DOI: 10.46991/JISEES.2025.SI1.035

Identification of Vaginal Lactic Acid Bacteria Using Classical Microbiological Techniques and MALDI-TOF MS

Ruzanna Harutyunyan[1], Tigran Aleksanyan[1*], Inesa Semerjyan[1], Edita Gharibyan[2], Margo Cnockaert[3], Peter Vandamme[3], Inga Bazukyan[1]

[1] Faculty of Biology, Yerevan State University, Yerevan, Armenia
[2] Department of Pregnancy Pathology, Erebuni Medical Center, Yerevan, Armenia
[3] Faculty of Science, Ghent University, Ghent, Belgium

ABSTRACT

The presence of lactic acid bacteria (LAB) in the vaginal microbiota is fundamental for reproductive health, primarily due to their role in sustaining an acidic pH and providing a defense against urogenital pathogens. The aim of this study was to identify six bacterial isolates (G 4.4 G 13.1, G 21.K.2, G38.0.1, G42.10.2 and G46.M.3) from the vaginal microbiome that had previously demonstrated strong antagonistic activity. The identification of the studied isolates was performed using classical bacteriological methods based on the analysis of their morphological, physiological, and biochemical characteristics. MALDI-TOF MS analysis was conducted in duplicate using the Bruker Microflex® LT/SH smart system (Bruker Daltonics, Bremen, Germany). All tested strains showed limited growth on the surface of MRS agar and produced lenticular or round, milky-white colonies measuring up to 1 mm in diameter within the depth of the agar. Research indicates that the optimal temperature for growth of the studied strains is generally 37°C, whereas strains G 13.1 and G46.M.3 exhibit optimal growth at 33°C. None of the isolates synthesized indole or deaminated arginine. All were negative for oxidase, catalase, urease, and amylase activities, exhibited homofermentative metabolism, and tested positive for gelatinase. The G46.M.3 isolate exhibits lipolytic and caseinolytic activities. The isolates demonstrated the ability to ferment a wide range of carbohydrates. According to MALDI-TOF MS analysis, strain G 4.4 was identified as *Lactobacillus delbrueckii* subsp. *lactis*, G 13.1 as *Lacticaseibacillus rhamnosus*, G 21.K.2 as *Lactobacillus delbrueckii* subsp. *lactis* DSM 20355, G 38.0.1 as *Lacticaseibacillus paracasei*, G 42.10.2 as *Lacticaseibacillus rhamnosus*, and G 46.M.3 as *Enterococcus faecalis* 20247_4 CHB. These results enhance the understanding of vaginal microbiota composition and highlight specific LAB with potential probiotic properties. Their demonstrated antagonistic activity suggests a promising role in supporting vaginal and reproductive health.

Keywords: vaginal microbiome, lactic acid bacteria, antibacterial activity, MALDI-TOF MS

Acknowledgement: The work was supported by the Science Committee of RA, in the frames of the research project No. 25RG-2I009 and No. 23AA-1F010.

References:

1. Neugent, M.L.; Hulyalkar, N.V.; Nguyen, V.H.; Zimmern, P.E.; De Nisco, N.J. Advances in understanding the human urinary microbiome and its potential role in urinary tract infection. *mBio* **2020**, *11*, e00218–220. DOI:10.1128/mBio.00218-20
2. Wieme, A.D.; Spitaels, F.; Aerts, M.; De Bruyne, K.; Van Landschoot, A.; Vandamme, P. Identification of beer-spoilage bacteria using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Int. J. Food Microbiol.* **2014**, *185*, 41–50. DOI:10.1016/j.ijfoodmicro.2014.05.003

***Corresponding Author:**

Tigran Aleksanyan, Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 1 Alex Manoogian str., Yerevan, 0025, Armenia.
Email: tigran.aleqsanyan@ysu.am