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Long-Term Stability of *Staphylococcus Aureus* and *Cutibacterium Acnes* Bacteriophages in Ethanol Based Solutions and Selected Buffers

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Extended Abstract

Phages, or bacteriophages, are viruses that infect bacteria and hold great potential for applications in medicine, biotechnology, and food safety. Understanding their stability under various conditions is crucial for ensuring efficacy and proper storage in both industrial and clinical settings.

This study investigates the long-term viability of two Staphylococcus aureus (myovirus) phages and Cutibacterium acnes (siphovirus) phage—used in some of the company's products—in ethanol solutions. As ethanol is one of the most commonly used preservatives, solvents, and stabilizers in cosmetics, pharmaceuticals, and research, it is essential to determine whether bacteriophages can be effectively incorporated into ethanol-based formulations without compromising their activity. While previous studies have evaluated phage viability in organic solvents, including ethanol, they have primarily focused on short-term exposure to high concentrations [1; 2] or on ethanol's use as a disinfectant [3]. Few studies have addressed the effects of long-term storage of phages in ethanol solutions sofar.

All three phages are part of the company's bacteriophage collection, propagated in the appropriate bacterial hosts, and purified and concentrated using tangential flow filtration (TFF) with deoxycholate. For each phage, two markedly different titers (10^7 and 10^{10} PFU/mL) were prepared to evaluate the effect of initial titer on stability during storage. The purified phages were stored at 4 °C in Tris-Cl buffer with varying ethanol concentrations (20%, 30%, 40%, and 50% (v/v)). Phage viability (PFU) was assessed at regular intervals using the double agar overlay plaque assay over a period of at least five months. In parallel, phage stability in commonly used biological buffers was also examined.

Preliminary data suggest that all tested phages are generally tolerant to lower ethanol concentrations, consistent with previous findings, and in some cases, ethanol may even enhance stability. At higher ethanol concentrations, however, results varied between phages and also depended on the initial titer.

Understanding the long-term survivability of phages in ethanol and other solvents contributes not only to the development and quality control of the company's product portfolio but also highlights the importance of such efforts for advancing future therapeutics and cosmetic formulations, where sustained phage activity is essential.

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