

Lactobacillus crispatus M247: a possible tool to counteract CST IV

Francesco Di Pierro¹, Alexander Bertuccioli², Daniela Cattivelli³, Sara Soldi³, Marina Elli³

Correspondence to: Francesco Di Pierro - f.dipierro@vellejaresearch.com

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Abstract

Bacterial CST (community state type) I is characterized by *Lactobacillus crispatus* dominance and is associated with a healthy vagina and a reduced risk of vaginosis, vaginitis, preterm birth, infertility and vaginal atrophy. On the other hand, CST IV is characterized by the absence of lactobacillus and is associated with unhealthy gynaecological conditions. Unfortunately, very few *L. crispatus* strains have been properly studied and documented for human use. Here we discuss the genetic, probiotic and vaginal colonization properties of strain M247, isolated in 1989 from infant faeces, and describe its possible gynaecological uses especially in woman characterized by CST IV.

Introduction

Culture-independent investigation of the human vaginal microbiota has revealed five distinct community state types (CSTs), four of which are mostly dominated by a single *Lactobacillus* species (CST I: *Lactobacillus crispatus*; CST II: *L. gasseri*; CST III: *L. iners*; CST V: *L. jensenii*), while CST IV is characterized by a paucity of *Lactobacillus* species and consists of a diverse group of facultative and strict anaerobes, including bacteria associated with bacterial vaginosis (BV) [1]. CST IV has been linked to BV, candidiasis, chlamydia, HPV persistence, *sine causa* infertility and spontane-

ous preterm birth. These conditions seem to be particularly correlated with CST IV, especially when in the presence of increased bacterial richness and vaginal microbiota diversity [2]. CST IV has also been associated with the menopause and vulvovaginal atrophy [3]. Research has revealed that the lactobacilli inhabiting the human vagina provide the first line of defence in the female urogenital and reproductive tracts. However, not all lactobacilli are equally protective and recent research indicates that of the four *Lactobacillus*-based CSTs, the vaginal microbiota CST I is most closely correlated with a healthy status, thereby suggesting *L. crispatus* as a biomarker of a healthy vaginal tract [4]. The healthy role played by *L. crispatus* seems to also affect urinary infections. As a matter of fact, eight different urotypes (UTs) have been described in humans, all of which are found in both men and women, except for UT 7, which occurs in healthy women only and has a relative abundance of *L. crispatus* [5]. Consequently, probiotics containing *L. crispatus* strains might be useful for prophylactic purposes in the uro-gynaecological context. Unfortunately, very few *L. crispatus* strains have been described in the scientific literature, and fewer than 30 genomes of this species have been sequenced and deposited in bacterial collections. However, one of these strains has recently been investigated carefully for possible use in gynaecological conditions.

Genetic features of *L. crispatus* M247

L. crispatus M247 (IDA: LMG-P-23257) was isolated in 1989 from the faeces of a healthy baby. Its genome is 2,112,063 bp (2.1 Mbp) long and it has 2,187 coding and 55 ribosomal genes [6]. The most abundant genes are those involved in carbohydrate metabolism (236 genes), followed by pro-

¹Scientific Department, Velleja Research, Milan, Italy

²D.I.S.B., Urbino, Italy

³Advanced Analytical Technologies, Piacenza, Italy

tein metabolism (203), DNA (107) and RNA (84) genes, and genes involved in cell wall and capsule biosynthesis (83). The strain appears to be safe as it does not demonstrate virulence factors and is unlikely to contain plasmids. From a phenotypic perspective, data confirm that the strain is responsive to all tested antibiotics, according to EFSA guidance. Analysis performed using CRISPRFinder identified four possible CRISPR (clustered regularly interspaced short palindromic repeats) in its genome; two of these CRISPR have been confirmed, while the other two are still under investigation. Genetic analysis also identified a gene encoding for a *Lactobacillus epithelium* adesin (LEA). This gene, previously characterized in *L. crispatus* ST1, was shown to play a major role in vaginal epithelium colonization and in determining competition with *Gardnerella vaginalis* [7, 8]. Moreover, two fibronectin-encoding genes (fibronectin type III domain and fibronectin-binding protein A N-terminus FbpA) were also identified in strain M247. These genes have been associated with the ability to colonize the vaginal mucosa and also contribute to controlling *Gardnerella vaginalis* through a competitive colonization mechanism [8].

The production of bacteriocins is an important feature of beneficial bacteria in helping combat pathogenic bacteria. At the gene level, a number of bacteriocins have been identified in strain M247: two helveticins, one penocin, two enterolysins and one bacteriocin of the LS2 group. These results are in agreement with previous analyses of other *L. crispatus* genomes and suggest possible exploitation of the strain for the control of microbial pathogens [8]. Although the phenotypic presence of bacteriocins released by M247 has not yet been demonstrated, antagonism against some uro-pathogenic strains of *Escherichia coli* has been observed as well as against *Staphylococcus epidermidis* but not against *Enterococcus faecium* [6]. As is well-known, *Lactobacillus* strains can produce different types of exopolysaccharides (EPS), facilitating bacterial adhesion and protecting against antibiotics and drying [9]. As regards strain M247, 18 genes encoding for enzymes of the glycosyltransferase group, which are involved in the biosynthesis of EPS, were identified [6].

This suggests that, in suitable environmental conditions, strain M247 can produce EPS.

Probiotic and colonizing properties of M247

Regarding its probiotic features, strain M247 survives in gastric juice (pH 3; 90 minutes), bile salts (0.5%; 48 hours) and

when subject to the pancreatin tolerance test (about 0.2%; 3 hours) losing 3 logs in acid conditions, 1 or 2 logs in bile medium (depending on the bile salts used) and increasing by 1 log in experimental pancreatic juice. Regarding its adhesion properties, strain M247 adhered, eight times better, to human ileostomy glycoproteins and to Caco2 cells (model of colonic epithelium) than did its spontaneous isogenic non-aggregating mutant (called MU5). Administered in double-blind conditions to healthy volunteers at a dose of at least 10 billion live cells for 8 consecutive days, the strain was demonstrated to be a strong colonizer as it was found in all faecal samples and in most biopsy samples [10]. These results (Table 1) were not observed when the strain was administered in poor/non-viable conditions (as shown by trial 2 in Table 1).

Biosynthesis and role of hydrogen peroxide produced by strain M247

Hydrogen peroxide production by vaginal lactobacilli was long thought to be an important defence mechanism against vaginal colonization by undesirable microorganisms. Its production and release by *L. jensenii* strains is especially strong, while varying among *L. crispatus* and *L. gasseri* strains [11]. M247 has been shown experimentally to be a very good producer of hydrogen peroxide (Table 2). However, recent papers [12, 13] have shown that, in contrast to lactic acid, hydrogen peroxide does not have a very strong killing ability, which has prompted some authors to consider as implausible the in vivo role of hydrogen peroxide as an antimicrobial agent produced by vaginal microbiota [14]. It has been recently observed that *L. crispatus* M247 instead uses hydrogen peroxide as a signal transducing molecule to induce PPAR-γ activation in intestinal epithelial cells, directly modulating epithelial cell responsiveness to inflammatory stimuli [15]. The same mechanism increases the amount of TLR-2 while reducing the amount of TLR-4 [16], thereby improving the strength of the tight junctions and reducing the ability of

Trial	Number of subjects in the tested group		Number of subjects with <i>L. crispatus</i> identified on day 8			
	M247	MU5	Faeces		Biopsies	
	M247	MU5	M247	MU5	M247	MU5
1	5	5	5	ND	2	ND
2	3	3	ND	ND	ND	ND
3	2	0	1		1	

Table 1 - Results of human colonization trials with *Lactobacillus crispatus* strain M247 and its non-aggregating isogenic mutant MU5

Incubation time (hours)	H ₂ O ₂ released (mg/10 ³ CFUs)						
	<i>L. johnsonii</i> ATCC 33200T	<i>L. gasseri</i> DSM 20243T	<i>L. fermentum</i> DSM 20052T	<i>L. plantarum</i> ATCC 21028	<i>L. paracasei</i> NCDO 151T	<i>L. crispatus</i> M247	<i>L. crispatus</i> MU5
1	22.10	15.67	0.18	0	0.00	32.02	47.44
2	24.50	17.04	0.17	0.06	0.03	32.15	35.33
4	30.16	22.56	0.15	0.06	0.06	37.39	8.33
6	35.22	25.98	0.15	0.07	0.06	42.44	9.27
8	37.40	28.55	0.13	0.13	0.08	41.69	7.98

Table 2 - *Lactobacillus crispatus* strain M247 released hydrogen peroxide in final concentrations higher than those measured for other producing strains

Gram-negative LPS-endowed bacteria to determine TNF- α -mediated inflammatory processes (Fig. 1).

This anti-inflammatory action exerted by M247 after colonization is evident when tested in a model of murine experimental colitis [17] where the strain can reduce most signs and symptoms of the condition (Fig. 2).

Vaginal presence after oral use of M247

As lactobacillus can be transferred from the gut to the vagina, it is thought this is also possible following probiotic administration of a mixture of lactobacilli. The literature indicates that oral administration of lactobacillus is required for 4–5 weeks before any effects on the vagina are seen [18]. We therefore evaluated the ability of M247 to colonize the vagina after oral administration of 10 billion live cells daily for 2 weeks to healthy women. Preliminary results from PCR analysis showed that M247 was present in the colon of 70% of subjects and in the vagina of 40% of subjects (Fig. 3). Other tests have further demonstrated that strain M247 can be recovered alive from the vagina of almost 100% of subjects following topical application for only 3 days by vaginal douche, suggesting M247 can colonize the va-

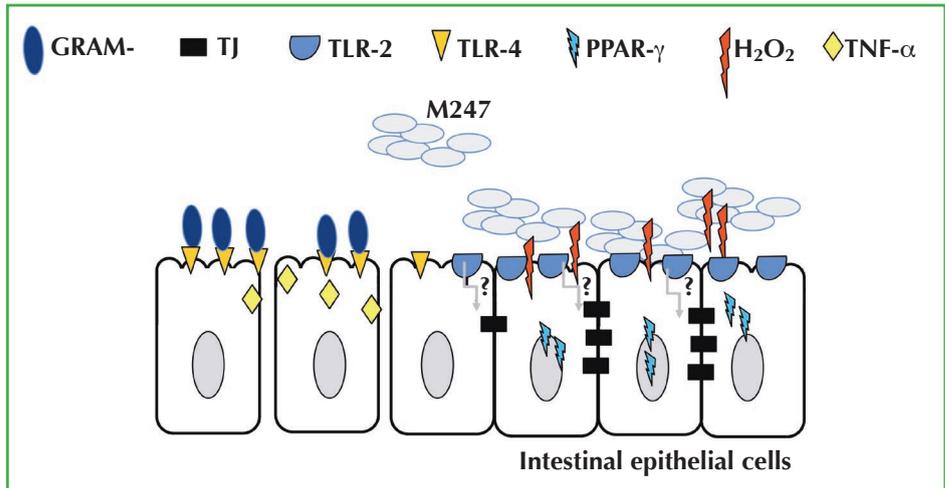


Figure 1 - *Lactobacillus crispatus* strain M247 releases hydrogen peroxide which induces up-modulation of PPAR- γ in intestinal epithelial cells. As a direct consequence, TLR-2 increases, TLR-4 decreases and tight junctions become more structured. These events together produce an anti-inflammatory response

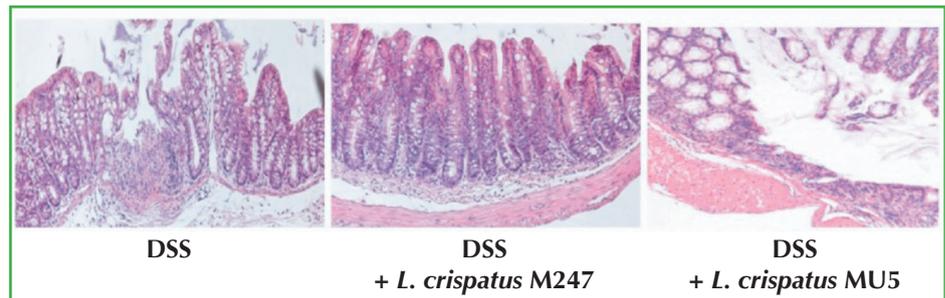


Figure 2 - Beneficial effect of *Lactobacillus crispatus* strain M247 on a murine experimental model of colitis induced by dextran sodium sulfate (DSS). Reproduced from Gastroenterology 2008;135(4):1216-1227

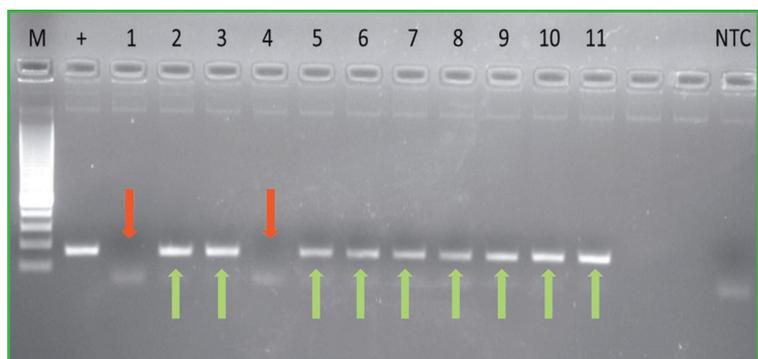


Figure 3 - *Lactobacillus crispatus* strain M247 in the vagina after oral administration. Green arrows indicate positive sampling, while red arrows indicate the negative sampling

gina very quickly using this method. A pilot trial evaluating vaginal colonization after 3 and 4 weeks of oral use of M247 is currently ongoing and results will soon be available.

Conclusions

L. crispatus M247 is one of the few *L. crispatus* strains which have been properly documented for human use. A good colonizer of the human gut, where it exerts strong anti-inflammatory effects, and the human vagina, where it combats dysbiosis, the strain could be used orally and/or topically to restore the normal vaginal microbiota. The current literature suggests strain M247 can be used in:

- 1) women with CST IV;
- 2) women with CST II, CST III or CST V and recurrent infections, a risk of preterm birth or *sine causa* infertility;
- 3) women with persistent HPV;
- 4) women with an HIV-positive partner or engaging in risky sexual behaviour;
- 5) women undergoing the menopause and/or with initial signs of vaginal atrophy;
- 6) women in whom other therapies against recurrent urinary infections have failed.

Conflict of interest

Francesco Di Pierro is the owner of Velleja Research.

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