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IPC20-0097: Revealing Antibiotic Resistance Profile of the Novel Probiotic Candidate Faecalibacterium prausnitzii DSM17677

Topic: **Regulatory and Claims**

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Introduction

Faecalibacterium prausnitzii, a common inhabitant of healthy human intestine, has been proposed as a novel probiotic with high application potential in the food and pharmaceutical markets. Despite its beneficial effects, little is known regarding its antibiotic susceptibility profile. Nevertheless, this information is an important requirement in terms of safety assessment, since probiotic strains must be free of risk of antimicrobial resistance transferability, in order to be included in Qualified Presumption of Safety list. In this study, the antibiotic susceptibility profile of *F. prausnitzii* DSM17677 strain was characterised by integrating phenotypic and *in silico* data.

Methods

Growth conditions: *Faecalibacterium prausnitzii* was grown in sBHI for 16h at 37°C, under an atmosphere of 85% N2, 5% H2 and 10% CO2. Afterwards, 0.1mL of this culture was transferred to 10mL of sBHI medium, and this bacterial suspension was incubated anaerobically for 9-10h at 37ºC. **Phenotypic antibiotic susceptibility testing:** Minimum inhibitory concentration (MIC) was determined for 9 clinically relevant antibiotics recommended by EFSA-FEEDAP, using broth microdilution technique, in accordance with guidelines from Clinical and Laboratory Standards Institute, and E-test® method, following supplier's recommended instructions. Microdilution and E-test® assays were performed, in three times independently with technical duplicates, using sBHI broth and sBHI agar, respectively. MIC values were read after 48h of incubation. ***In silico* analysis of resistance genes:** Antimicrobial resistance genes (ARG), genomic islands (GI) and mobile genetic elements (MGE) were predicted in *F. prausnitzii* DSM17677 whole genome (=A2-165; accession number: NZ_CP022479.1) using several available databases and bioinformatics tools.

Results

MIC is defined as the lowest antibiotic concentration that inhibits visible bacterial growth. Phenotypically, *F. prausnitzii* DSM17677 exhibited resistance against ampicillin, gentamycin, kanamycin and streptomycin. In contrast, this strain was susceptible to vancomycin, clindamycin, tetracycline and chloramphenicol. Only erythromycin failed categorical agreement of the results obtained by broth microdilution and E-test® methods. *Faecalibacterium prausnitzii* genome contains 24 annotated genes putatively involved in antibiotic resistance, including aminoglycosides (such as gentamycin, kanamycin and streptomycin) and macrolides (erythromycin). Antimicrobial resistance

genes for tetracycline and lincosamides (clindamycin) are also annotated. Only streptomycin related ARG is encoded within a GI and MGE, and consists in a specifically target mechanism of resistance. A broader homology search also revealed the presence of putative ARG that include β-lactamases, among others. Most of these genes are related with general resistance mechanisms and are not included within GI or MGE. No plasmids were reported for this strain.

Discussion

Categorical agreement was observed between broth microdilution and E-test® results, except for erythromycin. Furthermore, the phenotypic resistance profile is in accordance with the genomic context. The genomic analysis also identified other ARG that might not be expressed under the conditions tested. To be considered safe for human consumption, a potential probiotic strain must not contain acquired resistance. The only potential concern identified relates with the streptomycin-specific ARG, which is encoded within an MGE. Additionally, the same region belongs to a GI, meaning that this specific ARG could have been laterally acquired. To conclude, this study provides relevant information regarding the antibiotic resistance profile of the probiotic candidate *F. prausnitzii*.

Keywords: Antibiotic Resistance;Faecalibacterium prausnitzii;Probiotic;Resistance Genes;Safety Profile