Methodology article

Inhibition of spontaneous induction of lambdoid prophages in *Escherichia coli* cultures: simple procedures with possible biotechnological applications

Agata Czyz[1], Marcin Los[2], Borys Wrobel[3,4] and Grzegorz Wegrzyn*[2,3]

Address: [1]Laboratory of Molecular Biology (affiliated with the University of Gdansk), Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Kladki 24, 80-822 Gdansk, Poland, [2]Department of Molecular Biology, University of Gdansk, Kladki 24, 80-822 Gdansk, Poland, [3]Marine Biology Center, Polish Academy of Sciences, Sw. Wojciecha 5, 81-347 Gdynia, Poland and [4]Department of Molecular Genetics and Biotechnology, The Hebrew University - Hadassah Medical School, Ein Kerem, Jerusalem, Israel

E-mail: Agata Czyz - czyz@biotech.univ.gda.pl; Marcin Los - mlos@biotech.univ.gda.pl; Borys Wrobel - wrobel@biotech.univ.gda.pl; Grzegorz Wegrzyn* - wegrzyn@biotech.univ.gda.pl
*Corresponding author

Published: 18 April 2001

BMC Biotechnology 2001, **1**:1

Received: 13 March 2001
Accepted: 18 April 2001

This article is available from: http://www.biomedcentral.com/1472-6750/1/1

Abstract

Background: Infections of bacterial cultures by bacteriophages are serious problems in biotechnological laboratories. Apart from such infections, prophage induction in the host cells may also be dangerous. *Escherichia coli* is a commonly used host in biotechnological production, and many laboratory strains of this bacterium harbour lambdoid prophages. These prophages may be induced under certain conditions leading to phage lytic development. This is fatal for further cultivations as relatively low, though still significant, numbers of phages may be overlooked. Thus, subsequent cultures of non-lysogenic strains may be infected and destroyed by such phage.

Results: Here we report that slow growth of bacteria decreases deleterious effects of spontaneous lambdoid prophage induction. Moreover, replacement of glucose with glycerol in a medium stimulates lysogenic development of the phage after infection of *E. coli* cells. A plasmid was constructed overexpressing the phage 434 cI gene, coding for the repressor of phage promoters which are necessary for lytic development. Overproduction of the cI repressor abolished spontaneous induction of the λ*imm*434 prophage.

Conclusions: Simple procedures that alleviate problems with spontaneous induction of lambdoid prophage and subsequent infection of *E. coli* strains by these phages are described. Low bacterial growth rate, replacement of glucose with glycerol in a medium and overproduction of the cI repressor minimise the risk of prophage induction during cultivation of lysogenic bacteria and subsequent infection of other bacterial strains.

Background

Bacteria are widely used hosts for production of many biotechnologically important substances. Among these microorganisms, *Escherichia coli* is one of the most frequently used hosts for expression of recombinant genes.

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. This 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 [1,2,3]. Even under standard cultivation conditions, a spontaneous prophage induction occurs with low frequency [1,2,3]. However, this rare prophage induction results in the appearance of infecting phage particles in amounts ranging from 10^{-8} to 10^{-5} pfu (plaque forming units) per bacterial cell [1,2,3]. 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 may be present 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}.

Although spontaneous prophage induction should not be dangerous for the culture of lysogenic bacteria due to the immunity phenomenon, i.e. resistance of lysogenic cells to infection by the same type of the phage [1,2], overlooking the presence of phages may be fatal for further cultivations. Phage contamination may cause infection of non-lysogenic cells, and subsequent lytic development of the phage may destroy the culture and cause spreading of large amounts of phages throughout the whole laboratory.

Although bacteriophages have been considered as models in genetic and biochemical studies [1,2,3,4,5], many physiological aspects of bacteriophage growth have not been sufficiently investigated relative to the extensive studies directed at other aspects of phage biology (most notably genetic regulation). However, recent reports have indicated that development of bacteriophages largely depends on the physiology of the host cells [6,7,8]. Although the above mentioned studies concerned basic research rather than applications, we assumed that the results of such investigations should be useful in reducing the effects of prophage induction, and possible subsequent bacteriophage infection, on bacterial cultures. Here we present simple procedures which should be helpful in alleviation of problems caused by lambdoid prophage induction and possible phage contamination of bacterial cultures, especially in biotechnological laboratories.

Results

Effect of bacterial growth rate on spontaneous prophage induction

It was found previously that bacterial growth rate significantly influences lytic development of bacteriophages T4 and λ [6,7,8,9]. We asked whether the efficiency of spontaneous λ prophage induction is also dependent on this parameter. Therefore, we measured the average number of phages per cell in cultures of lysogenic bacteria cultivated at different growth rates. A strain lysogenic for phage λ *c*I857 *S7* was used in these studies as the *S7* mutation prevents cell lysis of non-suppressor bacteria. After a sample was withdrawn, cellular membranes were disrupted by chloroform, allowing liberation of phages. Therefore, we could avoid a potential problem with adsorption of liberated phages on other cells in the culture and the resultant underestimation of the efficiency of prophage induction.

We found that efficiency of spontaneous λ prophage induction is significantly decreased at low growth rates of lysogenic bacteria relative to higher growth rates (Table 1). In fact, we could detect infecting phages in the cultures only when bacterial growth rate was higher than 0.6 doublings per hour.

Table 1: Spontaneous induction of λ *c*I857 *S7* prophage in *E. coli* cells cultivated in media supporting different growth rates

Medium	Growth rate (doublings per hour)	Frequency of spontaneous prophage induction (phages per cell)[a]
LB	1.0	6.6×10^{-7}
M9GluCaa	0.8	4.0×10^{-8}
M9Glu	0.6	$< 1 \times 10^{-9}$
M9Gly	0.4	$< 1 \times 10^{-9}$
M9Suc	0.3	$< 1 \times 10^{-9}$

[a]The results are average values from three measurements. In all cases, the standard deviation was below 10%

A plasmid for abolition of spontaneous prophage induction

Spontaneous induction of lambdoid prophages usually results from a decrease in the expression of the repressor gene (cI), whose product blocks transcription from promoters indispensable for lytic development [1,2,3]. Therefore, one could imagine that efficient overproduction of the repressor should impair spontaneous induction of the corresponding prophage. On the other hand, it was demonstrated previously that moderate overexpression of the λ *c*I gene did not abolish spontaneous

prophage induction [10]. Thus, we aimed to construct a plasmid system for overexpression of the repressor gene sufficiently effective to block spontaneous prophage induction.

Plasmid pQE30-434CI was constructed (see Materials and methods) which contains a gene coding for phage 434 repressor under control of an IPTG-inducible promoter. We found that in the *E. coli* strain lysogenic for λ*imm*434, the frequency of spontaneous prophage induction in LB medium was 5.0×10^{-7} phages per cell, whereas in bacteria overproducing the *c*I repressor of phage 434 the efficiency of prophage induction was below detectable levels (i.e. below 10^{-9} phages per cell). Therefore, we conclude that overproduction of the *c*I repressor of a lambdoid phage can prevent spontaneous prophage induction and eliminate further problems caused by subsequent phage lytic development.

Growth conditions of bacterial cultures and efficiency of lysogenisation by λ

In the preceding paragraphs we reported procedures for impairment of spontaneous lambdoid prophage induction in lysogenic cells. However, if for any reason induction occurred, one should consider a possibility to minimise problems arising from subsequent infection of non-lysogenic strains by contaminating phages. The effects of infection by a temperate phage (like λ) on a bacterial culture can be reduced by increasing the frequency of lysogenisation and decreasing the frequency of initiation of phage lytic developmental pathway. It is known that the 'lysis-versus-lysogenisation' decision depends on the physiology of the host and that starvation and low temperature favour lysogenisation rather than lytic development [1,2,3]. Since starvation and low temperature are parameters that could not be used effectively in bioreactors designed for efficient production of desired substances, we investigated the efficiency of lysogenisation of bacteria growing in different media at low (i.e. 1) and high (i.e. 10) m.o.i. The efficiency of lysogenisation was moderate in LB medium (though higher at the higher m.o.i.). This parameter decreased dramatically when minimal medium was supplemented with glucose, or glucose and Casamino acids were used (Table 2). Apparently, this was due to a negative effect of glucose on production of cAMP, which normally stimulates lysogenisation by indirect inhibition of the degradation of the activator of lysogenic promoters, the *c*II gene product [1,2,3,11]. Replacement of glucose with another carbon source, glycerol, significantly increased the efficiency of lysogenisation (Table 2).

Table 2: Efficiency of lysogenisation by phage λ *c*I857 *S7* of *E. coli* cells cultivated in different media

Medium	Efficiency of lysogenisation[a]	
	m.o.i. = 1	m.o.i. = 10
LB	0.44	0.72
M9GluCaa	0.06	0.14
M9Glu	0.02	0.14
M9Gly	0.82	0.96

[a] An efficiency of lysogenisation of 1 would represent 100% lysogens among survivors and presented values reflect this value. The results are average values from three experiments. In all cases, the standard deviation was below 10%

Discussion

Bacteriophages cause serious problems in biotechnology laboratories. Both prophage induction and phage infection are dangerous for ongoing and subsequent cultivations. Unfortunately, many commonly used *E. coli* strains harbour lambdoid prophages. We were looking for simple procedures which could alleviate the problems caused by lambdoid bacteriophages.

Although effects of bacterial growth rate on phage lytic development have been investigated previously [7,8,9], the results of these studies were used in basic research rather than in biotechnological applications. Here we report that deleterious effects of spontaneous lambdoid prophage induction are significantly decreased at low growth rates of lysogenic bacteria relative to high growth rates. These differences could be caused either by a lower frequency of prophage induction in slowly growing cells or by a less efficient lytic development after excision of the phage DNA from the host chromosome under conditions supporting low bacterial growth rates. In fact, low burst sizes of λ phage in slowly growing bacteria have already been reported [7,8,9]. Nevertheless, irrespective of the specific mechanism of the observed phenomenon, it is clear that the number of phages per cell in cultures of slowly growing lysogenic bacteria was below the detection limit (Table 1). Thus, cultivation of lysogenic bacteria in bioreactors at low growth rates should prevent contamination of the culture with induced phages.

We demonstrated that overproduction of the *c*I repressor from a multicopy plasmid efficiently reduces the risk of lambdoid prophage induction. Furthermore, overproduction of *c*I in non-lysogenic strains should make bacteria resistant to infection by a corresponding lambdoid

phage due to repression of phage early promoters, necessary for lytic development. Interestingly, even moderate overproduction of *c*I was also inhibitory for lysogenization, most probably due to indirect impairment of the *int* gene expression [10].

Lambdoid prophage induction can be provoked by factors stimulating the SOS response [1,2]. One could imagine that certain conditions in bioreactors stimulate the SOS response. The SOS-induced RecA* protein triggers self-cleavage of the *c*I repressor, causing prophage induction [2]. To avoid this problem, one can clone an ind- allele of *c*I (e.g. *c*I*857*), whose product, the *c*I857 protein, is resistant to RecA*-mediated self-cleavage.

Obviously, for achieving both large amounts of a desired product from a cloned gene and prevention of lambdoid prophage induction, one should often use a pair of compatible plasmids, one bearing a recombinant gene and second overexpressing the *c*I gene. However, this should not be a serious problem as many vectors bearing different origins of replication (thus being compatible) are currently available [12].

Glucose is the most commonly used carbon source in biotechnological cultivations of *E. coli*. However, in accordance with previous reports [1,2,11], we demonstrated that replacement of glucose with another carbon source results in a significant increase in the efficiency of lysogenization after infection of the host cells by λ. Therefore, we propose to replace glucose with glycerol in the culture medium when lambdoid phage contamination is plausible.

In conclusion, we have demonstrated that relatively low growth rates of bacteria and replacement of glucose with glycerol in the medium should significantly reduce deleterious effects of lambdoid prophage induction in *E. coli* cultures. These simple procedures should alleviate problems with lamdoid phages contamination in bacterial cultures. Moreover, spontaneous induction of lambdoid prophage is impaired by efficient overproduction of the *c*I repressor from a multicopy plasmid. If induction of the SOS response during the cultivation is possible, we recommend to overexpress an ind- allele of the *c*I gene.

Materials and methods

Bacteria, phages and plasmids

Escherichia coli wild-type strain SG20250 [13] and the *supF (tyrT)* indicator strain QD5003 [14] were used. Bacteriophages λ *c*I857 *S*7 (bearing a temperature-sensitive mutation in the λ repressor gene, and a nonsense mutation in one of the phage genes responsible for host cell lysis) [14] and λ*imm*434 (a λ phage bearing the *immunity* region from lambdoid phage 434; such derivatives of λ are frequently used in the host strains for controlled expression of cloned genes) (from A. B. Oppenheim) were employed. Plasmid pQE30-434CI is a pQE30 (Qiagen)- based construct, which was made by cloning of the PCR fragment (produced as described below) digested with *Bam*HI and *Xma*I. For PCR amplification, primers 434-L (5'-GGG GGG GAT CCT GCA CTA GTA TTT CTT CCA GGG) and 434-R2 (5'-GGC CCC GGG TTA GGG CCC GAA TTT TAC CCT CGC) were used and phage λ*imm*434 DNA served as a template. The pQE30-434CI plasmid allows for IPTG-inducible production of His6-tagged phage 434 repressor as evidenced by Western-blotting using the Qiagen anti-His6 antibody (data not shown). The structure of pQE30-434CI was confirmed by restriction mapping and DNA sequencing.

Culture media and growth conditions

Luria-Bertani (LB) and minimal M9 media [12] were employed, but the M9 medium contained different carbon sources as follows (the resultant media were renamed correspondingly): 2 g glucose l^{-1} and 10 g Casamino acids l^{-1} (M9GluCaa medium), 2 g glucose l^{-1} (M9Glu medium), 2 g glycerol l^{-1} (M9Gly medium) or 6 g sodium succinate l^{-1} (M9Suc medium). Cultivation of the SG20250 strain in these media at 30°C resulted in different growth rates as presented in Table 1.

Efficiency of spontaneous prophage induction

Efficiency of spontaneous λ prophage induction was estimated as described previously [10]. Briefly, samples of cultures of lysogenic bacteria grown at 30°C were shaken vigorously with chloroform and, following centrifugation, liberated phages were titrated on the QD5003 strain at 37°C. Number of bacterial cells in the culture was estimated by titration on LB plates at 30°C.

Efficiency of lysogenisation

Efficiency of lysogenisation of *E. coli* cells by bacteriophage λ *c*I857 *S*7 was estimated as described previously [10]. Briefly, bacteria were grown at 30°C in indicated medium to mid- log phase. A phage lysate was added to a sample of the culture to indicated multiplicity of infection (m.o.i.) and the mixture was incubated for 30 min at 30°C. Then, serial dilutions of the mixture in the TM buffer (10 mM Tris-HCl, 10 mM $MgSO_4$, pH 7.4) were spread on LB plates and incubated overnight at 30°C. Lysogens were identified among survivors by a temeperature-sensitivity assay (cells lysogenic with λ *c*I857 *S*7 are temperature-sensitive due to thermal induction of the prophage) and by testing sensitivity to superinfection as described previously [10].

Acknowledgements

We thank Dr. Peter Neubauer for discussions, and Katarzyna Potrykus, Joanna Potrykus and Dr. Mark S. Thomas for critical reading of the manuscript. This work was supported by the European Commission (contract

no. QLK3-CT-1999-00533) and the Polish State Committee for Scientific Research (grant no. 127/E-335/SPUB-M-5PR-UE/DZ 177/2000). A.C. and G.W. acknowledge financial supports from the Foundation for Polish Science (the fellowship for young scientists and subsidium 14/2000, respectively). B.W. acknowledges first FEBS and then EMBO long-term fellowship and is grateful to Prof. Amos B. Oppenheim for hosting him in his laboratory.

References

1. Ptashne M: **A genetic switch: phage λ and higher organisms.** *Cambridge, MA: Cell Press and Blackwell Scientific Publications,* 1992,
2. Taylor K, Wegrzyn G: **Regulation of bacteriophage λ replication.** *In Molecular Microbiology. Edited by Busby SJW, Thomas CM, Brown NL. Berlin-Heidelberg: Springer,* 1998, 81-97
3. McAdams HH, Shapiro LL: **Circuit simulation of genetic networks.** *Science* 1995, **269**:650-656
4. Thomas R: **Bacteriophage λ: transactivation, positive control and other odd findings.** *Bioessays* 1993, **15**:285-289
5. Gottesman M: **Bacteriophage λ: the untold story.** *J Mol Biol* 1999, **293**:177-180
6. Hadas H, Einav M, Fishov I, Zaritsky A: **Bacteriophage T4 development depends on the physiology of its host Escherichia coli.** *Microbiology* 1999, **143**:179-185
7. Gabig M, Obuchowski M, Wegrzyn A, Szalewska-Palasz A, Thomas MS, Wegrzyn G: **Excess production of phage λ delayed early proteins under conditions supporting high Escherichia coli growth rates.** *Microbiology* 1998, **144**:2217-2224
8. Wegrzyn A, Czyz A, Gabig M, Wegrzyn G: **ClpP/ClpX-mediated degradation of the bacteriophage λ O protein and regulation of λ phage and λ plasmid replication.** *Arch Microbiol* 2000, **174**:89-96
9. Echols H, Green L, Kudrna R, Edlin G: **Regulation of phage lambda development with the growth rate of host cells: a homeostatic mechanims.** *Virology* 1975, **66**:344-346
10. Wegrzyn G, Glass RE, Thomas MS: **Involvement of the Escherichia coli RNA polymerase α subunit in transcriptional activation by the bacteriophage lambda CI and CII proteins.** *Gene* 1992, **122**:1-7
11. Grodzicker T, Arditti RR, Eisen H: **Establishment of repression by lambdoid phage in catabolite activator protein and adenylate cyclase mutants of Escherichia coli.** *Proc Natl Acad Sci USA* 1972, **69**:366-370
12. Sambrook J, Fritsch EF, Maniatis T: **Molecular cloning: a laboratory manual.** *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press,* 1989,
13. Gottesman S, Clark WP, Crecy-Lagard V, Maurizi MR: **ClpX, an alternative subunit for the ATP-dependent Clp protease of Escherichia coli. Sequence and in vivo activities.** *J Biol Chem* 1993, **268**:22618-22626
14. Goldberg AR, Howe M: **New mutations in the S cistron of bacteriophage lambda affecting host cell lysis.** *Virology* 1969, **38**:200-202