PHAGEinLYON / PHAG-ONE : Optimization of pharmaceutical production of anti-S. aureus therapeutic phages using in silico and experimental approaches

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Résumé

Introduction. Treatment of multi-drug resistant bacteria is a major health challenge for the next decade. Phage therapy is a promising therapeutic alternative to antibiotics and the "Hospices Civils de Lyon" (HCL) have acquired a significant clinical and microbiological experience in this field. Through PHAGE*in*LYON program, and PHAG-ONE project, HCL are implementing the first public platform for production of therapeutic phages. After one year of development, a bank of 17 phages anti-*S. aureus* was built and 3 of these phages were selected for their high therapeutic potential. The development of pharmaceutical production processes of these 3 therapeutic phages must address several major challenges: i) the risks associated to the use of a pathogenic bacterial strain for phage production inducing potential contamination of therapeutic products by virulence/resistance factors or lysogenic phages, ii) the need for high production yield suitable for clinical use. Here, we report the development of these processes in compliance with regulatory agency requirements.
Methods.

i) We performed an *in silico* analysis of bacterial genomes of the large collection (n> 2000 genomes) of the Centre National de Référence des Staphylocoques using a proprietary algorithm/software to select the strains best suited for phage production by excluding all bacteria harboring major virulence /resistance factors and temperate phages.

ii) Experimental parameters (bacterial growth media, inoculum ratio phage/bacteria, kinetic of phage production) were optimized in a pharmaceutical scale up.

*Intervenant

Results.

i) With the *in silico* approach, we selected 6 *Staphylococcus aureus* candidate strains, over more than 2000 clinical strains. Only 3 of them allowed to reach an amplification yield acceptable for pharmaceutical production.

ii) Production optimization experiments allowed us to identify amplification conditions in a medium usually used to industrially produce recombinant proteins, to obtain phage titers $\geq$ 1.1011 PFU/mL in only 4 hours for the 3 selected therapeutic phages.
Conclusion. We report here the first steps of the pharmaceutical process for therapeutic phage production in compliance with regulatory requirements. The next ongoing step is the development of purification processes and specific quality controls to ensure the safety of therapeutic phages.

Mots-Clés: Phage production optimization yield safety Staphylococcus