Preliminary screening of sulfur vents for antibiotic-producing microorganisms

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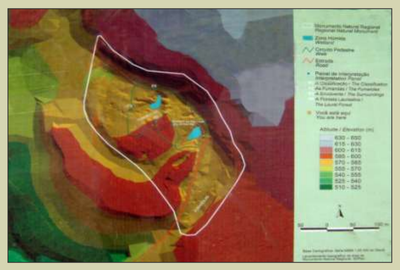

Introduction

Sulfur vents like the ones found in the island of Terceira, Azores (North Atlantic; 37–40°N, 25–31°W), are home to extremophilic microorganisms adapted to survive in harsh ecological niches with stressing conditions in terms of temperature, pH and sulfur concentrations. It has been suggested and demonstrated that adaptation responses of microorganisms under stress may include the production of metabolites or enzymes useful to humans. Sulfur vents are an under explored source of potential antibiotic producing microorganisms. In this work, we collected a small sample from microorganisms living in soil around sulfur vents and tested their activity against food-borne human pathogens (*Proteus* sp., *Salmonella* sp., *Escherichia coli* ATCC 25922, *Listeria monocytogenes*, *Staphylococcus aureus* 3DA, *Staphylococcus aureus* ATCC 9144, *Pseudomonas aeruginosa*) and a non-pathogenic *Listeria innocua*. Isolates displaying a large-spectrum of inhibition against the target microorganisms were identified by molecular biology methods.

Methods

Soil samples were collected aseptically from hot sulfur vents in the Furnas do Enxofre fumarolic field located in the interior of Terceira island. It is a 64,746 m² area within a range of 583m and 620m of altitude above sea level with annual precipitation values around 2000 mm. The area covers a part of the Galhardo trachytic lava dome and extends beyond its crater being located at the intersection of NW-SE, E-W and NNE-SSW faults. Due to the low permeability soils, the area is prone to flooding. Vegetation is diverse with a huge number of mosses and liverworts. CO_2 is the main constituent of the total gas in the steam discharges, representing 94 to 99.6 mol%. H_2S and H_2 are the other major components contributing 0.1 to 3 mol% of the total gas. Nitrogen concentration is below 1.5 mol%. CH_4 values are in general lower than 0.8 mol%. Sampling was carried out in three sites, with different temperatures (45ºC,69ºC and 73ºC). Isolation of microorganisms was carried out by swabbing an extraction of diluted soils (1g soil/10 ml saline) on ½ R2A plates that were subsequently incubated in the laboratory at 45ºC for several days, after which microbial growth was analyzed. Morphophysiological characteristics of the isolates (Gram status, colony colour, pigment production, cell morphology, catalase, oxidase, and nitrate reduction activities) were recorded. The antimicrobial spectrum of the isolated microorganisms was evaluated by the streak assay, using the human pathogenic microorganisms *Proteus* spp., *Salmonella* spp., *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 9144 and 2 LAQ, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Listeria innocua* as target strains.

DNA was extracted and purified using the UltraClean® Microbial DNA Isolation Kit using the manufacturer's protocol (MoBio, Carlsbad, CA). Extracted DNA was amplified with universal bacterial primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). DNA fragments were cleaned using the ExoSAP-IT ® for PCR product (Affymetrix, Inc. Santa Clara, CA), and were Sequenced using Big Dye kit and an ABI-3100 automated sequencer (Applied Biosystems, Carlsbad, CA). Sequences analysis were done through Ribosomal Database Project software (http://rdp.cme.msu.edu/) and Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Twenty-five bacterial isolates were obtained. Culturability was achieved from all of the sulfur vents sampled, independent of their temperature. These isolates were classified according to different morphophysiologic characteristics (fig.1). All isolates were rod-shaped bacteria, 88% of them presenting Gram positive reaction. Isolates showed relatively low percentages of oxidase and catalase activities, 28% and 40% respectively, while nitrate reduction activity was detected in 60% of them. Almost two-thirds of the bacteria showed some kind of antibiotic activity against food-borne human pathogens (fig. 2). Two isolates with a wide spectrum of antibiotic activity (P16-B, P2-5B) were sequenced and phylogenetically analyzed (fig. 3). Using a 97% identity cutoff, the two isolates were found to have closest relatives of *Paenibacillus elgii* and *Bacillus licheniformis,* organisms in the *Bacillales* order in the *Firmicutes* Phylum (fig.4).

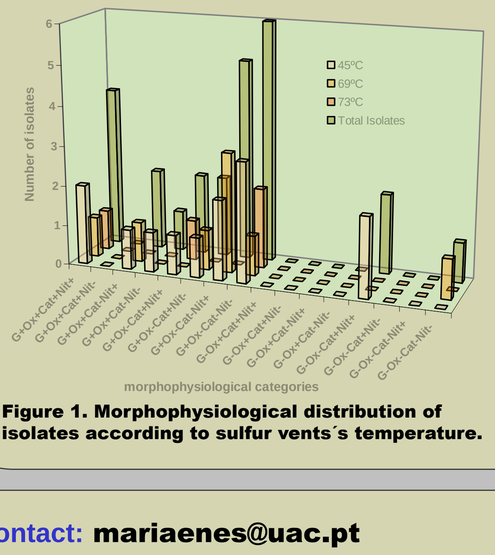

Figure 1. Morphophysiological distribution of isolates according to sulfur vents´s temperature.

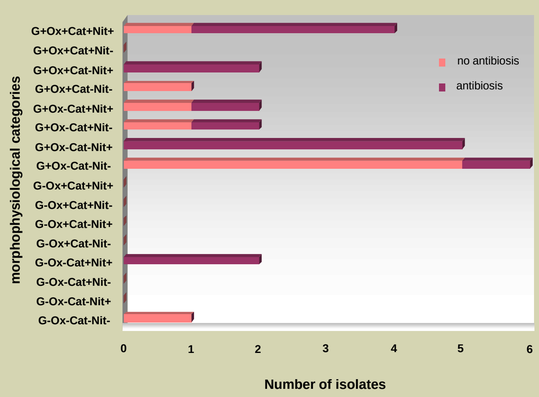

Figure 2. Antibiotical activity found in the isolates classified according to morphophysiological categories.

Figure 3 – Cross-steak tests with antibiotic producing isolates a) and b) and a negative one c) towards the selected pathogens.

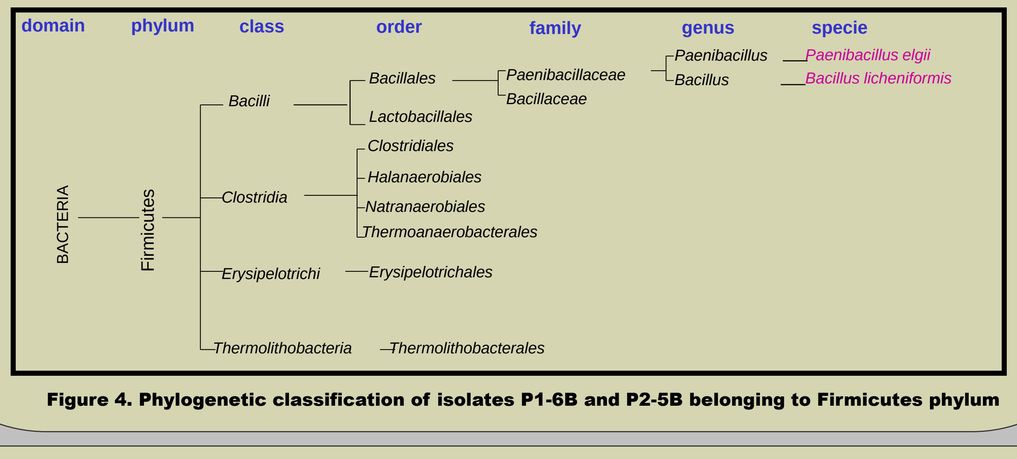

Figure 4. Phylogenetic classification of isolates P1-6B and P2-5B belonging to Firmicutes phylum

Conclusions

- **A high percentage of catalase negative isolates (60%) were found in comparison to microorganisms living in other extreme environments in the archipelago, including lava tubes.**
- **High occurrence of antibiotic producer microorganisms in these environments.**

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