Exploring Skin Microbiome Interactions for Antimicrobial Discovery in Diabetic Foot Ulcers

Luísa R. Baptista [1,2]; Susana Alarico [1,2]; Igor Tiago [3,4]; Nuno Empadinhas [1,2]; Ana Maranha [1,2]
1 - CNC - Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, 3004-504 Coimbra, Portugal 2 - CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, 3004-504 Coimbra, Portugal 3 - Department of Life Sciences, University of Coimbra, Coimbra, 3000-456 Coimbra, Portugal 4 - TimeUp Lda, IPN incubator, Coimbra, 3030-199 Coimbra, Portugal

23 skin isolates produced diffusible antimicrobial molecules, with 4 requiring competitive conditions for metabolite expression. Chromatographic techniques enabled the partial purification of active compounds, which will be structurally characterized in future studies.

BACKGROUND

Diabetes mellitus (DM) is a growing global health issue, with a fourfold increase in cases over the past 40 years (WHO, 2023). DM patients present delayed wound healing and consequent common complication Diabetic foot ulcers (DFUs). Additionally, the skin commensal microbes that play specific roles in wound healing, are also altered (Byrd et al., 2018). DFUs antibiotic treatments are often ineffective, contributing to the global antimicrobial resistance (AMR) crisis (Uberoi et al., 2024).

METHODS

Swab samples were collected from 234 diabetic patients and 21 non-diabetic controls (**CT**). Samples were collected from **DFU**, from their contralateral non-ulcerated foot (**NDFU**), from diabetic patients without any skin ulcerations (intact diabetic skin - **IDS**). Bacterial strains were isolated and identified via 16S rRNA sequencing. Antimicrobial activity was assessed using spot-on-lawn agar diffusion assays against several DFU-derived strains. To exclude contact-dependent effects, bacterial extracts were tested against five indicator bacteria isolated from DFU (MDR and non-MDR S. aureus and P. aeruginosa and Staphylococcus CoNS). Active extracts were fractionated using organic solvents (methanol, ethanol, acetone, chloroform:methanol [2:1], and 1-butanol) and purified via C18 chromatography. Whole-genome sequencing and genome mining using Antismash (Blin et al, 2025) identified biosynthetic gene clusters (BGCs) with therapeutic potential.

CONCLUSION

This study demonstrates that skin-associated bacteria, particularly from diabetic patients, can serve as a valuable source of novel antimicrobial compounds through their microbial interactions.

RESULTS

A total of 539 bacterial strains were identified: 112 from DFU, 72 from NDFU, 365 from IDS, and 180 from CT. Dominant genera included *Staphylococcus* (74.0%), *Pseudomonas* (3.8%), and *Kocuria* (2.5%) (**Figure 1**).

Overall, 6.0% of isolates exhibited antimicrobial activity, primarily from *Staphylococcus* (50%), *Pseudomonas* (25%), and *Aerococcus* (11.3%). Activity was most frequent in DFU isolates (8.9%), followed by NDFU (7.0%), IDS (6.0%), and CT (3.9%) (**Figure 2**).

Producers of diffusible antimicrobial compounds included *Pseudomonas* (11 isolates), *Staphylococcus* (8), and one isolate each of *Aerococcus*, *Bacillus*, *Enterobacter*, and *Pseudoxanthomonas*. Four isolates required competitive conditions to induce antimicrobial activity.

Acetone was the most effective solvent for compound extraction, and C18 chromatography further refined active fractions (**Figure 3**). Genome mining of the 8 most promising isolates identified 60 biosynthetic gene clusters (BGCs) with <50% homology to known clusters. These included putative cyclodipeptides (CDPs) in the *Bacillus* B1 strain, a lassopeptide, as well as several ribosomally synthesized and post-translationally modified peptides (RiPPs, n=12) and non-ribosomal peptides (NRPs, n=27) (**Figure 4**).

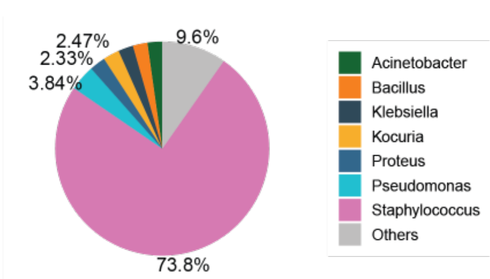

Fig 1. Relative abundance of bacterial genera isolated from skin samples. Genera at lower frequencies (<2%) are grouped under Others.

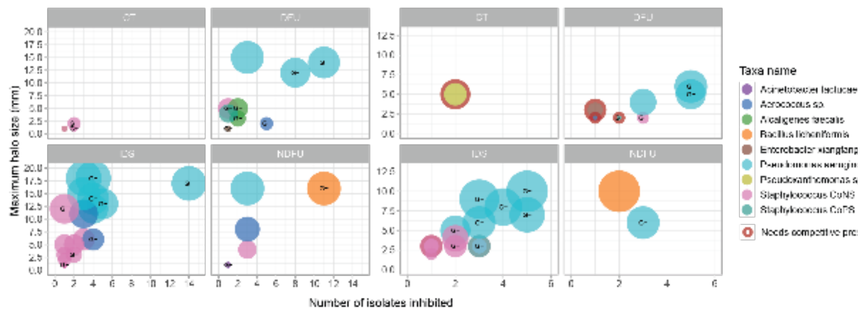

Fig 2. Antimicrobial activity of skin isolates by genus and sample source. **(A)** Distribution of antimicrobial-producing isolates by genus **(B)** Distribution of difusable counpunds. A subset of isolates (n=4) required competitive conditions to express antimicrobial activity.

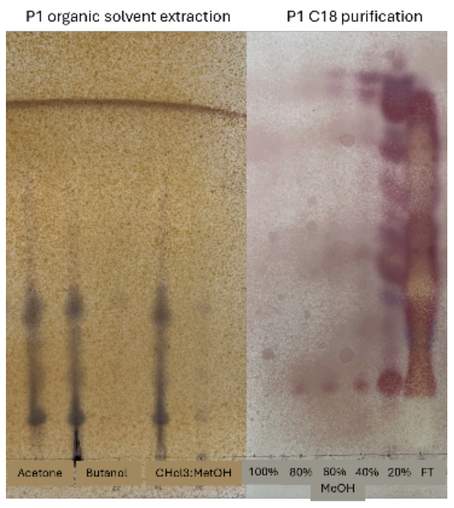

Fig 3. Extraction and purification of antimicrobial compounds from active isolates. **(A)** Thin-layer chromatography (TLC) analysis of organic solvent extraction of bacterial extracts stained with α-naphthol **(B)** C18 chromatography fractions visualized by TLC and stained with ninhydrin

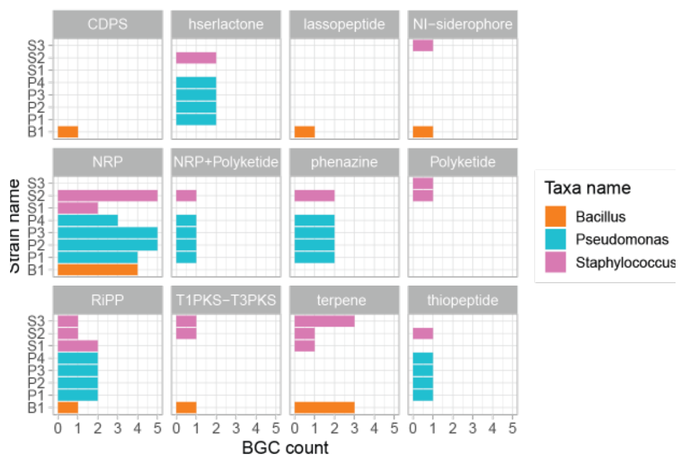

Fig 4. Biosynthetic gene clusters (BGCs) identified from genome mining of eight antimicrobial-producing isolates.

ACKNOWLEDGEMENT / REFERENCES

Funding: 2022.06809.PTDC_AMRFight, UIDB/04539/2020, UIDP/04539/2020, LA/P/0058/2020, DL57/2016-SFRH/BPD/108299/2015

Blin K. (2025), Nucleic Acids Research, Nucleic Acids Res gkaf334; Byrd, A.L. (2018). Nat. Rev. Microbiol. 16, 143; Uberoi, A. (2024). Nat. Rev. Microbiol. 22, 507-521; WHO (2023) Global Report On Diabetes.

CENTER FOR NEUROSCIENCE AND CELL BIOLOGY