

## Nonculturable forms of bacteria in lyophilized probiotic preparations

<sup>1</sup>Larisa Blinkova, <sup>2,3</sup>Danik Martirosyan, <sup>1</sup>Yury Pakhomov, <sup>1</sup>Olga Dmitrieva, <sup>2</sup>Rachel Vaughan, and <sup>1</sup>Michael Altshuler

<sup>1</sup>FGBU “Mechnikov Research Institute of Vaccines and Sera”, Russian Academy of Medical Sciences, Moscow, 105064 Russia; <sup>2</sup>Functional Food Center Inc., Richardson, TX 75390, USA; <sup>3</sup>Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390, USA

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**Corresponding author:** Larisa Blinkova, PhD, FGBU “Mechnikov Research Institute of Vaccines and Sera”, Russian Academy of Medical Sciences, Moscow, 105064 Russia

### **ABSTRACT**

**Background:** Nonculturable cells are formed under stress. These viable but nonculturable (VBNC) cells retain the ability to revert to active growth and division when conditions become favorable, or after treatment with resuscitating factors. Information about the possible presence of VBNC in bacterial lyophilized probiotic preparations, foodstuffs, live vaccines, etc., indicates that human as well as animal intestines are a significant area for research.

**Methods:** Samples were stored for different periods of time (up to 30 years) according to the manufacturers’ manuals. Total counts were conducted using the Goryaev-Thoma counting chamber and actual viability was assessed by luminescence microscopy after staining with Live/Dead® (Baclight™). CFU/ml counts were made using solid or semisolid media. Viable cells that lacked the ability to form colonies were considered VBNC.

**Results:** We studied 11 batches of commercial probiotics (Russia) from different sources, containing lyophilized *E. coli*, lactobacilli, or bifidobacteria, in ampoules or vials. In *E. coli* preparations, depending on storage periods, the amounts of VBNC varied from 4.1% (3 years) to 99.7% (30 years) and showed different total viability (52.2 – 91.3%), as well as the percentage of VBNC cells. A different sample that had been expired for 11 years was 79.5% NC. It is also noteworthy that the 5-dose vials, 4 years past expiration, from yet another source, showed a higher amount of VBNC cells (85.5%). Two different batches that had been expired for three years contained 4.1 and 21.3% VBNC cells. 4 of the 5-dose vials of lyophilized lactobacilli were not expired and contained 58.8 – 80.4% VBNC cells. Total viability varied from 92.9 to 100%, and there was an unmistakable positive correlation between total viability and culturability. The last batch, which had expired 6 years earlier, has 23.7% viable cells and about 98% VBNC. Non-expired bifidobacterial samples contained 70.7 and 95.5% of viable cells and were 50 and 100% culturable.

**Conclusion:** We demonstrated the presence of VBNC cells in lyophilized probiotic preparations

that contained live bacteria. Probiotics stored past their expiration date may retain a high potential medical effect because they contained high numbers of viable cells. VBNC cells in studied preparations may have the potential to return to an actively growing state.

**Keywords:** nonculturable forms of bacteria, probiotics