Microbial Cell Factories

Poster Presentation

A simple emergency procedure to be used if biotechnological protein production is endangered by bacteriophage infection of *Escherichia coli* cultures: effective inhibition of bacteriophage lytic development in infected cultures by removing a carbon source from the medium

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Background

Bacteriophage infections cause serious problems in both research laboratories and large biotechnological companies. Once infected by bacteriophages, bacterial cultures are usually completely destroyed, as phage lytic development in a bacterium ends up with cell lysis and liberation of progeny phages that infect neighbor bacterial cells. A possibility of spreading of bacteriophages throughout a laboratory is even more dangerous than a loss of a single culture. Namely, subsequent cultures may be infected, which can lead to cultivation problems lasting even several months or longer. Therefore, a method for inhibition of bacteriophage lytic development in infected cultures would be useful. Perhaps it is not difficult in small cultures (e.g. flask cultures), when simple sterilization of the whole material and a flask should be sufficient. However, phage contamination in bioreactors is a serious technical problem indeed.

Escherichia coli is one of the most widely used bacterium in genetic engineering and biotechnology. This bacterium is, however, a host for many bacteriophages and thus, it is endangered by phage infections. Bacteriophages have been considered as models in genetic and biochemical studies for a long time. However, many physiological aspects of bacteriophages' growth were not sufficiently investigated relative to extensive molecular biology studies. On the other hand, recent reports indicated that development of bacteriophages largely depends on the physiology of the host cells. In laboratories, the physiological status of a cell depends, in turn, on cultivation conditions. Therefore, we aimed to find cultivation conditions that may result in inhibition of bacteriophage development and are not deleterious for bacterial cells. Previous studies indicated that development of phages T4 and λ is significantly less effective in slowly growing host cells than in rapidly growing bacteria. Thus, we aimed to test whether induction of starvation, caused be depletion of a carbon source from the culture medium, may inhibit phage development effectively. Growth of bacteriophages in bacterial cells cultured on solid (agar) media supporting various growth rates and at different temperatures was also investigated.

Results

We found that a decrease in temperature of an infected bacterial culture might impair development of some bacteriophages (e.g. λ) but not others (e.g. T4). Therefore, usefulness of a method of inhibition of phage development in infected bacterial cultures based on changes of temperature would be limited.

The presence of various carbon sources in the same kind of medium results in different bacterial growth rates. We investigated phage plaque formation on lawns of bacteria growing in media containing various carbon sources (glucose, glycerol, succinate or acetate) at the same temperature. The changes in plaque morphology of both phages were significant, though more pronounced in λ, as this phage was not able to form plaques when host grew on the medium with acetate as a carbon source, i.e. at the lowest growth rate. Removing the carbon source induces starvation conditions and minimal bacterial growth rate. Therefore, we asked whether removing the carbon source can lead to inhibition of formation of progeny phages in infected bacterial cultures.

We found that formation of phage progeny was completely inhibited in infected cultures devoid of the carbon source. This was true for all tested bacteriophages (λ, P1 and T4). Addition of glucose to infected cultures of starved bacteria resulted in restoration of phage progeny production, indicating that depletion of the carbon source was the sole reason for inhibition of development of phages λ, P1 and T4.

Conclusion

Development of bacteriophages λ, P1 and T4 is completely inhibited after removing a carbon source from infected *E. coli* cultures. Therefore, to minimize deleterious effects of phage contamination, especially in high-cell density and/or fed-batch cultivations, it may be recommended to stop feeding bacteria immediately after observation of first signs of phage infection. Such a procedure should lead to starvation of bacteria and inhibition of production of phage progeny. Although unambiguous detection of phage contamination at early stages of infection may be difficult using traditional methods, a newly developed technology of electric DNA chips allows for early detection of phages in bacterial cultures, even a few generations before they cause visible lysis of host cells.

An interesting side aspect of the performed study is the reduced ability of T4 and the inability of λ to form plaques on medium with acetate as the only carbon source. Although it remains unclear whether this effect is due to the very low growth rate of *E. coli* under these conditions or to the specific effect of acetate on the ΔpH and consequently on the proton motive force, or osmotic pressure of the host cell, this effect is interesting in connection to the proposed strategy to avoid phage propagation by stop of the feeding. Most *E. coli* fed-batch cultivations, kept at glucose limitation, would immediately stop phage growth, even if acetate is still available.