

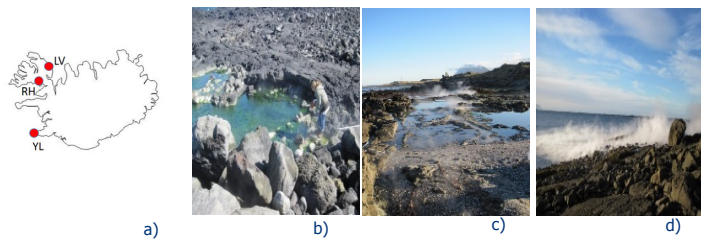
# Novel bacterial species from intertidal, geothermal areas in Iceland

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## Introduction

Intertidal areas are highly dynamic environment, subject to constant periodic disturbances with steep gradients of temperature, mineral composition and salinity. Temperature gradients are manifested most clearly in the contrast between the hot fluid in geothermal coastal hot areas and the cold seawater.

The objective of this study project was to study the composition and diversity of species that tolerate the extreme changes of environmental factors during tidal cycle.



**Figure 1.** Intertidal geothermal areas in Iceland. a) A schematic picture of Iceland and the sampling sites. b) Sampling site Yngingarlindir (YL) c) Laugarvík (LV) d) Reykhólar (RH).

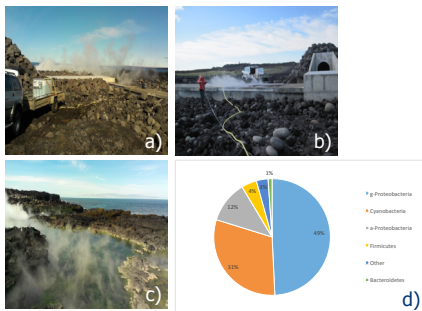
## Materials and methods

Samples were collected from three separate coastal areas in Iceland (Fig 1a). At the coastal ponds Yngingarlindir (YL) (Fig 1b) the hot water upstream is an affluent from a geothermal powerplant. The temperature at the sampling sites ranged from 22 – 43°C.

At Laugarvík (LV) (fig 1c) hot water emerges from hot springs, at the bottom of narrow fissures. Sea water flows at high tide and during low tide the hot springs emerge. The temperature at the sampling sites ranged from 48 - 70°C.

At sampling site, Reykhólar (RH) (fig 1d) an affluent from a seaweed processing plant flows in streams down a rocky shore. The temperature where the samples were collected ranged from 25 – 65 °C.

Biodiversity was estimated with metagenomic sequencing and purified strains. Isolation of strains was done by cultivating at aerobic and anaerobic conditions using different media, temperatures and pH. Identification of species or closest relatives was done by sequencing of the 16S rDNA and by BLAST in the NCBI database.



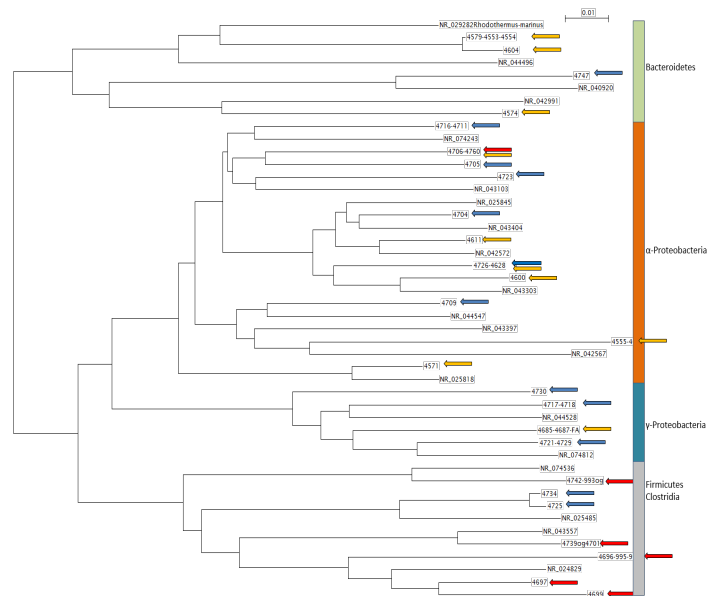
**Figure 2.** The majority of species found in YL belong to  $\gamma$ -Proteobacteria and Cyanobacteria. Metagenomic sequencing was done on a 1000 liters water-sample collected from YL, figure a), b) and c). More than 5 different phyla were found at the sampling site, figure d).

## Conclusions

The intertidal biotopes described in this project create a unique environment which enhance growth of an unusually high ratio of novel bacterial species. Bacteria that undergo such extreme environmental changes are still fairly unknown and need to be studied further for better understanding of these highly dynamic ecosystems.

## Results

- Metagenomic studies showed that the majority of the bacteria from YL belonged to *Proteobacteria* and *Cyanobacteria* (figure 2).
- A total of 176 strains belonging to 63 species were isolated from all three intertidal areas.
- Thereof 38 species represent novel species or genera, 86-97% similarity to the closest relative (figure 3).
- From the total of 38 novel species isolated, 12 have already undergone whole genome sequencing (454 FLX) (table 1).



**Figure 3.** A phylogenetic tree of 31 isolated strains from YL (blue arrows), LV (yellow arrows) and RH (red arrows) and their closest relative according to NCBI database. 63 species were cultivated from the three sampling sites. Of those 38 belonged to novel species or genera. Bar shown in the upper right corner represents relative distance.

**Table 1.** The 12 novel strains that have undergone 454 FLX sequencing. Of the 38 novel strains that were collected in this project 12 have already been sequenced and 7 strains are in process.

Strain	Origin	Closest relative	Sim	Phyla
4553	LV	Rhodothermus marinus	92%	CFB group bacteria
4555	LV	Geminicoccus roseus	87%	$\alpha$ -proteobacteria
4685	LV	Mariniobacter lutaoensis	96%	$\gamma$ -proteobacteria
4716	YL	Ochrobacterium anthropi	91%	$\alpha$ -proteobacteria
4717	YL	Mariniobacter nitratreductens	92%	$\gamma$ -proteobacteria
4696	RH	Clostridium clariflavum	92%	Firmicutes
4784	RH	Geobacillus leptophilus	99%	Firmicutes
4705	YL	Rhodopseudomonas julia	89%	$\alpha$ -proteobacteria
4699	RH	Clostridium straminisolvens	93%	Firmicutes
4721	YL	Hahella chejunensis	92%	$\gamma$ -proteobacteria
4725	YL	Caminicella sporogenes	93%	Firmicutes
4726	YL	Rhodovulum strictum	94%	$\alpha$ -proteobacteria