmicutes strains correspond to order Bacillales, considered as fast-growing bacteria. These genera are represented among the most abundant cultured and widely distributed species (Al-Amoudi et al., 2016). Overall, evidence suggest that Gram-positive bacteria constitute a significant proportion of deep-sea communities. Among them, members of order Bacillales are known for their versatility and ability to form endospores that can survive as resistant forms (Gontang et al., 2007; Ettoumi et al., 2009, 2013). Despite their ability to survive under varied conditions and their marked phylogenetic diversity, marine *Bacillus* strains have attracted less interest with respect to their terrestrial counterparts (Ettoumi et al., 2009).

The phylogenetic analysis (**Figure 5**) agrees with a previous study carried out by Ferrera et al. (2015) that monitored the waters surrounding the volcano in El Hierro during the eruptive and post-eruptive phases by culture-independent methods. The analysis by flow cytometry and 454-pyrosequencing of 16S rRNA gene amplicons, exhibited that most sequences were related to the phyla Proteobacteria (68.4%), with Alphaproteobacteria (54.8%), and Gammaproteobacteria (10.0%) as the most abundant classes. Subsequently, González et al. (2020) characterized and identified the microbial biomineralization in the hydrothermal vent of volcano Tagoro which are involved in the iron, sulfur, and methane cycles. Most of the ubiquitous bacterial taxa were those commonly found in the water column. Around 50% of the bacterial DNA relative abundance is related to *Marinomonas* sp. and *Pseudomonas* sp., both

TABLE 2 | Antiproliferative activity of 33 selected bacterial strains isolated from environmental samples of Tagoro submarine volcano against human solid tumor cell lines.

			GI_{50} (μg/mL)											
			A549		HBL-100		HeLa		SW1573		T-47D		WiDr	
Phylum/*genus*	**Strain ID**	**Culture condition***	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**
Bacteroidetes														
Salinimicrobium	AR29-06	S	37		42		37		37		29		40	
		A	45		51		41		36		27		44	
Actinobacteria														
Micrococcus	AR07-11A	S	38	42	47	43	35	43	37	43	35	43	40	46
		A	41	48	42	39	40	45	44	44	39	42	47	48
Mycobacterium	AR07-15C	S	250	48	250	53	250	50	250	46	250	48	250	50
		A	32	51	54	56	41	53	41	52	41	48	37	49
Glutamicibacter	AR10-02	S	42	87	39	52	44	87	38	54	48	89	42	88
		A	51	106	44	250	45	83	34	82	49	192	47	175
Pseudonocardia	AR14-11D	S	45	44	44	49	44	51	49	48	44	52	48	53
		A	48	45	50	53	47	48	47	46	45	50	52	57
Micromonospora	AR22-02	S	6	99	51	187	48	82	46	90	54	142	53	117
		A	47	250	50	250	48	250	50	250	46	250	50	250
Firmicutes														
Halobacillus	AR01-01	S	14		29		22		6.3		39		39	
		A	<0.6		9.4		2		<0.6		3.1		3.9	
	AR14-06	S	44	32	74	47	69	39	77	37	250	68	117	79
		A	3.3	29	23	49	6	42	1.7	45	7.7	42	11	44
Bacillus	AR06-01	S	43	42	38	41	55	58	49	56	50	49	48	51
		A	43	42	41	45	49	46	47	63	48	54	47	58
	AR06-02	S	40	43	43	44	50	46	45	47	49	47	45	47
		A	42	35	42	47	51	48	55	46	47	42	51	52
	AR07-04	S	54	111	56	62	48	49	52	57	49	102	65	132
		A	52	57	59	57	45	48	48	58	48	51	63	67
	AR11-01	S	39	69	45	43	44	46	44	48	43	63	44	73
		A	42	111	41	250	43	99	45	148	43	104	45	108
	AR13-02	S	37	45	48	48	40	51	50	49	40	46	50	48
		A	32	250	52	242	47	221	43	185	50	250	44	226
	AR14-07B	S	33	71	46	87	39	72	41	92	42	110	43	140
		A	35	35	50	41	40	34	47	38	44	41	49	47
	AR19-02	S	35		52		49		44		52		47	
		A	36		46		36		37		98		83	
Proteobacteria														
Arcobacter	AR37-04B	S	47	45	44	43	47	44	44	47	42	41	44	46
		A	44	44	45	45	46	43	46	46	41	43	45	46

(Continued)

TABLE 2 | Continued

			GI_{50} (μg/mL)											
			A549		HBL-100		HeLa		SW1573		T-47D		WiDr	
Phylum/*genus*	**Strain ID**	**Culture condition***	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**	**MB**	**MM1**
Hyphomonas	AR21-05	S	250	52	250	60	168	45	250	50	250	48	250	49
		A	40	58	58	65	45	45	49	50	48	48	49	47
Limimaricola	AR29-03	S	37	40	46	41	51	58	45	47	50	45	51	53
		A	40	43	48	42	43	50	51	53	49	54	52	50
Ruegeria	AR07-02B	S	46	250	52	250	46	250	47	250	51	250	52	250
		A	54	250	53	250	47	250	50	250	49	250	56	250
	AR07-16	S	44	42	37	55	34	39	45	36	35	37	28	36
		A	54	53	47	44	32	42	45	40	39	46	37	39
Aliivibrio	AR26-02	S	46	42	45	47	35	34	32	34	42	45	40	43
		A	35	38	46	47	37	34	32	31	43	43	44	39
Halomonas	AR07-13	S	44	52	51	50	39	41	43	38	43	37	29	37
		A	49	54	41	47	38	46	41	44	38	45	37	41
	AR21-02	S	41	40	50	46	37	31	36	32	43	45	48	46
		A	40	38	47	40	36	34	33	34	42	45	45	46
Kangiella	AR14-04	S	32	35	48	42	41	41	41	41	40	40	41	41
		A	26	16	52	50	39	37	43	38	37	39	45	43
Photobacterium	AR07-02A	S	46	250	48	250	46	250	55	250	54	250	64	250
		A	58	43	55	50	49	44	50	48	49	49	56	48
	AR27-02	S	38	37	40	40	35	31	29	35	34	40	44	43
		A	38	50	47	46	30	34	33	36	41	41	42	46
Pseudoalteromonas	AR04-01	S		11		53		13		2.5		27		18
		A		250		250		250		250		250		250
	AR05-06	S	44	66	50	51	46	48	47	56	47	81	54	68
		A	46	49	48	46	49	51	49	51	49	52	56	58
Psychrobacter	AR21-04	S		94		74		86		72		105		72
		A		86		103		85		42		56		24
Shewanella	AR05-05B	S	53	201	56	231	53	126	46	250	55	250	55	250
		A	47	250	45	250	48	250	52	250	53	250	55	250
	AR07-11B	S	50	50	52	50	48	42	41	44	39	42	40	39
		A	46	55	46	46	39	42	40	43	37	39	36	42
Vibrio	AR05-02	S	39	36	44	45	45	43	48	52	49	50	46	49
		A	42	72	45	50	41	48	47	93	51	130	46	101
	AR07-03B	S	44	250	50	250	42	173	43	250	47	250	57	220
		A	46	44	53	55	44	42	49	47	49	53	61	67

GI_{50} < 20 μg/mL ; *20 ≤ GI_{50} ≤ 39 μg/mL* ; *40 ≤ GI_{50} ≤ 60 μg/mL* ; *GI_{50} < 60 μg/mL.*

**MB and MM1 media were diluted in a 1:5 ratio for oligotrophic conditions.*

A, agitation conditions; S, static cultures.

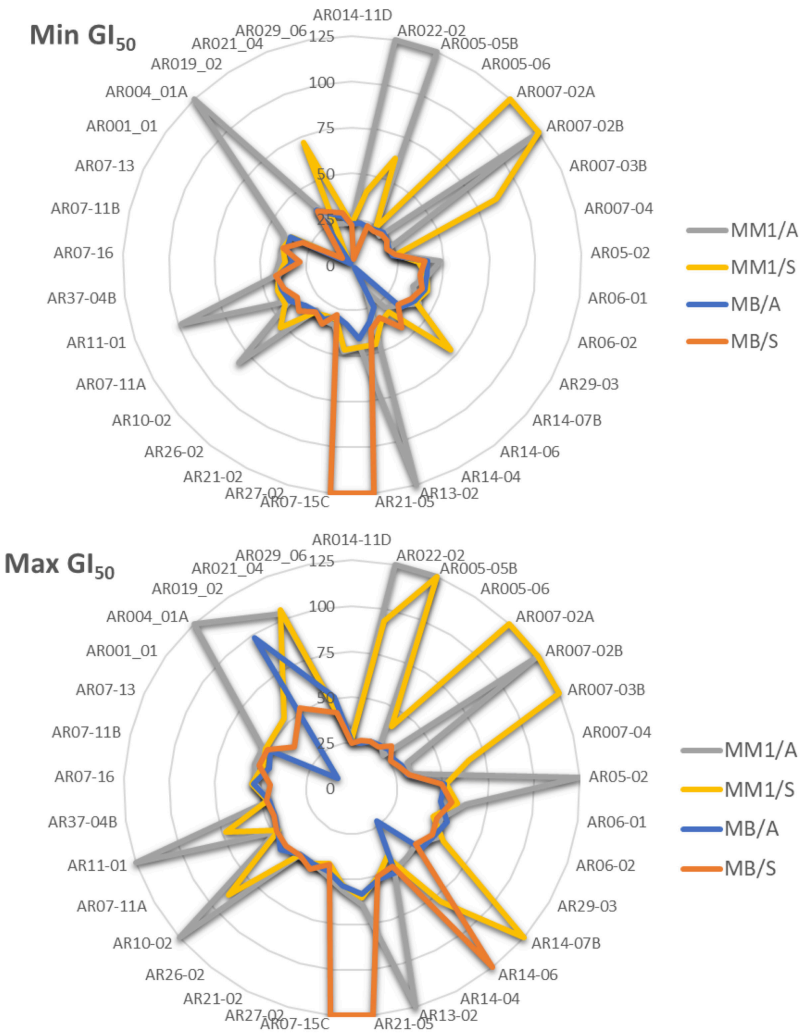

FIGURE 6 | Radar chart of the effect of culture conditions in the antiproliferative activity (Min and Max GI_{50}, μg/mL). MB and MM1 media were diluted in a 1:5 ratio for oligotrophic conditions. A, agitation conditions; S, static cultures.

corresponding to the Gammaproteobacteria class. Other taxa detected were Acidobacteria and Bacteroidetes. Even though we did not use specific media nor the addition of antibiotics, our results show an important proportion of the common bacterial community in the marine environment and reinforces the findings reported by Sanz-Sáez et al. (2020), who developed a marine culture collection of heterotrophic bacteria covering a variety of oceanographic regions from different seasons and years, using a standard marine medium with the aim to describe the fraction of the bacterioplankton community that can be commonly isolated under laboratory conditions. The strains were mainly affiliated with the classes Alphaproteobacteria (35.9%), Gammaproteobacteria (38.6%), and phylum Bacteroidetes (16.5%).

Natural products have been an important source of pharmacologically active compounds and more than 10% of them have a microbial origin. However, even when rediscovery of known compounds led to reduced interest in natural sources, currently, in the light of an increased appreciation for the exceeding rich and unique biodiversity in the oceans, has been a renewed interest on marine natural products (Lyu et al., 2021). Nonetheless, the supply limitation, which is the well-known bottleneck on natural products, led scientists to put the view on microbial resources supported by the special characteristics of marine environment (Jensen and Fenical, 2000; Desbois, 2014; Rotter et al., 2021).

During the last decades, active compounds has been tested and discontinued, meanwhile other antitumor drugs has been successfully completed either obtained by synthesis, cultivation or by production in other organism such as microorganisms (Stonik, 2009). Especially, anticancer compounds represent more than one half of the new marine natural products discovered from 1985 to 2012. In this sense, marine microbes have been considered a hot spot in natural products research and among them actinomycetes (47.01%) and bacteria (46.38%)

represents the highest proportion of bioactive compounds (Hu et al., 2015).

Our results reveal the extracts of *Micromonospora* AR22-02 (Actinobacteria), *Kangiella* AR14-04 and *Pseudoalteromonas* AR04-01 (Gammaproteobacteria), and *Halobacillus*, AR14-06 and AR01-01 (Firmicutes) as the most active under the studied conditions, and support the previous suggestion that most anticancer molecules produced by marine bacteria are derived from Actinobacteria, Proteobacteria (Gammaproteobacteria), and Firmicutes (Gavriilidou et al., 2021). In this sense, a large group of anticancer peptides and some small molecules have been obtained from bacteria occurring in marine sediments comprising genera as *Bacillus*, *Halobacillus*, and *Paenibacillus* (Karpiński and Adamczak, 2018).

Marine actinobacteria are well-known to have the potential to produce biomolecules with interesting novel bioactivities and it is assumed that each strain has the genetic potential to produce among 15–25 secondary metabolites (Ul Hassan et al., 2017). Within this phylum, *Micromonospora* and *Streptomyces* are considered the most prolific producers of secondary metabolites. Nevertheless, properties of each strain can be widely diverse. For example, Davies-Bolorunduro et al. (2019) evaluated the anticancer activity of 32 actinomycetes strains against a panel of cancer cell lines and the isolates of genera *Micromonospora* and *Streptomyces* exhibited cytotoxicity with IC_{50} values ranging from 0.03 to 4.4 mg/mL. Among other Phyla, some extracts of *Halomonas* and *Bacillus* have showed cytotoxicity with $IC_{50} < 50$ μg/mL (Aboul-Ela et al., 2012; Sagar et al., 2013; Chen et al., 2019). It is noticeable that many secondary metabolites have been isolated from *Bacillus*, including peptides, terpenoids, polyketides, and isocumarins, however, a few marine *Bacillus* strains have been examined for pharmaceutical activities (Chen et al., 2019).

Furthermore, it has been possible to establish a correlation between the effects of the use of different culture conditions and the antiproliferative activity for each single strain. In this case, lower GI_{50} values were observed when strains are grown under low nutrient conditions (1:5 diluted MB medium). Aside from nutrient requirements, the importance of agitation, which allows the renewal of nutrients (Bonnet et al., 2020) and a higher diffusion coefficient for oxygen into water (Somerville and Proctor, 2013) may explain the observed effects. These results reinforce the implementation of strategies that stimulate the production of secondary metabolism with potential biological activity (Pan et al., 2019). Once the experimental conditions have been established for the strains of interest, dereplication studies, and scale-up cultures will be developed to characterize the chemical entities responsible for the antiproliferative activity observed.

During the history of marine natural products research, several metabolites extremely toxic against tumor cells have been described, many of them belonging to new structural series of antitumor substances that opened new prospects for synthetic modeling. Until those substances were discovered, all the natural products applied in chemotherapy were referred to not more than 4–5 structural types (Stonik, 2009; Khalifa et al., 2019). For this reasons, bioprospection studies of singular marine ecosystems will open new opportunities for biodiscovery of novel specimens as well as novel lead compounds and its potential biological applications based on a sustainable supply.

CONCLUSION

Bioprospecting studies of marine microorganisms have been focused on certain phyla or classes and several microbial genera had been minimized, in part because of the expectation to the discovery novel strains that produce different metabolites and mechanisms of action compared to terrestrial microorganisms (Martín-Rodríguez et al., 2014; de Vera et al., 2018). Nonetheless, our results exhibit the antiproliferative potential of genus such as *Halobacillus*, *Kangiella*, *Photobacterium*, *Halomonas*, among others, isolated from a singular ecosystem as Tagoro submarine volcano. Their biotechnological development provides an excellent starting point to access novel secondary metabolites and enzymes with potential for pharmaceutical and industrial applications.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank) databank, accession numbers MW826671–MW826754.

AUTHOR CONTRIBUTIONS

AD-M and JF designed, conceived and supervised the research study, and experiments. SG-D and AD-M developed the experimental work of isolation and taxonomic identification of bacteria, culture, and extraction of bacterial strains. SG-D and CR performed the phylogenetic analysis. IL and JP achieved the antiproliferative analysis. EF-N was responsible for oceanographic cruise design. JF, AD-M, EF-N, and JP were project administration and funding acquisition. All authors performed the analysis and data compilation, contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.687701/full#supplementary-material

Supplementary Figure 1 | Radar chart of the antiproliferative activity (GI_{50}) expressed in μg/mL for each cancer cell line.

Supplementary Table 1 | Physical–chemical parameters at sampling stations, including pH (ΔpH) and temperature (Δθ) anomalies around the main and secondary crater (DA04–06), sampling data, and identification.

Supplementary Table 2 | Summary of the bacterial isolates and phylogenetically closely related species from GenBank according to 16S rRNA gene sequence similarity organized by sampling location.

Supplementary Table 3 | Antiproliferative activity (GI_{50}, μg/mL) of the marine bacterial extracts against human solid tumor cell lines.

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