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Rapid detection of bacteriophage infection and prophage induction using electric biochips

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Background

Bacteria are widely used hosts for production of many biotechnologically important substances. Bacteriophages are viruses that infect bacterial cells. Thus, infection of bacterial cultures by bacteriophages may lead to serious problems, including complete loss of a desired bioproduct and spreading of bacteriophages throughout the whole laboratory. Although phage contamination, and resultant phage infection of bacterial cultures, may cause serious problems in all types of microbiological laboratories, it is especially dangerous when cultivations are performed on a large scale. Moreover, a number of commonly used strains of *E. coli* contain lambdoid prophages that often bear some regulatory genetic elements useful in the control of the expression of cloned genes. However, under certain conditions a prophage induction occurs that may have similar effects on a bacterial culture as phage infection. Even under standard cultivation conditions, a spontaneous prophage induction occurs with low frequency. However, this rare prophage induction results in appearance of infecting phage particles in amounts ranging from 10^{-8} to 10^{-5} pfu (plaque forming units) per bacterial cell. These numbers seem to be low, but when cultivations are performed on a large scale, e.g. reaching 10^{10} cells per ml, this means from 10^{2} to 10^{5} phages per ml. Considering even a very small bioreactor containing one litre of the culture, this adds up to 10^{8} infecting phage particles. If we consider a 100-litre bioreactor, the number of phages in the medium may reach 10^{10}. The potential (but, in fact, very real) problems described above, indicate a need for rapid detection of phage infection or prophage induction. However, using traditional methods we can detect the presence of phages in a bacterial culture unambiguously after several hours after infection, at best. Unfortunately, this is usually too late to save at least a part of the infected culture, and to avoid phage spreading throughout the laboratory. Therefore, it seems that development of new methods for rapid detection of bacteriophages in bacterial cultures became crucial.

Results

We present novel methods for detection of bacteriophages, which are based on the use of electric biochips. The principle of this method is to capture the target molecules (either nucleic acids or proteins) on the chip, and the use of the secondary detection probe which is coupled with an enzyme catalyzing a red-ox reaction. The electric signal appearing as a result of this reaction is then measured by an micro-electrode. This method gives relatively quick and quantitative results. Two kinds of electric biochips were used. One was desired for detection of bacteriophage genetic material (with DNA probes), and the second was desired for detection of phage virions (with specific antibodies). In both cases, we were able to detect the presence of bacteriophages (phages lambda and its derivatives, M13, P1 and T4 were used as models in this study) in amounts between 10^{4} to 10^{7} particles/molecules within as short time as 25–50 min (the values differed depending on the specific method used) from sample withdrawal. The results were quantitative in a wide spectrum of concentrations of bacteriophage DNA and virions.

Conclusion

We suggest that electric bio-chips may provide a potentially useful technique for rapid and quantitative detection of the presence of bacteriophages and for monitoring bacteriophage infection and prophage induction. It is worth noting that very often recurrent infections with the same phage occur in particular laboratories. This is because many virions can survive in a laboratory even in a dry form. Thus, although for detection of phage DNA or virions by means of electric bio-chips it is necessary to prepare specific DNA probes or specific antibodies, this method may be especially useful in the case of recurrent infections with the same bacteriophage. In such a case single isolation of the bacteriophage strain should be enough to prepare specific probes and/or serum.

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