

ACTA MICROBIOLOGICA BULGARICA



Short Communication

Antibacterial activity of Lactobacillus bulgaricus G-LB-44

Andrew B. Onderdonk¹, Rositsa Tropcheva^{2,3}, Haralabia Boleti⁴, Petko Petkov⁵

¹Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA;

²Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Acad. Sci., 26, Acad. G. Bontchev, str, 1113, Sofia, Bulgaria;

³Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski, 8, Dragan Tsankov Blvd., 1164, Sofia, Bulgaria.

⁴Intracellular Parasitism group & Light Microscopy Unit, Hellenic Pasteur Institute, 127 Vas. Sofias, 11521 Athens, Greece;

⁵ProViotic AD, Bratia Buckston 58, Sofia, Bulgaria.

Lactobacillus bulgaricus G-LB-44 was screened for antibacterial activity against (i) different pathogenic species - Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Acinetobacter baumanii, Enterococcus faecalis, Pseudomonas aeruginosa and (ii) 16 lactic acid bacteria strains and 2 commercially available probiotics using plate counting. All counts were recorded as colony-forming unit per millilitre (CFU/mL). Lactobacillus bulgaricus inhibition of H. pylori has been initially manifested by Boyanova et al. (2009).

The results showed that *Lactobacillus del-brueckii* subsp. *bulgaricus* G-LB-44 could reduce the growth of potentially harmful bacteria that cause diseases in humans. In most cases, the reduction in the numbers of pathogenic bacteria was greater than 99%. The broad based activity of *Lac-*

tobacillus delbrueckii subsp. bulgaricus G-LB-44 is unusual among Lactobacillus species and suggests that there are unique cellular components that may account for this activity. Such compounds are common in nature, however they tend to be directed to very specific strains of other bacteria that compete for nutrients with the probiotic bacteria in a natural setting such as the gastrointestinal tract. It is, therefore, unusual to find a single strain of Lactobacillus that produces an inhibitory effect for a broad array of harmful bacteria, since these bacteria would rarely be found at the same time within the same natural setting. Moreover, with regard to other lactic acid bacteria strains and commercial probiotics, no inhibitory activity of Lactobacillus delbrueckii subsp. bulgaricus G-LB-44 was observed. The study confirms that G-LB-44 is a specific strain

Table 1. Pathogen inhibition by L. bulgaricus G-LB-44

Organism	Strain	Inhibition rate; [%]
Acinetobacter baumanii	ATCC 19606	>99
Enterococcus faecalis	ATCC 29200	>99
Enterococcus faecalis	ATCC 29212	>99
Enterococcus faecium	ATCC 51559	>95
Esherichia coli	ATCC 25922	100
Esherichia coli	ATCC 35150	>99
Listeria monocytogenes	ATCC BAA-751	100
Listeria monocytogenes	Clinical Isolate	100
Psuedomonas aeruginosa	ATCC 27853	100
Salmonella typhimurium	Clinical isolate	100
Shigella sonnei	ATCC 325931	100
Staphylococcus aureus	ATCC 12600	>99
Staphylococcus aureus	ATCC 25923	>99
Staphylococcus aureus	ATCC 29213	>98

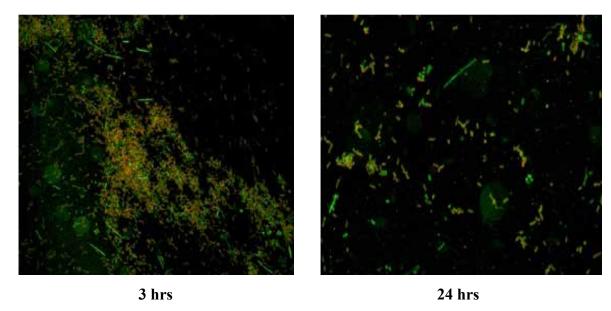


Fig. 1. Comparison of the whole biofilm structure and the cell density at different time points of the test

of the *L. bulgaricus* and that its inhibitory power against pathogens surpasses other *L. bulgaricus* strains. G-LB-44 is an example of an effective probiotic with suitable scientific substantiation of health benefits.

Lactobacillus bulgaricus G-LB-44 is able to significantly decrease the cell density of *E. coli* biofilm as well as to destroy completely the *E. coli* biofilm structure within a 24 hourperiod as demonstrated in Figure 1, in images obtained by confocal laser scanning microscopy.

E. coli biofilms growing on glass slides were incubated with L. bulgaricus G-LB-44 for 3 and 24 hrs. The bacteria populations were stained with Live/Dead viability kit (Invitrogen) at the end of the incubation period, following the manufacturer's instructions. Fluorescence microscopy images were acquired by a Leica CLSM TCS-SP confocal microscope with a 63.0 X lense. Green short cells: live E. coli; red short cells: dead E. coli; green long cells: live L. bulgaricus G-LB-44.

Since the sub-species of *Lactobacillus bulgaricus* has been safely used in foods for over 100 years with no indications of overdose or side effects, we

believe that the above results indicate the potential of this strain to be used both as a natural preservative capable of inhibiting pathogens in unpasteurized food and as away to replenish the healthy bacteria found in the human digestive system.

Acknowledgements

The authors thank Prof. Angel S. Galabov, MD, DSc for the special contribution and guidance during this study, Genesis Laboratories for providing their proprietary strain of *L. bulgaricus* G-LB-44 for the purpose of this study, the Section of Morphology of Microorganisms and Electron Microscopy of the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences for the preparation of the biofilm samples and the Light Microscopy unit of the Hellenic Pasteur Institute, Athens, Greece for the production of the microscopy images.

References

Boyanova, L., M. Stephanova-Kondratenko, I. Mitov (2009). Anti-Helicobacter pylori activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* strains: preliminary report. *Lett. Appl. Microbiol.* **48**: 579-584.