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Short communication

Molecular and phenotypic traits of in-vitro-selected mutants of *Bifidobacterium* resistant to rifaximin

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Abstract

Nucleotide mutations inside a core region of the *rpoB* gene, encoding the β subunit of RNA polymerase, were found in rifaximin-resistant mutants of *Bifidobacterium*. Five different missense mutations of codons 513, 516, 522 and 529 were identified. Further aspects of rifaximin resistance were investigated, using *Bifidobacterium infantis* BI07 as a model strain. Partial resistance of RNA polymerase of a BI07 mutant at a rifaximin concentration >10 μg/mL was observed by cell-free transcription assay. Mass spectrometry detection of rifaximin in the cellular pellet of the BI07 resistant mutant, as well as changes in biosynthesis of saturated and cyclopropane fatty acids during growth, suggested a reduction in membrane permeability for the antibiotic moiety.

Keywords: *Bifidobacterium*; Rifaximin; Resistance mechanisms; *rpoB* gene; RNA polymerase; Membrane permeability

1. Introduction

The bacterial flora of the gastrointestinal tract plays a major role in human physiology, modulating metabolic and immunological processes and preventing overgrowth of opportunistic and pathogen microorganisms. Administration of antimicrobial agents, whether therapeutically or prophylactically, alters the ecological balance between the host and the normal microbiota [1]. Probiotic bacteria belonging to *Lactobacillus* and *Bifidobacterium* genera are commonly used to alleviate the possible gastrointestinal side effects caused by drugs [2].

Rifaximin is a product of synthesis experiments designed to modify the parent compound, rifamycin, to achieve low gastrointestinal absorption and good antibacterial activity. Since the drug remains virtually unabsorbed after oral administration, it is used locally to treat disorders mediated by susceptible bacteria within the gastrointestinal tract. Rifaximin, in common with its structural analogue rifampicin and other members of the rifamycin class, acts on the β subunit of the bacterial RNA polymerase to inhibit RNA synthesis [3]. The effects of rifaximin on the composition of the intestinal microbiota were evaluated by Brigidi et al. [4]. Interesting results emerging from that study concerned the frequent selection of *Bifidobacterium* rifaximin-resistant mutants during antibiotic treatment.

Point mutations inside the *rpoB* gene, which encodes the β subunit of RNA polymerase, have been indicated as the principal factor determining rifampicin resistance in *Escherichia coli* and *Mycobacterium tuberculosis* [5,6]. In a recent study, a missense mutation in the *rpoB* core region, containing the 81 bp fragment where >95% of hot-spot codons were identified in *E. coli* and *M. tuberculosis* rifampicin-resistant mutants, was found in a rifaximin-resistant mutant of the probiotic strain *Bifidobacterium infantis* BI07. This point mutation resulted in the substitution of Gln with

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Arg in the protein sequence of the β subunit of RNA polymerase [7].

In the present paper, we characterised the *rpoB* gene sequence of several probiotic and intestinal *Bifidobacterium* strains. Furthermore, using the model strain *B. infantis* BI07, we analysed the functionality of RNA polymerase and changes of membrane permeability.

2. Materials and methods

2.1. Antimicrobial agent, bacterial strains and culture conditions

Rifaximin was provided by Alfa Wassermann (Bologna, Italy). A stock solution of 10 mg/mL rifaximin in methanol was used. The bifidobacterial strains used in this study are listed in Table 1. Spontaneously resistant mutants at a rifaximin concentration of 100 μg/mL were selected using the method described by Vitali et al. [7]. All bifidobacteria were grown anaerobically in Man–Rogosa–Sharpe (MRS) medium (Difco, Detroit, MI) containing 0.05% L-cysteine at 37 °C for 18–36 h. Anaerobic conditions were achieved in anaerobic jars supplemented with Anaerocult A (Merck, Milan, Italy).

2.2. rpoB gene analysis

Isolation of genomic DNA and sequencing of the *rpoB* gene of the wild-type and rifaximin-resistant *Bifidobacterium* clones were performed as described by Vitali et al. [7]. The sequences comprised the codons 508–550 and were deposited in the DDBJ database under the accession numbers reported in Table 1. Mutations in the *rpoB* gene of the resistant mutants were searched using the ClustalW program [8].

Table 1
rpoB gene mutations identified in *Bifidobacterium* rifaximin-resistant mutants

Strain (*rpoB* sequence accession no.)[a]	Codon position[b]	Nucleotide change[c]	Amino acid change[d]
B. longum biovar *infantis* ATCC 15697^T (wt: AB198724; res: AB198725)[e]	529	C**G**T/CA**T**	**Arg/His**
	530	CT**G**/CT**T**	Leu/Leu
	532	GC**T**/GC**A**	Ala/Ala
	533	**T**TG/**C**TG	Leu/Leu
B. breve ATCC 15700^T (wt: AB198726; res: AB198727)	522	**TCC/GCT**	**Ser/Ala**
	546	GA**G**/GA**A**	Glu/Glu
	548	CG**C**/CG**A**	Arg/Arg
B. longum ATCC 15707^T (wt: AB198728; res: AB198729)	529	C**G**T/CA**T**	**Arg/His**
	530	CT**C**/CT**T**	Leu/Leu
	532	GC**T**/GC**A**	Ala/Ala
	541	GA**T**/GA**C**	Asp/Asp
	547	GT**C**/GT**G**	Val/Val
B. adolescentis ATCC 15703^T (wt: AB198732; res: AB198733)	508	AC**C**/AC**T**	Thr/Thr
	522	**GCT/TCC**	**Ala/Ser**
	523	GG**C**/GG**T**	Gly/Gly
	536	GG**T**/GG**C**	Gly/Gly
	546	GA**A**/GA**G**	Glu/Glu
B. longum biovar *infantis* BI07 (wt: AB198734; res: AB198735)[e]	513	C**A**G/C**G**G	**Gln/Arg**
B. breve BBSF (wt: AB198736; res: AB198737)	516	**G**AC/**T**AC	**Asp/Tyr**
B. longum BL04 (wt: AB198738; res: AB198739)	508	AC**G**/AC**T**	Thr/Thr
	511	CT**C**/CT**G**	Leu/Leu
	512	TC**G**/TC**C**	Ser/Ser
	521	**T**TG/**C**TG	Leu/Leu
	522	**GCG/TCT**	**Ala/Ser**
	524	GT**C**/GT**G**	Val/Val
	525	AC**G**/AC**C**	Thr/Thr
	528	CG**C**/CG**T**	Arg/Arg
	530	CT**C**/CT**G**	Leu/Leu
	532	GC**G**/GC**T**	Ala/Ala
	539	TC**G**/TC**C**	Ser/Ser
	546	GA**G**/GA**A**	Glu/Glu

ATCC, American Type Culture Collection (Rockville, MD); ^{T}, type strain.

[a] *B. infantis* BI07, *B. breve* BBSF and *B. longum* BL04 are included in the probiotic preparation VSL#3 (VSL Pharmaceuticals, FL, USA).

[b] Codon numbering system described by Telenti et al. [6].

[c] Wild-type/resistant nucleotide sequence (the mutated base is indicated in bold type).

[d] Wild-type/resistant amino acid (the mutated amino acid is indicated in bold type).

[e] These strains are designated *B. infantis* throughout the paper.