Analyzing the potential of selected gut commensal strains to produce antimicrobial peptides: phenotypic and *in silico* approaches

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Introduction

The World Health Organization estimates that the number of antibiotic resistance-related deaths could reach 10 million by 2050 [1]. Given the dynamics and high diversity of microorganisms that inhabit the **gastrointestinal tract**, this is the ideal place to discover **new antimicrobial peptides (AMP)** to replace **traditional antibiotics** [2].

Among the most extensively studied members are the commensal strains ***Akkermansia muciniphila* DSM 22959** and ***Faecalibacterium duncaniae* DSM 17677**, which are reported to have a beneficial impact on intestinal health [3;4].

Antimicrobial peptides are small molecules that act as the first line of defense against microbial invaders, playing a vital role in the innate immune system [5]. One approach to identify new strategies to combat **antimicrobial resistance** is to evaluate the ability of these bacteria to produce AMP.

Objectives

Evaluate the potential of the commensal probiotic candidates ***Akkermansia muciniphila* DSM 22959** and ***Faecalibacterium duncaniae* DSM 17677** to produce **antimicrobial peptides**, using both **phenotypic** (against **nine selected bacterial pathogens**) and ***in silico*** approaches.

For this, the well-studied and commercial probiotic strain, *Lacticaseibacillus rhamnosus* GG (LGG), was used for comparative purposes.

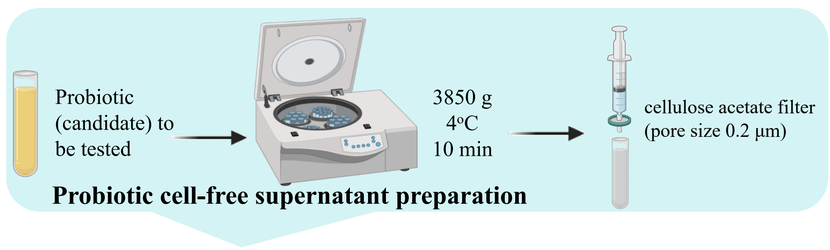
Methods

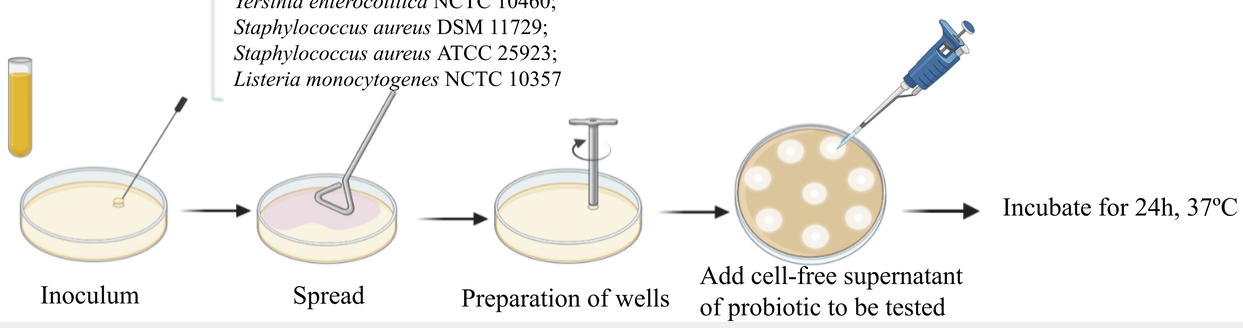
1) Antimicrobial activity potential

(a) Phenotypic:

- **Agar well diffusion method**

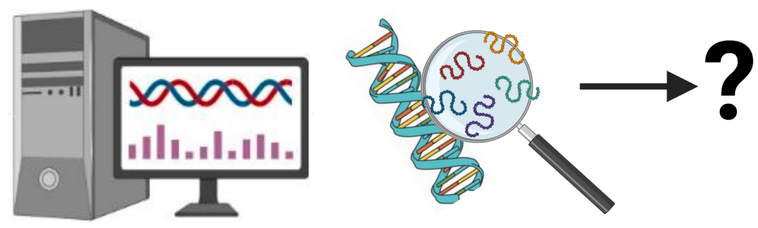
Pathogen to be tested:
- *Streptococcus intermedius* 2567;
- *Escherichia coli* O157:H7;
- *Escherichia coli* ATCC 25922;
- *Klebsiella pneumoniae* ESB;
- *Salmonella enterica* ATCC 14028;
- *Yersinia enterocolitica* NCTC 10460;
- *Staphylococcus aureus* DSM 11729;
- *Staphylococcus aureus* ATCC 25923;
- *Listeria monocytogenes* NCTC 10357





(b) *In silico* analysis

- **BAGEL4**
- **antiSMASH**



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Main findings

(a) Phenotypically, probiotic strains supernatant showed **no antimicrobial activity** against the nine selected pathogens.

(b) Regarding ***in silico*** analysis:

Table 1. Summary results of *in silico* analysis for *F. duncaniae* DSM 17677, *A. muciniphila* DSM 22959 and LGG using BAGEL4 and antiSMASH tools.

Bacterial strain	Accession number	BAGEL4 hits	antiSMASH hits
***F. duncaniae* DSM 17677**	NZ_CP048437.1	0	3
***A. muciniphila* DSM 22959**	NZ_CP042830.1	1	2
LGG	NZ_CP031290.1	1	2

(1.1) ***F. duncaniae*** has a low potential to produce bacteriocins and other bioactive peptides.

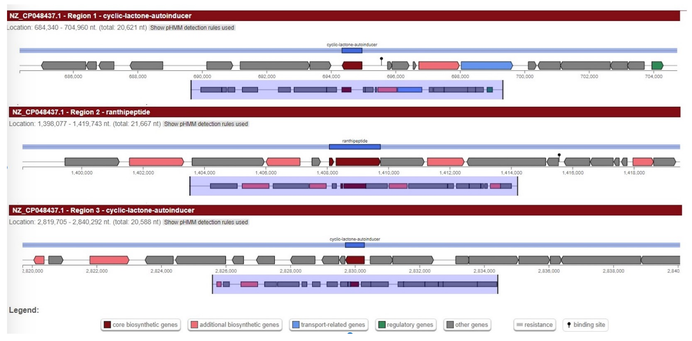
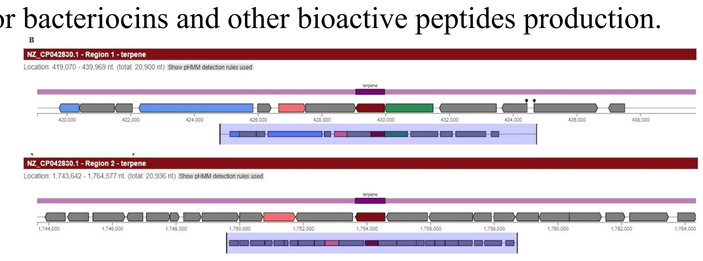
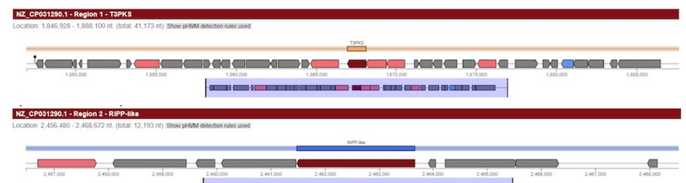


Fig. 1 Results obtained by antiSMASH analysis for *F. duncaniae* (1.1); *A. muciniphila* (1.2) and LGG (1.3)

(1.2) ***A. muciniphila*** DSM 22959 also has a low potential for bacteriocins and other bioactive peptides production.



(1.3) LGG showed that it may be a potential producer of bioactive peptides – complete gene clusters (carnocin and type III polyketide synthetase).



Conclusions

Despite the **absence of phenotypic evidence** of antimicrobial activity in the cell-free supernatants of LGG, *F. duncaniae* DSM 17677 and *A. muciniphila* DSM 22959 against a spectrum of 9 pathogenic strains, it cannot be definitively concluded that they lack genes responsible for antimicrobial compound biosynthesis within their genomes, and consequent production potential.

The *in silico* analysis revealed that ***F. duncaniae* and *A. muciniphila* exhibited low potential to produce bacteriocins** and other bioactive peptides, corroborating the observed phenotype, due to the absence of complete gene clusters homologous to existing ones. Conversely, LGG was identified as a promising candidate for the production of bacteriocins and other bioactive peptides, namely carnocin and a type III polyketide synthetase.

In conclusion, this study offers enhanced robustness and novel insights into the antimicrobial potential of *F. duncaniae* DSM 17677 and *A. muciniphila* DSM 22959.

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