Establishment of the staphylococcal bacteriophage collection characterized by a reticulometric classification system in the Czech Collection of Microorganisms (CCM)

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Introduction

Staphylococcal bacteriophages of the family Siphoviridae have been widely used in shape typing of human strains of Staphylococcus aureus subsp. aureus as well as in fundamental genetic studies of this species. Prophages integrated in the staphylococcal chromosomes are the most widespread mobile genetic elements and they play an important role in pathogenicity of this species, changing its phenotype as a result of lysoytic conversion associated with production of virulence factors such as enterotoxins, exfoliative toxin A (ETA), Panton-Valentine leukocidin (PVL) and immune evasion factors and/or mediate generalized transduction [1].

The bacteriophage genes are organized into functional genomic modules. A genomic module found in a phage can be replaced in another phage by a sequence-unrelated module that frequently fulfills similar function. In the recent work we have designed a multiplex PCR strategy for typing the major modules of the staphylococcal siphophage genome and the complete characterization of S. aureus family Siphoviridae. The nine PCR assays designed for the sequences distinctive for 50 module types were designed to be capable to identify the bacteriophage gene pool present in phage genomes. Here we propose updating the phage classification describing reticulate relationships among phages to better reflect the modular structure and extensive mosaic pattern of their genomes. Although, the reticulate approach was already abandoned for general phage taxonomy it is excellent for diagnostics and classification of S. aureus siphophages [2].

Given the great biological and clinical importance of staphylococcal siphoviruses, the staphylococcal bacteriophage collection was established in 2010 by extending the CCM in the Czech Republic. About forty S. aureus siphophage strains coding for ETA or PVL, phages exhibiting transducing properties or applicable for phage typing were characterized in detail up to now and being maintained.

Material and Methods

Bacteriophage strains. Thirty-three Staphylococcus aureus bacteriophages of the International Typing Set (a) and nine induced PLV- converting phages (b) were selected from the collection of the National Collection of Type Cultures (Health Protection Agency, London, UK) and from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. The bacteriophages were stored at 4°C. Staphylococcus aureus strains of different serotypes (S. aureus methicillin-resistant strain MRSA 252, MRSE 305, MRSA 306, S. aureus USA 300 strain MW2, S. epidermidis CCM 7732, S. epidermidis CCM 7731, S. epidermidis CCM 7734 and S. epidermidis CCM 7735) were used as recipient strains for lysogenization with phages of the International Typing Set (a) and nine induced PLV- converting phages (b). Phages were induced from fresh cultures of recipient strains with chloroform in a ratio of 1:10000.

Phage isolation. Phages were isolated from long-term stored stocks by DNA isolation. Tenfold washes of crystals isolated in 10 ml of bacterial cells were made with 10 ml of sterilized water. Then, the resulting phages were directly used for DNA extraction. DNA was isolated using Qiagen kit. DNA was analyzed by agarose gel electrophoresis.

Phage propagation. The bacteriophage strain data was designed to allow storage a variety of data and cross-reference with that concerning propagating, selection, host and lysogenic bacterial strains records in MINE (Microbial Information Network Europe). The database system for computer storage and handling bacteriophage strain data has been developed on the basis of standardized PVT format.

Conclusions

a. Multiplex PCR based multilocus diagnostic scheme convenient for rapid and reliable S. aureus phage and prophage classification and for the study of bacteriophage evolution has been established.

b. Modular genome structure of more than 40 S. aureus siphophages was characterized.

c. The database system for computer storage and handling bacteriophage strain data has been developed on the basis of standardized PVT format.

References


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Fig. 2. Experimental characterization of modular genome structure of the International Typing Set (a) and nine induced PLV- converting phages (b).

Fig. 3. Sample of record documenting the genetic modular structure of S. aureus phage 11 in the CCM internal database.

Acknowledgements

We gratefully acknowledge the financial support of the Czech Science Foundation (3100606495) and Ministry of Education, Youth and Sports of the Czech Republic (MSHI0021622415 and MS00021622416).

Fig. 1. Agar gel electrophoresis showing multiplex PCR patterns for the bacteriophages in genome sequencing and control S. aureus strains. (A) multiplex PCR assay 1 for the integrase locus; (B) multiplex PCR assay 2 for lytic control region; (C and D) multiplex PCR assays 3 and 4 for DNA replication module; (E) multiplex PCR assay 5 for transcription regulation module; (F) multiplex PCR assay 6 for DNA packaging module of serogroup B phages; and (G) multiplex PCR assay 9 for phage typing module.

Fig. 3. Sample of record documenting the genetic modular structure of S. aureus phage 11 in the CCM internal database.

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