ISOLATION AND CHARACTERIZATION OF POTENTIALLY PATHOGENIC BACTERIA FROM MOUNTAINOUS REGIONS IN FRANCE

Masoumeh (Nasim) Kashiri
Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Bremen, Germany
Masoumeh.kashiri@brillhygiene.com

DR. BRILL + DR. STEINMANN
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE

ICEBIO

AARHUS UNIVERSITET

BACKGROUND AND RESEARCH APPROACH

Background

- Cold alpine environments host diverse microbial communities that have adapted to extreme conditions. With climate change accelerating glacial melt, understanding these microbes is critical to assess potential public health risks.

- **This study focuses on** Isolating and identifying bacterial strains from alpine environments and assessing their pathogenic potential.

Research approach

1. Snow samples collected and cultured

2. Isolation and identification of bacteria:
Peribacillus simplex
Sphingomonas faeni

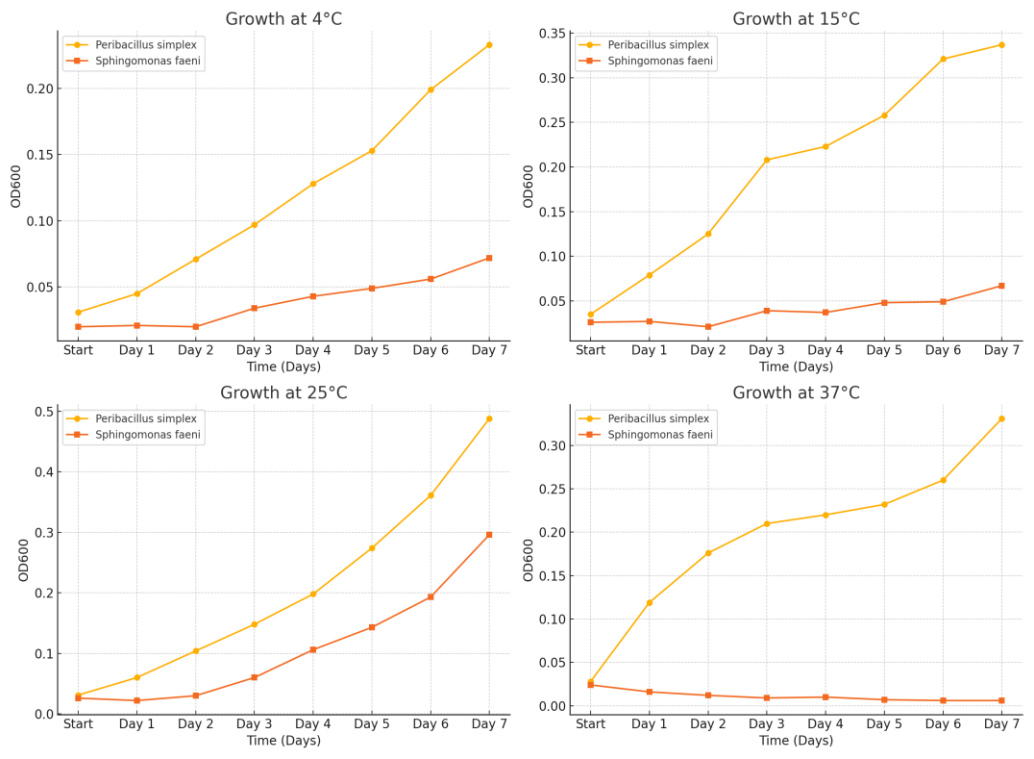
3. Growth assessment at different temperatures

4. Hemolytic activity testing

5. Whole genome sequencing and ARG detection

KEY FINDINGS AND IMPORTANCE

Peribacillus simplex isolate can survive and grow at higher temperatures (37 °C)



Both *Peribacillus simplex* and *Sphingomonas faeni* isolates carry antibiotic resistance genes involved in multiple mechanisms.

Gene	Mechanism of Resistance
blaZ	Beta-lactamase
vanY	Cell wall modification
emrY1, expZ, tetA2	Efflux pump systems
vat	Antibiotic inactivation
acrB, mexA, oqxB15, ttgB	Multidrug efflux pumps
rpoB	Target site modification

I will be at **PICO3.2.** screen for any questions or discussions

Studying cold-adapted microorganisms is essential to understand emerging public health risks linked to antibiotic resistance and environmental changes

INTRODUCTION

- Cold-adapted microorganisms inhabit a large proportion of Earth's ecosystems.
- Over time, bacterial communities evolve genes aimed at providing fitness advantages to the producing organisms
- This is probably the case with virulence factors and antimicrobial resistance genes.
- Stressful conditions brought about by a changing climate can heavily influence competition within bacterial communities.
- Some of these bacteria can also adapt to the new conditions and higher temperatures increasing their chances of interaction with human populations and ecosystems.
- However, the pathogenicity potential of these bacteria is very poorly understood.

In the present study we investigated bacterial isolates from snow samples of the mountains in Grenoble for their temperature tolerance, hemolytic activity and antibiotic resistance genes.

SAMPLING, ISOLATION AND MOLECULAR IDENTIFICATION

sampling

- Snow samples were collected from Chamrousse ski resort in Grenoble
- The samples were transferred to the home lab in a cool box.

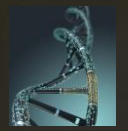
Cultivation

- Samples were melted at 4°C and cultured on R2A plates
- Plates were incubated at 4°C, 15°C and 37°C.
- 2 negative control plates were incubated at each temperature

Isolation

- 2 Morphologically different colonies from the plates were streaked onto fresh R2A plates to obtain pure cultures.
- The plates were incubated at the initial isolation temperature

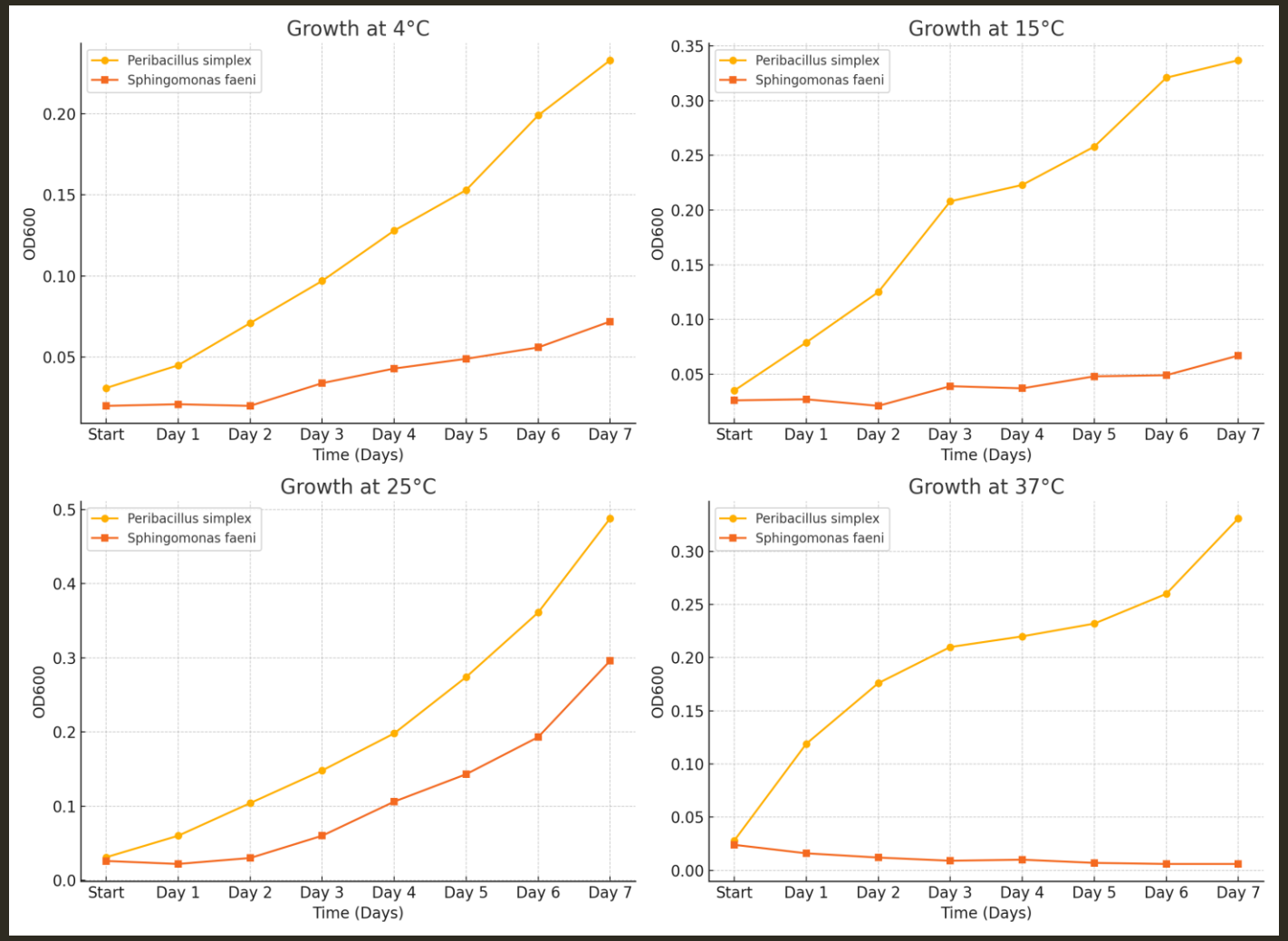
Molecular identification



- Genomic DNA was extracted and 16S rRNA was amplified via PCR with universal primers, then purified and sanger sequenced.
- Isolates 1 showed 100% homology with *Peribacillus simplex* in the NCBI database.
- Isolate 2 showed 100% homology with *Sphingomonas faeni* in the NCBI database.

Growth assessment based on OD600 measurements

OD600 measurements were taken over 7 days **at 4 different temperatures** to assess the growth of isolates



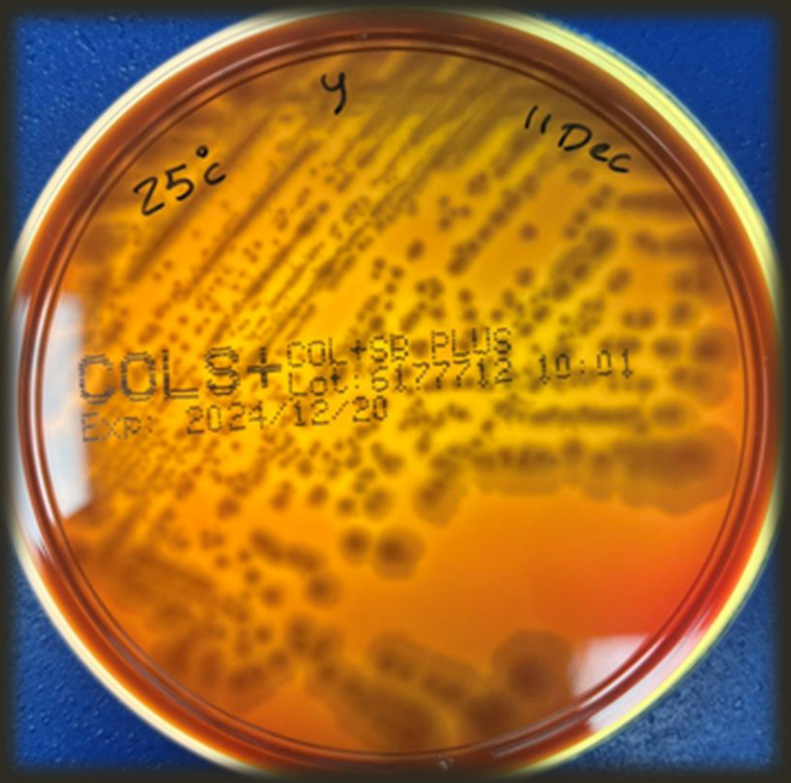
Peribacillus simplex:

Peribacillus simplex behaves as a mesophilic bacterium with the ability to survive moderate cold but thrives better at warmer, temperate conditions (25–37°C). Its survival at 37°C suggests a potential for adaptation to human body-associated temperatures, which may have implications for its pathogenic potential.

Sphingomonas faeni:

Sphingomonas faeni is characterized as a psychrotolerant microorganism — it can grow at low temperatures (15°C) but cannot survive at human body temperatures (37°C). Therefore, its direct pathogenic potential for humans under normal conditions seems limited, although its ability to carry ARGs remains concerning from a public health perspective.

HEMOLYTIC ACTIVITY ASSESSMENT



Methodology:

- Bacterial isolates were cultured on sheep blood agar and horse blood agar plates.
- Plates were incubated at 15°C, 25°C, and 37°C for up to 7 days.

Results:

1. *Peribacillus simplex* showed alpha hemolysis (partial hemolysis, greenish discoloration) on both sheep and horse blood agar.

2. *Sphingomonas faeni* did not grow on blood agar plates at any tested temperature.

WHOLE GENOME SEQUENCING TO IDENTIFY GENES RELATED TO PATHOGENICITY

- Nanopore sequencing method

(used for long-read sequencing to capture the entire genome more efficiently)

- Process raw data

(used for assembly, annotation, and downstream analysis)

- BLASTn analysis with CARD database

(preferred over VFDB for its relevance to antibiotic resistance genes)

- MegaBLAST analysis of the most relevant genes

(to identify organisms that carry the same genes)

Antibiotic Resistance Genes detected

Isolate	Gene	Resistance Mechanism
Peribacillus simplex	**blaZ**	Beta-lactamase production: hydrolyzes beta-lactam antibiotics (e.g., penicillins).
	vanY	Cell wall alteration: modifies peptidoglycan to resist vancomycin.
	emrY1, expZ	Efflux pumps: actively export multiple antibiotic classes.
	tetA2	Tetracycline efflux pump: removes tetracycline antibiotics from the cell.
	vat	Inactivates streptogramin A antibiotics via acetylation.
Sphingomonas faeni	**acrB, mexA**	Multidrug efflux systems: expel beta-lactams, fluoroquinolones, macrolides.
	oqxB15	Efflux pump: resistance to quinolones, chloramphenicol.
	ttgB	Efflux of antibiotics and organic solvents.
	rpoB	Mutation-based target modification: resistance to rifampin.

CONCLUSION

- *Peribacillus simplex* grew at 25°C and 37°C, showed alpha hemolytic activity on blood agar, and carried multiple antibiotic resistance genes (ARGs), suggesting mesophilic adaptation and potential virulence traits.

- *Sphingomonas faeni* thrived only at 15°C and 25°C, indicating a psychrotolerant profile, and carried several ARGs.

- These microorganisms need to be studied further to better understand their potential impact on public health and ecosystems under changing environmental conditions.

THANKS FOR YOUR ATTENTION!