

## Abstract

Extremophiles may "love" or tolerate extreme conditions (Rothschild & Mancinelli 2001), such as those in the hydrothermal vents of the Kolumbo-Santorini volcanic complex and the Milos island. Previous studies have shown that these areas host an impressive microbial variety (Oulas et al. 2016, Christakis et al. 2018), while bacteria of the genus *Pseudomonas* isolated from the active area of the Kolumbo volcano, they appear to be resilient at low prices of low prices pH, at high concentrations of heavy metals as well as antibiotics (Mandalakis et al. 2019, Bravakos et al. 2021). The purpose of the present work was to isolate microorganisms from these hydrothermal vents to control the presence of gene resistance to a series of antibiotics. Microbial cultures were performed, isolation of genomic DNA, amplification and sequencing of the 16S rRNA gene. Representatives of the Pseudomonadota phylum were found in all three sampling areas, while bacteria of the Bacillota phylum were isolated from the Santorini caldera and the Kolumbo crater. Six antibiotic resistance genes (ARG) were also investigated (INT, Sul1, TetA, bla<sub>CTX-M</sub>, bla<sub>OXA-48</sub> and QnrS). In Santorini the most common gene was bla<sub>OXA-48</sub> (38%), while Kolumbo dominated Sul1 (34%) and there was a single strain carrying the resistance gene bla<sub>CTX-M</sub>.

## Results 1 - ISOLATES

**Santorini:** the phyla Pseudomonadota (79%) and Bacillota (21%), were identified. In the phylum of Pseudomonadota, members affiliated to the genera *Vibrio* (63%), *Pseudomonas* (22%) and *Halopseudomonas* (15%) were identified. The majority of Bacillota was covered by the genera *Niallia* (72%), *Lysinibacillus* (14%), *Bacillus* (7%) and *Neobacillus* (7%).  
**Kolumbo:** The phyla of Bacillota (74%) and Pseudomonadota (26%) were identified. In the phylum of Bacillota, members affiliated to the genera *Cytobacillus* (44%), *Bacillus* (25%), *Niallia* (20%), *Mesobacillus* (7%), *Lysinibacillus* (2%) and *Brevibacillus* (2%) were identified. The Pseudomonadota phylum were represented entirely by the genus *Vibrio* (Figure 2).  
**Milos:** Three strains were identified from the Milos sample affiliated to the genus *Vibrio*.

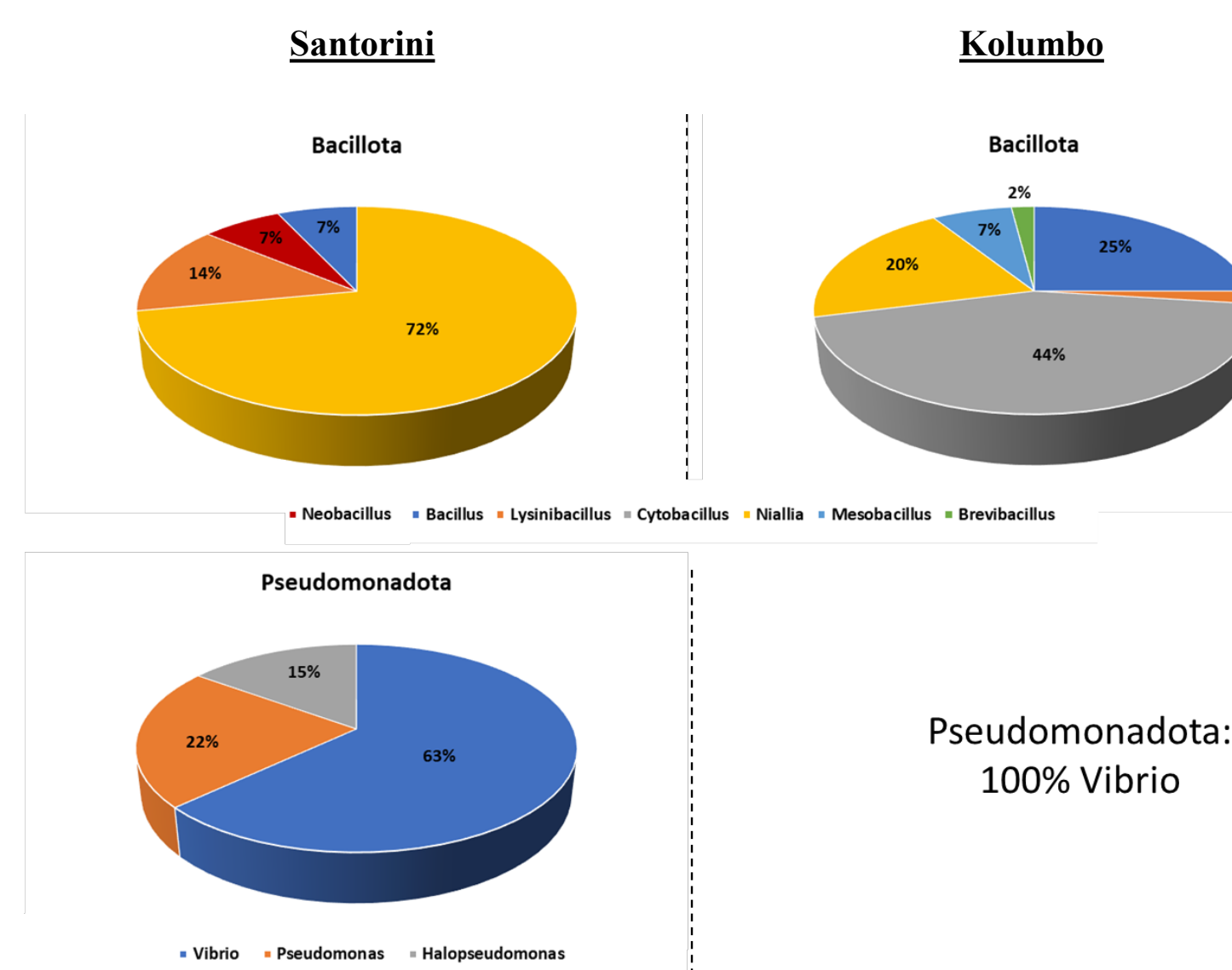


Figure 2. The genera of the bacterial strains isolated from Santorini (left) and Kolumbo (right).

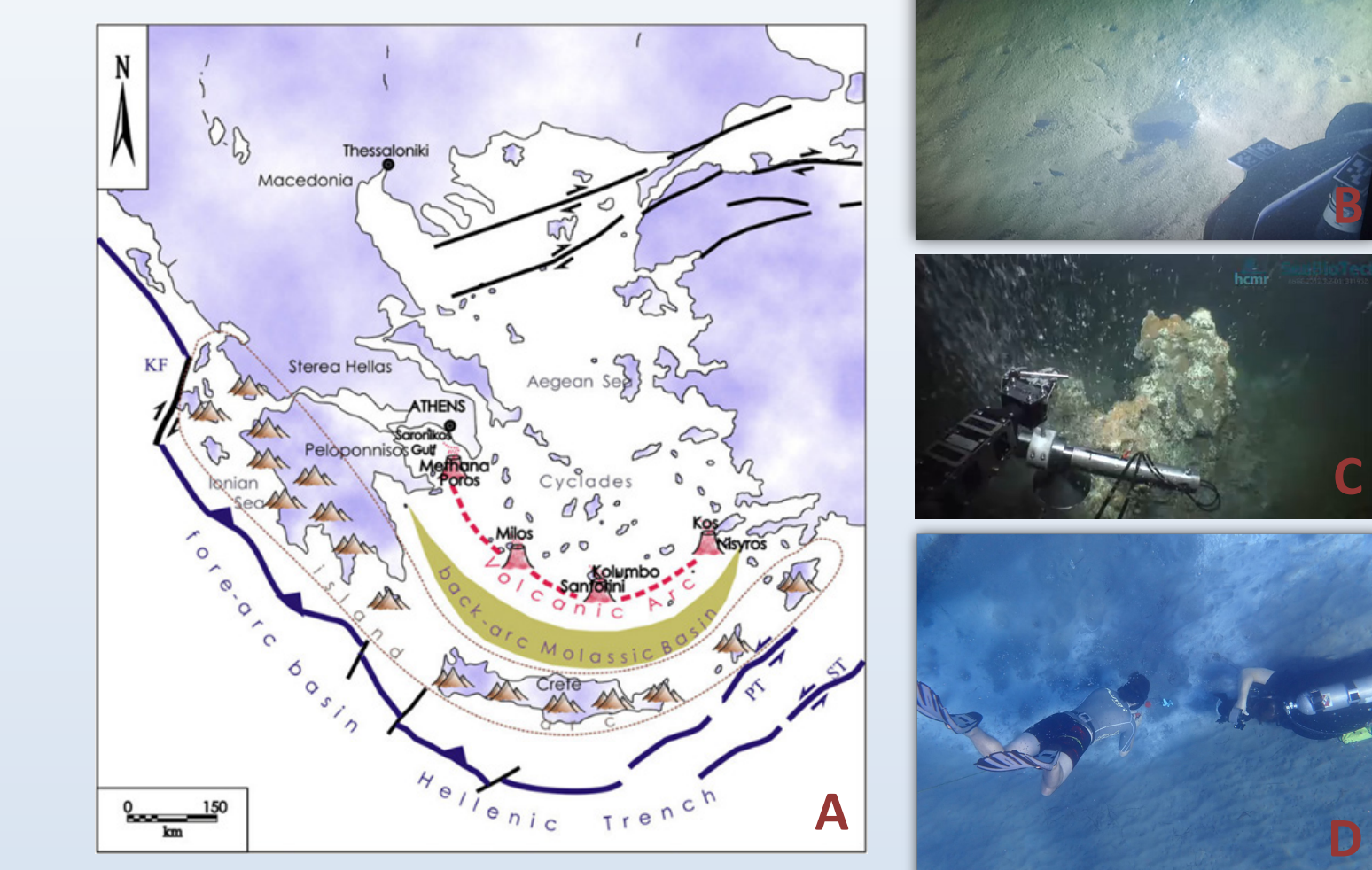


Figure 1. (A) The Hellenic Volcanic Arc (Nomikou et al. 2012) and the hydrothermal vents in (B) Santorini (credit: R. Camilli, NASA PSTAR), (C) Kolumbo (credit: P. Polymenakou, Seabiotech) and (D) Milos (credit: S. Le Moine Bauer, CarDHynAI).

All samples were collected from previous sampling expeditions.  
1) the "R/V POSEIDON cruise P510" conducted in 06/03-29/03/2017  
2) the "NASA PSTAR" expedition with vessel (cable layer) Ocean Link and the Nereid Under Ice (NUI) robotic explorer that was carried out in November 2019 and  
3) the "CarDHynAI" project's sampling campaign that was carried out in September 2019 at the shallow hydrothermal vents of Paleochori Bay in Milos Island.  
This was followed by laboratory analyzes in which cultivation and re-cultivation was done in LB and Marine broth.  
Microbial characterization was performed by 16S rDNA analysis.  
The presence of six ARG was investigated i.e. INT, Sul1, TetA, bla<sub>CTX-M</sub>, bla<sub>OXA-48</sub> and QnrS.

## DISCUSSION

Bacterial strains of Pseudomonadota, Bacteroidetes, Bacillota, Chloroflexi and Planctomycetes have been found in the Santorini-Kolumbo hydrothermal system using molecular approaches (Oulas et al. 2016, Christakis et al. 2018). In the present research, refining the cultivation method, cosmopolitan species of the genera Pseudomonadota and Bacillota were isolated. In previous studies done by Mandalakis et al. (2019) with culture experiments, strains of the genus *Pseudomonas* from Kolumbo volcano with resistance to antibiotics were identified. In the present study, we identified ARG in pristine environments such as the Kolumbo volcano. These genes have been previously identified in clinical studies (Lazar et al. 2021). In fact, 50% of the bacterial strains tested were found to have at least one ARG.  
A reasonable question that arises is the following: "**Based on what mechanisms did genes from clinical microorganisms manage to find themselves in remote extreme environments?**". A possible evolutionary mechanism is high concentrations of metals that act as stress mechanisms and confer resistance to a range of stressors such as antibiotics.

## Introduction

There are organisms that succeed and specialize in environments with extreme values of physicochemical parameters and are therefore characterized by the anthropocentric term "**extremophiles**" (Rothschild & Mancinelli 2001, Rampelotto 2013, Gurunathan et al. 2021). The hydrothermal vents have been identified in the Hellenic Volcanic Arc (HVA) and mainly in Santorini, Kolumbo and Milos (Figure 1). The extremophiles of the HVA are of particular interest as according to recent publications by Mandalakis et al. (2019) and Bravakos et al. (2021), strains of the genus *Pseudomonas* were found to be resistant to low pH values, high concentrations of heavy metals and a series of antibiotics.  
**The purpose of this work was, beyond the identification of the bacteria, to check for the presence of six antibiotic resistance genes, bla<sub>CTX-M</sub> (β lactam resistance), bla<sub>OXA-48</sub> (β lactam resistance), INT (integrase gene), Sul1 (sulfonamide resistance), TetA (tetracycline resistance) and QnrS (quinolone resistance).**

## Results 2 - ARG

**ARG:** At least one ARG was found in 50% of bacterial strains identified. All six ARG investigated in the present work were detected in the Santorini caldera samples (Figure 3). It should be pointed out that the TetA gene was absent from the Kolumbo sample. Unlike Santorini caldera and Kolumbo, a single ARG was detected in Milos (bla<sub>CTX-M</sub> gene). In half of the samples (74/148) no resistance gene was observed. 33% of samples had one resistance gene, 14% had two resistance genes and three resistance genes were identified in 3% of samples.

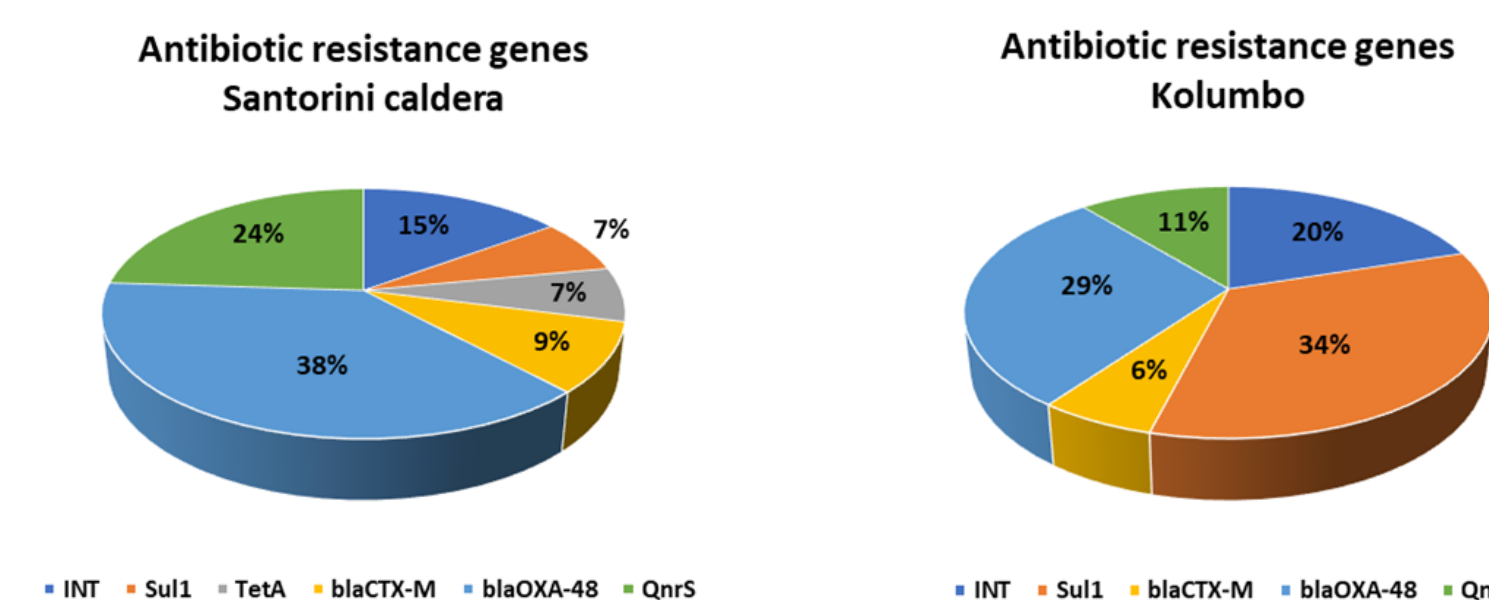


Figure 3. The antibiotic resistance genes found in the Santorini caldera and Kolumbo samples.

## SUMMARY

With this research effort:  
✓ about 350 bacterial strains were isolated  
✓ identified and screened for ARG 148 strains (Pseudomonadota and Bacillota)  
✓ 74 strains had at least 1 gene  
✓ 5 strains carried 3 genes  
**For this reason, it is proposed to further investigate both the bacterial composition and the presence of resistance genes in the hydrothermal system of the Hellenic Volcanic Arc.**

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