Species-Specific Bacteriophage Amplification for Enhanced MALDI-TOF MS Bacterial Diagnostics

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ABSTRACT

Effectively negating the need for overnight bacterial enrichment required by most conventional diagnostic methods.

Background. Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) is emerging as a sensitive approach for rapid bacterial diagnostics. Most bacterial identification techniques (conventional MALDI-TOF MS included) rely on toxic culture practices and/or costly molecular methods, which render them impractical for rapid bacterial identification. With a near-tolerable assessing some of these shortcomings, we employed the evolutionarily-conserved interactions between a bacteriophage (phage) and its bacterial host to develop a new, rapid, and species-specific diagnostic methodology.

Methods. Bacteriophage phage MS2 and plague diagnostic phage A1122 were used as well-characterized biomarkers for identification of E. coli and Y. pestis, respectively. Infections were monitored by MALDI-TOF MS over a 5-hour period to assay for an increase in phage major capsid protein concentrations in comparison to bacterial concentrations as a means of signal amplification. This allowed for production of phage-specific protein mass profiles and output. Further, phage infections were modeled in silico using a modified Payne and Jansen algorithm to predict the approximate time during which peiphage MS2-phage amplification would begin and expand beyond the MALDI-TOF MS limit of detection (LOD).

Results. MS2-derived signal amplification resulting from infection of 5x10^6 cfu/ml E. coli, a bacterial concentration that was initially below the limit of detection, was detectable within one hour. Similarly, a A1122 amplification signal resulting from infection of 5x10^6 cfu/ml Y. pestis was detectable within two hours. A modified computational model for predicting phage concentration as a function of time and significant agreement between mathematically calculated growth curves and those experimentally obtained by MALDI-TOF MS was obtained.

Conclusions. Novel and reproducibly predictable phage-based signal amplification readily allowed for MALDI-TOF MS bacterial detection and identification in vivo as one hour for E. coli, and within two hours for Y. pestis. Overall, this event led to the need for overnight bacterial enrichment required by most conventional diagnostic methods.

INTRODUCTION

• MALDI-TOF MS is a useful tool for rapid bacterial detection and identification.
• Several shortcomings have been identified with regards to its relative sensitivity and need for bacterial samples grown under strict conditions. Indeed, while a majority of bacterial monomers as biomarkers are often detectable by MALDI-TOF MS on their own, this requires time-intensive overnight culture under controlled growth conditions.
• To address these issues, highly species-specific phage amplification was applied and determined to significantly improve the overall utility of MALDI-TOF MS for bacterial analysis, effectively shortening detection times by removing the need for overnight bacterial enrichment, while at the same time lowering the amount of bacteria required for detection.

To this end, the real-time monitoring of phage amplification was performed with an initial phage concentration ranging from 10^2 to 10^7 pfu/ml and was determined using a modified computational model of the Mal-threads, phage and culture conditions.

Bacterial strains, plaque, and culture conditions

• Bacterial strains were utilized for a variety of MALDI-TOF MS in bacteriologic diagnostics can be problematic, particularly when investigating extensive time-course studies requiring multiple simultaneous analyses.

In order to order the requirement for repeated parallel analyses, a modified phage therapy model was investigated as a means of predicting the time during which a phage infection when a detectable signal would occur.

Phage amplification is the process whereby a burst of phage proteins are produced as a result of a species-specific infection and replication in a bacterial host.

When applied in an in vitro fashion, this event leads to an exponential increase in the number of detectable phage proteins in an infection reaction.

This increase in phage proteins in comparison to bacterial heat concentration can be exploited using MALDI-TOF MS as a method for signal amplification during bacterial detection and identification.

METHODS

Bacteriophage amplification

• Phage amplification was observed to increase the utility of MALDI-TOF MS-based bacterial detection by eliminating the need for extensive culture prior to analysis.

• A phage amplification-mediated detection of E. coli was reproducibly and predictably observed within 1 hour of infection.

• Using a modified phage therapy modeling algorithm, the point in time at which phage concentrations exceeded MALDI-TOF MS LOD could be accurately, and reproducibly predicted prior to experimentation.

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