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Isolation and characterization of potentially pathogenic bacteria from mountainous regions in France

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Introduction:

High-altitude snowy regions are recognized as unique biomes hosting diverse microbial communities. Microorganisms in these environments have evolved adaptations to survive extreme conditions, such as low temperatures, high UV radiation, and limited nutrient availability. These adaptations may include antibiotic resistance and virulence factors, which could pose ecological and public health risks if transferred to human pathogens or clinically relevant ecosystems.

This study aimed to isolate and identify bacterial strains from these environments, assess their temperature tolerance, hemolytic activity, and potential antibiotic resistance profiles and to investigate the presence of antibiotic resistance genes (ARGs) and their potential public health risks.

Methods:

Snow samples were collected from Chamrousse Ski Resort (Grenoble, France) and cultivated on R2A agar at 4°C, 15°C, and 37°C, with morphologically distinct colonies isolated and purified. Growth was monitored over 7 days at 4°C, 15°C, 25°C, and 37°C by measuring OD600 at 24-hour intervals to assess temperature tolerance. Hemolytic activity was evaluated on sheep and horse blood agar plates incubated at 15°C, 25°C, and 37°C, with patterns of alpha, beta, or gamma hemolysis recorded. Genomic DNA was extracted, and 16S rRNA sequencing was used to identify the isolates at the species level. Whole genome sequencing was conducted using the Oxford Nanopore method, and antibiotic resistance genes (ARGs) were identified via the CARD database. Minimum inhibitory concentration (MIC) testing is planned as a follow-up to validate resistance profiles and assess the functional expression of the identified ARGs.

Results:

Sanger sequencing of the 16S rRNA gene identified *Peribacillus simplex* for isolates 1, 2, and 3, and *Sphingomonas faeni* for isolate 4, with 100% sequence homology. Growth monitoring revealed that *Peribacillus* isolates grew best at 25°C, with *Peribacillus 1* showing moderate growth at 37°C, while *Sphingomonas* exhibited psychrotolerant traits, thriving at 15°C and 25°C but performing poorly at other temperatures. Hemolytic activity tests showed that *Peribacillus 1* exhibited alpha hemolysis on both sheep and horse blood agar, whereas *Peribacillus 2 and 3* showed gamma hemolysis, and *Sphingomonas* did not grow on blood agar. Whole genome sequencing identified several antibiotic resistance genes (ARGs) linked to multidrug resistance and virulence, including **blaZ** and **vanY** in *Peribacillus* spp., and **acrB** and **mexA** in *Sphingomonas* spp.

Conclusion:

This study highlights the adaptability of microbial communities in snowy alpine environments to changing climates and their potential to spread ARGs and hemolytic features into ecosystems. The presence of such traits in these microorganisms underscores their possible role as reservoirs of antibiotic resistance and virulence factors in natural habitats. Further studies, including MIC testing and pathogenicity assessments, are crucial to fully understanding the ecological and public health implications of these findings.