

Bacteriophages

Receptor-binding Proteins

Diagnosis

Viral proteins to improve the detection of secondary bacterial infections (SBI) associated to COVID-19

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Bacteriophages (phages) are characterized for their high specificity being able to discriminate their host up to the strain level. This feature is largely dependent on specific structural proteins encoded on the phage's genomes. These proteins recognize specific receptors on the bacterial cell surface and are known as phage receptor binding proteins (RBP). The ability to specifically recognize and bind to certain bacteria make RBP valuable biorecognition elements with high potential for the development of new diagnosis methods.

Considering the slow turnover of the conventional culture methods and the limitations of the immune and molecular assays, it is crucial and urgent the development of new diagnostic methodologies able to rapidly and accurately detect and identify the etiological agent of important bacterial infections. This is exacerbated in COVID-19 patients for which a high rate of deaths was attributed to secondary bacterial infections (SBI).

Through bioinformatics and functional analysis we identified RBP encoded in the genome of two lytic phages. These two RBP were able to specifically recognize and bind to 2 of the most important bacteria responsible for SBI associated with COVID-19: *Pseudomonas aeruginosa* and *Staphylococcus aureus*. By fusing the RBP to different fluorescent proteins we developed a method to detect and identify these bacteria in multiplex through epifluorescent microscopy and spectrofluorimetry. Fusion of the RBP to the NanoLuc luciferase improved the limit of detection 100 times when compared with the fluorescent proteins.

This new methodology was tested against more than 200 bacteria isolated from COVID-19 patients with a specificity of 100% and 90%, and a sensitivity of 44% and 96%, against *P. aeruginosa* and *S. aureus* respectively.

In conclusion, we developed here a new methodology based on viral proteins able to fast and accurately detect *P. aeruginosa* and *S. aureus* that will improve diagnosis of SBI associated with COVID-19.