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Bifidobacterium longum W11: an antibiotic-resistant probiotic

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Preface

Possible unwanted consequences of antibiotic use include: (a) the selection of antibiotic-resistant pathogenic bacteria; (b) increased susceptibility of the host to new infections; (c) gram-negative bacterial overgrowth; (d) diarrhoea; and (e) *Clostridium difficile* colonization [1]. Theoretically, except for antibiotic resistance, all these effects could be alleviated with probiotics. However, even a small delay between antibiotic administration and supplementation with probiotics severely reduces the positive impact of the probiotics as they are unable to integrate into the gut microbiota. The high sensitivity of probiotics to antibiotics prevents stable colonization of the gut, thus ensuring only non-significant and transient effects. However, the use of antibiotic-resistant bacteria could be beneficial. Of course, for safety reasons, this resistance must not be transferable and must not be located in plasmid DNA as probiotics could otherwise be responsible for dangerous horizontal gene transfer (Fig. 1) to pathogens [2]. Antibiotic-resistant probiotics sound very attractive, even tempting pharmaceutical companies to falsely claim some probiotic strains have antibiotic-resistant properties. Indeed, a brochure recently suggested that physicians could use *Bifidobacterium longum* BB536 in conjunction with antibiotics. This clearly suggests that BB536 is antibiotic resistant, even though it is known to be susceptible to antibiotics [3]. The questions are then: do we have any antibiotic-resistant probiotic strains for use with specific antibiotics? Are we sure that these antibiotic-resistant probiotics cannot transfer their resistance to pathogens? The answers to both questions are yes. Some species of lactic acid bacteria commonly used in the food industry or naturally found in raw food are resistant to vancomycin and include *Lactobacillus casei*, L. rhamnosus, *L. curvatus*, *L. plantarum*, *L. coryniformis*, *L. brevis*, *L. fermentum*, *Pediococcus pentosaceus*, *P. acidilactici*, *Leuconostoc lactis* and *L. mesenteroides*. This vancomycin resistance found in lactobacilli, leuconostocs and pediococci is intrinsic, chromosomally encoded and not transferable [4]. Unfortunately, vancomycin is an antibiotic frequently used in hospitals, and is rarely (if at all) prescribed by family physicians. As it is often used in combination with other antibiotics such as linezolid and meropenem, having vancomycin-resistant probiotics is not that relevant.

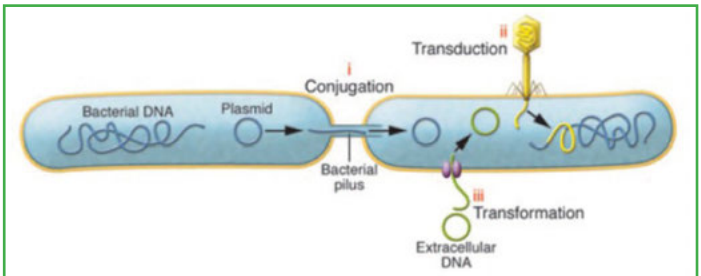

Figure 1 - Mechanisms for the acquisition of resistance genes. From: J Clin Invest 2014;124(10):4212–4218

Bifidobacterium longum W11

Bifidobacterium longum is a commensal bacterium present in the human gut. It is one of the 32 species belonging to the genus *Bifidobacterium*. It is an early colonizer of the gastrointestinal tract of infants and one of the major constituents of newborn intestinal microbiota, where it is predominant especially in the first 6 months of life. *Bifidobacterium longum* W11 (LMG P-21586) is of particular interest for use as a probiotic [5]. It tolerates low pH and is resistant to bile salts, two characteristics which allow it to reach and survive in the intestine. In addition, as is well known, it is important that probiotic bacteria are able to adhere to human intestinal

cells and then proliferate: adhesion enables probiotic strains to colonize the intestinal tract, stabilize the intestinal mucosal barrier, competitively exclude pathogenic bacteria, and provide improved metabolic and immune-modulatory activity. The W11 strain is able to colonize the gut and has impressive persistence (persistence indicates the length of time the strain is recoverable from faeces after wash-out). Indeed, several studies have shown that some strains of *Bifidobacterium* spp. can produce exopolysaccharides, sugar polymers which facilitate strong anchorage, and then create persistence, to intestinal epithelial cells (Fig. 2). Microscopy indicated that the W11 strain strongly adheres to human enterocytes (Fig. 3) and that the production of this exocellular polymers contributes to its adhesion and persistence properties [6]. Likely due to its production and release of exopolysaccharides, the W11 strain has been shown to be a strong colonizer also in severe conditions: in elderly patients on total enteral nutrition it increased the bifidobacterial count by more than 10-fold while simultaneously reducing the number of Clostridia [7]. In addition to its probiotic characteristics, the W11 strain also has important biological properties. From an immunity perspective, it seems to promote a Th1 response while lowering the Th2 response [8]. Clinically, the W11 strain has been shown to improve constipation in those following a low-calorie diet for the treatment of obesity [9], and to increase stool frequency (by 25% on average) in patients with constipation-variant IBS, reducing abdominal pain and bloating in those with moderate-severe symptoms [10].

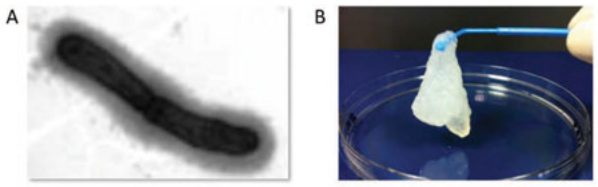

Figure 2 - Bifidobacterial exopolysaccharide is the grey area surronding the bacterium in A and the gel shown in B.
From: PLoS One 2016;11(9):e0162983

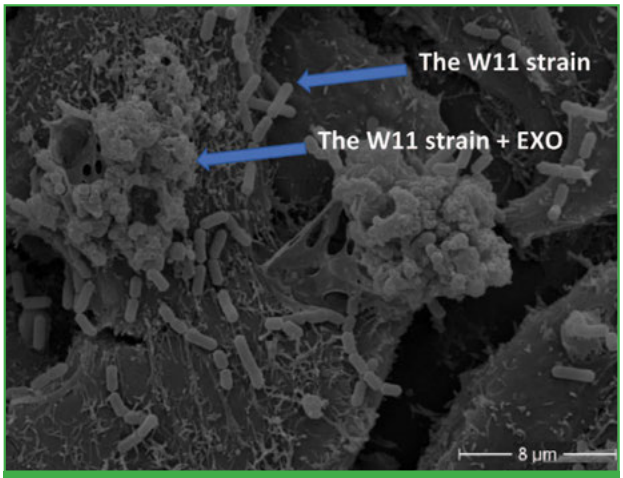

Figure 3 - Figure 3 W11 adhesion to enterocytes by exopolysaccharide (EXO)
Adapted from: Food Nutr Sci 2014;5:1787–1792

Resistance of W11 to rifaximin

It has recently been shown that the W11 strain is totally resistant to rifampicin, rifapentine, rifabutin and rifaximin at concentrations ranging from 32 to 256 mg/ml and is also partially resistant to the same drugs at a concentration of 512 mg/ml (Fig. 4) [11]. A mutation in the rpoB gene (DNA-mediated RNA polymerase subunit β) is responsible for this resistance and has already been described in *Staphylococcus aureus* and *Escherichia coli* [12, 13]. Analysis of W11 shows a chromosomal DNA mutation which causes a change in the triple of a specific amino acid (P564L) of the protein leading to resistance to rifamycin. In W11 the exact position of the rpoB gene on the chromosome was identified and a targeted search was conducted for transposable elements 200 kbp upstream and downstream of the rpoB gene, using TransposonePSI software. No transposable elements were identified, confirming that the rpoB gene is not flanked by mobile genetic elements [11].

Possible clinical uses of strain W11

In the last decade, the rifamycin-derivative rifaximin has been registered in many European countries and in the United States. It has attracted interest due to its pharmacological, toxicological and clinical characteristics. It has an excellent safety profile due to negligible intestinal absorption after oral administration [14]. Its wide antimicrobial spectrum covers gram-positive and gram-negative bacteria, including aerobes and anaerobes [15]. Rifaximin has been used successfully in the treatment of several intestinal disorders, including traveller's diarrhoea, diverticular disease, small intestinal bacterial overgrowth (SIBO), *C. difficile* infection, Crohn's disease, IBS, functional dyspepsia and hepatic encephalopathy. From a pharmacological perspective, rifaximin is not a bioavailable antibiotic and its mechanisms of action involve not only direct bactericidal activity but also alteration of the virulence factors of enteric bacteria, reduction of pathogen adhesion and internalization to the intestinal epithelium, and reduction of inflammatory cytokine release. Therefore, rifaximin could be used as a novel treatment for all those intestinal diseases mainly characterized by dysbiosis and inflammation. Consequently, a rifaximin-resistant probiotic

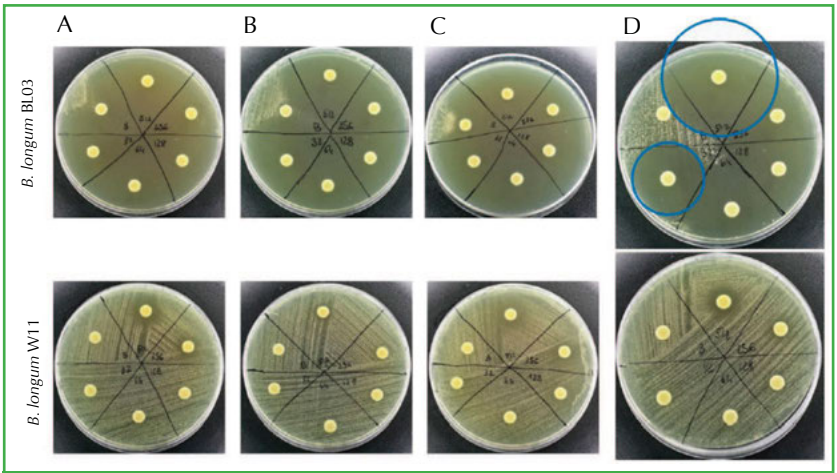

Figure 4 - W11 cultured in the presence of rifampicin (A), rifapentine (B), rifabutin (C) and rifaximin (D). The control test was performed with a susceptible strain (BL03). From: J Clin Gastroenterol 2016;50:S153–S156

strain, W11, could be used as adjuvant therapy when administered along with the antibiotic. Indeed, this association has been evaluated in IBS where patients administered rifaximin plus W11 reported greater improvement in symptoms than patients administered only rifaximin (plus placebo, of course) [16].

Conclusions

Bifidobacterium longum W11 is the first probiotic strain identified as having antibiotic-resistant properties. This characteristic is chromosomally based and not transferable. W11 can be safely used in combined therapy with rifaximin in conditions responsive to rifaximin and in dysbiosis. This would open new treatment approaches in the era of probiotics.

Conflict of interest

Francesco Di Pierro is owner of Velleja Research.

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