

## Research Article

# The Role of the *Exo-Xis* Region in Oxidative Stress-Mediated Induction of Shiga Toxin-Converting Prophages

Katarzyna Licznarska,<sup>1</sup> Aleksandra Dydecka,<sup>1</sup> Sylwia Bloch,<sup>1</sup> Gracja Topka,<sup>1</sup>  
Bożena Nejman-Faleńczyk,<sup>1</sup> Alicja Węgrzyn,<sup>2</sup> and Grzegorz Węgrzyn<sup>1</sup>

<sup>1</sup>Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

<sup>2</sup>Laboratory of Molecular Biology (Affiliated with the University of Gdańsk), Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Wita Stwosza 59, 80-308 Gdańsk, Poland

Correspondence should be addressed to Grzegorz Węgrzyn; [grzegorz.wegrzyn@biol.ug.edu.pl](mailto:grzegorz.wegrzyn@biol.ug.edu.pl)

Received 11 August 2015; Revised 22 September 2015; Accepted 28 September 2015

Academic Editor: Luciano Saso

Copyright © 2016 Katarzyna Licznarska et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Previous studies indicated that these genetic elements could be involved in the regulation of lysogenization and prophage induction processes. The effects were dramatic in Shiga toxin-converting phage  $\Phi 24_B$  after treatment with oxidative stress-inducing agent, hydrogen peroxide, while they were less pronounced in bacteriophage  $\lambda$  and in both phages irradiated with UV. The hydrogen peroxide-caused prophage induction was found to be RecA-dependent. Importantly, in hydrogen peroxide-treated *E. coli* cells lysogenic for either  $\lambda$  or  $\Phi 24_B$ , deletion of the *exo-xis* region resulted in a significant decrease in the levels of expression of the S.O.S. regulon genes. Moreover, under these conditions, a dramatic decrease in the levels of expression of phage genes crucial for lytic development (particularly *xis*, *exo*, *N*, *cro*, *O*, *Q*, and *R*) could be observed in  $\Phi 24_B^-$ , but not in  $\lambda$ -bearing cells. We conclude that genes located in the *exo-xis* region are necessary for efficient expression of both host S.O.S regulon in lysogenic bacteria and regulatory genes of Shiga toxin-converting bacteriophage  $\Phi 24_B$ .

## 1. Introduction

Infection of humans by enterohemorrhagic *Escherichia coli* (EHEC) strains causes hemorrhagic colitis, and in some patients it may result in various complications, including, the most severe of them, the hemolytic-uremic syndrome and neurological dysfunctions [1–3]. The main causes of EHEC-mediated complications are Shiga toxins, produced by the infecting bacteria [4]. The severity of EHEC infections and significance of the medical problem related to them are exemplified by local outbreaks, occurring in various geographical regions around the world. One of the most famous of them took place in 2011 in Germany, where over 4,000 patients developed severe symptoms, and 54 died [5–10].

In EHEC strains, Shiga toxins are encoded by genes (called *stx* genes) located in genomes of prophages [11, 12]. The phages bearing *stx* genes are referred to as Shiga toxin-converting bacteriophages, and all of them belong to the family of lambdoid phages (with phage  $\lambda$  serving as a paradigm)

[12]. *stx* genes are present between Q antiterminator gene and the genes coding for proteins causing cell lysis; thus, in the lysogenic state, these genes are not transcribed [13–15] and Shiga toxins are not produced. Their expression is possible only after prophage induction [11, 12] which usually requires activation of the bacterial S.O.S. response, mediated by RecA protein, though RecA-independent induction of Shiga toxin-converting prophages by EDTA has also been reported [16].

During infection of human intestine by EHEC, the oxidative stress appears to be the most likely condition causing the bacterial S.O.S. response and subsequent induction of Shiga toxin-converting prophages [17]. In fact, it was demonstrated that hydrogen peroxide (which is produced by neutrophils as a response to infection) enhanced production of Shiga toxins by EHEC [18] due to oxidative stress-mediated induction of Shiga toxin-converting prophages [19, 20].

Since many antibiotics not only kill bacteria and inhibit their growth but also induce prophage lytic development, their use is not recommended when EHEC infection is

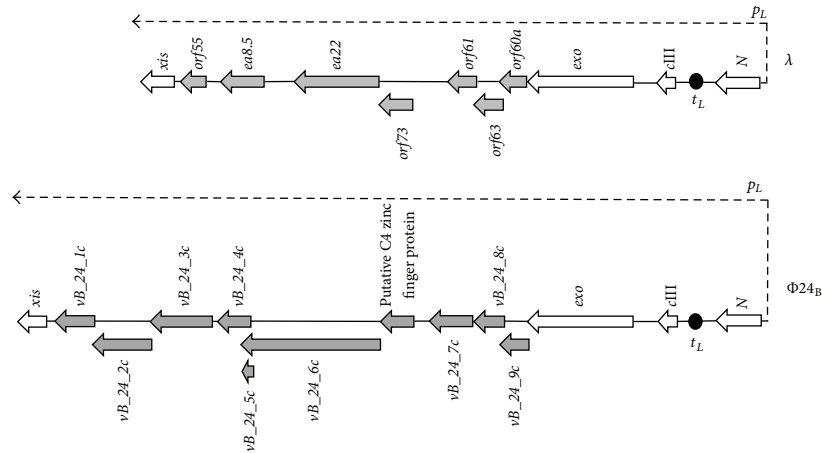


FIGURE 1: Schematic maps of the *exo-xis* regions of bacteriophages  $\lambda$  and  $\Phi24_B$ . Genes from the *exo-xis* region are marked as thick grey arrows, and other genes are shown as thick open arrows. Directionality of transcription from  $p_L$  promoter is indicated as thin dashed arrow.  $t_{L1}$  terminator is marked as black oval.

confirmed or even suspected (reviewed in [12]). Therefore, there are serious problems with treatment of patients, indicating that searching for new targets of potential therapies against Shiga toxin-producing bacteria is important. One might consider that such therapies should be focused on inhibition of Shiga toxin-converting prophage induction which would impair production of the EHEC virulence factor. All lambdoid phages, including Shiga toxin-converting bacteriophages, contain the *b* region in their genomes which is dispensable for the development under standard laboratory conditions [14, 15]. Inside this part of the phage genome, there is an evolutionarily conserved fragment, located between *exo* and *xis* genes and transcribed from  $p_L$  promoter, called the *exo-xis* region (Figure 1). This region encompasses several genes and open reading frames whose functions in phage development are largely unknown, and only a few articles are available in the literature that focused on them. Nevertheless, some interesting observations have been reported. Namely, induction of expression of genes from the *exo-xis* region resulted in synchronization of the host cell cycle [21] and inhibition of host DNA replication [22]. Moreover, overexpression of these genes impaired lysogenization of *E. coli* by bacteriophage  $\lambda$  [23] and enhanced induction of prophages  $\lambda$  and  $\Phi24_B$  (one of Shiga toxin-converting phages) [24]. Ea8.5 protein, encoded by a gene located in the *exo-xis* region, contains a fused homeodomain/zinc finger fold [25] which suggests a regulatory role for this protein. Interestingly, prophage induction with mitomycin C or hydrogen peroxide caused different expression patterns of genes from the *exo-xis* region; such differences were observed in both phages,  $\lambda$  and  $\Phi24_B$  [26]. In this work, we used the deletion mutants to investigate the role of the *exo-xis* region in induction of  $\lambda$  and  $\Phi24_B$  prophages under oxidative stress conditions.

## 2. Materials and Methods

**2.1. Bacteria and Bacteriophages.** *E. coli* MG1655 strain [27] and its derivatives, used in this work, are listed in Table 1. Bacteria were routinely cultured in the Luria-Bertani (LB)

medium at 30°C (most experiments) or 37°C (lysogenization and recombination procedures during construction of strains and SOS ChromoTest, according to the instructions of kits' manufacturers), under aerobic conditions. Where appropriate, the following antibiotics were added: chloramphenicol up to 20  $\mu\text{g}/\text{mL}$ , kanamycin up to 50  $\mu\text{g}/\text{mL}$ , and/or tetracycline up to 12.5  $\mu\text{g}/\text{mL}$ .

Bacteriophages  $\lambda$  papa (from our collection) [26] and  $\Phi24_B$  ( $\Delta\text{stx}2::\text{cat}$ ) [28] were employed in this study. Phage suspensions were stored in the TM buffer (10 mM Tris-HCl, 10 mM  $\text{MgSO}_4$ , pH 7.2) at 4°C.

The deletion mutants were constructed as described previously [29], by using the Quick and Easy *E. coli* Gene Deletion Kit (from Gene Bridges). The deletion of the indicated region was performed according to the manufacturer's protocol using primers listed in Table 2. In the first step, the targeted sequence has been replaced with the FRT-flanked kanamycin resistance cassette, and the selection marker was subsequently removed in the FLP-recombinase step, leaving only 87 nucleotides of the cassette in the place of the original sequence. Each deletion was confirmed by DNA sequencing.

Lysogenic strains were constructed according to the procedure described previously [24], with slight modifications. Briefly, host bacteria were cultured to  $A_{600}$  of 0.5 in LB medium supplemented with  $\text{MgSO}_4$  and  $\text{CaCl}_2$  (to final concentrations of 10 mM each) at 37°C with shaking. At this point, one milliliter of the culture was withdrawn and centrifuged (10 min, 2000  $\times g$ ). Pellet was washed twice with TCM buffer (10 mM Tris-HCl, pH 7.2, 10 mM  $\text{MgSO}_4$ , 10 mM  $\text{CaCl}_2$ ) and then suspended in 1 mL of the same buffer. Next, bacteria were incubated for 30 min at 30°C and mixed with phage suspensions at multiplicity of infection (m.o.i.) = 5. Mixtures of bacteria and phages were incubated in TMC buffer for 30 min at 30°C; then serial dilutions were prepared in TM buffer (10 mM Tris-HCl, 10 mM  $\text{MgSO}_4$ , pH 7.2), and the mixture was plated onto LB agar. Plates were incubated at 37°C overnight. Lysogens were verified by sensitivity to UV irradiation and confirmed by PCR with primers designed to amplify phage sequence (Table 3).

TABLE 1: *Escherichia coli* strains.

Strain	Genotype or relevant characteristics	Reference
<i>E. coli</i> MG1655	F <sup>-</sup> λ <sup>-</sup> <i>ilvG rfb-50 rph-1</i>	[27]
<i>E. coli</i> MG1655 (λ)	MG1655 bearing λ prophage	[24]
<i>E. coli</i> MG1655 (λΔ <i>exo-xis</i> )	MG1655 bearing λ prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>orf</i> s)	MG1655 bearing λ prophage with deletion of <i>orf60a</i> , <i>orf63</i> , <i>orf61</i> , and <i>orf73</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>orf60a</i> )	MG1655 bearing λ prophage with deletion of <i>orf60a</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>orf63</i> )	MG1655 bearing λ prophage with deletion of <i>orf63</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>orf61</i> )	MG1655 bearing λ prophage with deletion of <i>orf61</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>orf73</i> )	MG1655 bearing λ prophage with deletion of <i>orf73</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>ea22</i> )	MG1655 bearing λ prophage with deletion of <i>ea22</i>	This study, by recombination
<i>E. coli</i> MG1655 (λΔ <i>ea8.5</i> )	MG1655 bearing λ prophage with deletion of <i>ea8.5</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> )	MG1655 bearing Φ24 <sub>B</sub> prophage	[24]
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>exo-xis</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>orf</i> s)	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of 4 orfs being homologues of <i>lorf60a</i> , <i>lorf63</i> , <i>lorf61</i> , and <i>lorf73</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>orf60a</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of <i>vb.24<sub>B</sub>-9c</i> , the homologue of <i>lorf60a</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>orf63</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of <i>vb.24<sub>B</sub>-8c</i> , the homologue of <i>lorf63</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>orf61</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of <i>vb.24<sub>B</sub>-7c</i> , the homologue of <i>lorf61</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>orf73</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of the sequence of putative C4 zinc finger protein, the homologue of <i>lorf73</i>	This study, by recombination
<i>E. coli</i> MG1655 (Φ24 <sub>B</sub> Δ <i>ea22</i> )	MG1655 bearing Φ24 <sub>B</sub> prophage with deletion of <i>vb.24<sub>B</sub>-6c</i> , the analogue of <i>lea22</i>	This study, by recombination
<i>E. coli</i> MG1655 <i>recA13</i>	MG1655 but <i>recA13</i>	[33]
<i>E. coli</i> MG1655 <i>recA13</i> (λ)	MG1655 <i>recA13</i> bearing λ prophage	This study, by lysogenization
<i>E. coli</i> MG1655 <i>recA13</i> (λΔ <i>exo-xis</i> )	MG1655 <i>recA13</i> bearing λ prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by lysogenization
<i>E. coli</i> MG1655 <i>recA13</i> (Φ24 <sub>B</sub> )	MG1655 <i>recA13</i> bearing Φ24 <sub>B</sub> prophage	This study, by lysogenization
<i>E. coli</i> MG1655 <i>recA13</i> (Φ24 <sub>B</sub> Δ <i>exo-xis</i> )	MG1655 <i>recA13</i> bearing Φ24 <sub>B</sub> prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by lysogenization
<i>E. coli</i> PQ37	<i>sfjA::Mud(Ap lac) cts, lacΔU169, mal<sup>+</sup>, galE, galY, PhoC, rfa, F<sup>-</sup>, thr, leu, his, pyrD, thi, trp::MUC<sup>+</sup>, srl300::Tn10, rpoB, uvrA<sup>+</sup></i>	[31]
<i>E. coli</i> PQ37 (λ)	PQ37 bearing λ prophage	This study, by lysogenization
<i>E. coli</i> PQ37 (λΔ <i>exo-xis</i> )	PQ37 bearing λ prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by lysogenization
<i>E. coli</i> PQ37 (Φ24 <sub>B</sub> )	PQ37 bearing Φ24 <sub>B</sub> prophage	This study, by lysogenization
<i>E. coli</i> PQ37 (Φ24 <sub>B</sub> Δ <i>exo-xis</i> )	PQ37 bearing Φ24 <sub>B</sub> prophage with deletion of all orfs and genes localized between genes <i>exo</i> and <i>xis</i>	This study, by lysogenization

**2.2. Phage Lytic Development after Prophage Induction.** Bacteria lysogenic with tested phages were cultured in LB medium at 30°C to A<sub>600</sub> of 0.1. Two induction agents were tested: H<sub>2</sub>O<sub>2</sub> (1 mM) and UV irradiation (50 J/m<sup>2</sup>; this dose was achieved by 20 sec incubation of the bacterial suspensions in Petri dishes under UV lamp hanged 17 cm above the laboratory table). At indicated times after induction, samples of bacterial cultures were harvested, and 30 μL of chloroform was added to 0.5 mL of each sample. The mixture was vortexed and centrifuged for 5 min in a microcentrifuge.

Then, serial dilutions were prepared in TM buffer, and phage titer (number of phages per mL) was determined by spotting 2.5 μL of each dilution of the phage lysate on a freshly prepared LB agar (1.5%) or LB agar with 2.5 μg/mL chloramphenicol (according to a procedure described previously [30]), with a poured mixture of 1 mL of the indicator *E. coli* MG1655 strain culture and 2 mL of 0.7% nutrient agar (prewarmed to 45°C), supplemented with MgSO<sub>4</sub> and CaCl<sub>2</sub> (to a final concentration of 10 mM each). When full-plate titration was used, 0.1 mL of phage lysate dilutions was



TABLE 3: Primers used for PCR.

Primers for bacterial sequences		Primers for phage sequences	
Name	Sequence (5' → 3')	Name	Sequence (5' → 3')
pF_recA	AGATTTTCGACGATACGGCCC	pF_λ.int	TTTGATTTCAATTTTGTCCCCT
pR_recA	AACCATCTCTACCGGTTTCGC	pR_λ.int	ACCATGGCATCACAGTATCG
pF_lexA	ATGGATGGTGAAGTGGCTGGC	pF_λ.xis	TACCGCTGATTCGTGGAACA
pR_lexA	TTCGTCATCAATACGTGCGAC	pR_λ.xis	GGGTTTCGGGAATGCAGGATA
pF_ssb	ATCGAAGGTCAGCTGCGTAC	pF_λ.exo	TGCCGTCCTGCATAAACC
pR_ssb	CGACTTCTGTGGTGTAGCGA	pR_λ.exo	TCTATCGCGACGAAAGTATGC
pF_recF	CGATACCGGCGCTATACTCC	pF_λ.cIII	ATTCTTTGGGACTCCTGGCTG
pR_recF	TTACGAACAGCTACGCCCG	pR_λ.cIII	GTAAATTACGTGACGGATGGAAAC
pF_rpoD	GAATCTGAAATCGGGCGCAC	pF_λ.N	CTCGTGATTTTCGGTTTGC
pR_rpoD	GTCAACAGTTCAACGGTGCC	pR_λ.N	AAGCAGCAAATCCCCTGTTG
pF_rpoH	GCTTTGGTGGTTCGCAACTTT	pF_λ.cI	ACCTCAAGCCAGAATGCAGA
pR_rpoH	TCGCCGTTCACTGGATCAAA	pR_λ.cI	CCAAAGGTGATGCGGAGAGA
pF_rpoS	TTGCTCTGCGATCTCTTCCG	pF_λ.cro	ATGCGGAAGAGGTAAGGCC
pR_rpoS	GAACGTTTACCTGCGAACC	pR_λ.cro	TGGAATGTGTAAGAGCGGG
pF_uvrA	GTCCATATCCGCCACTACCG	pF_λ.cII	TCGCAATGCTTGGAAGTGA
pR_uvrA	TTACCCAACGTCTTGCCGAG	pR_λ.cII	CCCTCTTCCACCTGCTGATC
pF_ftsK	ACAAACCGTTTATCTGCGCG	pF_λ.O	AATTCTGGCGAATCCTCTGA
pR_ftsK	ATCTTTACCCAGCACCACGG	pR_λ.O	GAATTGCATCCGGTTT
pF_16SrRNA	CCTTACGACCAGGGCTACAC	pF_λ.Q	TTCTGCGGTAAGCACGAAC
pR_16SrRNA	TTATGAGGTCCGCTTGCTCT	pR_λ.Q	TGCATCAGATAGTTGATAGCCTT
		pF_λ.R	ATCGACCGTTGCAGCAATA
		pR_λ.R	GCTCGAAGTACCATAACCAG
		pF_Φ24 <sub>B</sub> .int	CAGTTGCCGGTATCCCTGT
		pR_Φ24 <sub>B</sub> .int	TGAGGCTTTCTTGCTTGTC
		pF_Φ24 <sub>B</sub> .xis	TATCGCGCCGGATGAGTAAG
		pR_Φ24 <sub>B</sub> .xis	CGCACAGCTTTGTATAATTTGCG
		pF_Φ24 <sub>B</sub> .exo	TGCCGTCCTGCATAAACC
		pR_Φ24 <sub>B</sub> .exo	TCTATCGCGACGAAAGTATGC
		pF_Φ24 <sub>B</sub> .cIII	ATTCTTTGGGACTCCTGGCTG
		pR_Φ24 <sub>B</sub> .cIII	GTAAATTACGTGACGGATGGAAAC
		pF_Φ24 <sub>B</sub> .N	AGGCGTTTCGTGAGTACCTT
		pR_Φ24 <sub>B</sub> .N	TTACACCGCCCTACTCTAAGC
		pF_Φ24 <sub>B</sub> .cI	TGCTGTCTCCTTTACACGA
		pR_Φ24 <sub>B</sub> .cI	GCGATGGGTGGCTCAAAATT
		pF_Φ24 <sub>B</sub> .cro	CGAAGGCTTGTGGAGTTAGC
		pR_Φ24 <sub>B</sub> .cro	GTCTTAGGGAGGAAGCCGTT
		pF_Φ24 <sub>B</sub> .cII	TGATCGCGCAGAAACTGATTTAC
		pR_Φ24 <sub>B</sub> .cII	GACAGCCAATCATCTTTGCCA
		pF_Φ24 <sub>B</sub> .O	AAGCGAGTTTGCCACGAT
		pR_Φ24 <sub>B</sub> .O	GAACCCGAAGTCTTACCG
		pF_Φ24 <sub>B</sub> .Q	GGGAGTGAGGCTTGAGATGG
		pR_Φ24 <sub>B</sub> .Q	TACAGAGGTTCTCCCTCCCG
		pF_Φ24 <sub>B</sub> .R	GGGTGGATGGTAAGCCTGT
		pR_Φ24 <sub>B</sub> .R	TAACCCGGTTCGATTTTTC

plated onto LB agar. Plates were incubated at 37°C overnight. Analogous experiment but without induction agents (control experiments), which allows estimation of effects of spontaneous prophage induction, was performed with each lysogenic strain. The relative phage titer, expressed as plaque

forming units (pfu)/mL, was calculated by subtracting the values obtained in the control experiment from the values determined in the main experiment, and as a consequence it represents the ratio of phage titers in induced and noninduced cultures. Each experiment was repeated three times.

**2.3. The S.O.S. Assay.** The S.O.S. assay was performed using the SOS-ChromoTest Kit (Environmental Bio-Detection Products Inc.), following the manufacturer's protocol and using provided 4-nitro-quinoline oxide (4-NQO) as a positive reference standard, and 1 mM H<sub>2</sub>O<sub>2</sub> and UV irradiation (50 J/m<sup>2</sup>) as tested inducers of the S.O.S. response [31, 32]. In the case of UV light irradiation, the production of  $\beta$ -galactosidase was evaluated immediately after the exposure, without 2 h incubation at 37°C (recommended by the manufacturer), as, without this modification, the visual detection of the blue color was not possible due to rapid S.O.S. response after UV irradiation. Before use, the SOS-ChromoTest bacterial strain (*E. coli* PQ37, provided with the kit) was lysogenized by following phages:  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi24_B$ , or  $\Phi24_B\Delta_{exo-xis}$ , according to procedure described above.

**2.4. Preparation of RNA and cDNA from Bacteria.** For the isolation of total RNA, the previously described [26] procedure was employed. Briefly, the prophage induction was performed with 1 mM H<sub>2</sub>O<sub>2</sub> or UV irradiation (at the dose of 50 J/m<sup>2</sup>). Following induction, the samples were withdrawn at indicated times and the growth of bacteria was inhibited by the addition of NaN<sub>3</sub> (Sigma-Aldrich) to a final concentration of 10 mM. Total RNA was isolated from 10<sup>9</sup> bacterial cells by using the High Pure RNA Isolation Kit (Roche Applied Science). Bacterial genomic DNA carryover was removed by incubation with TURBO DNase from TURBO DNA-free Kit (Life Technologies) for 60 min at 37°C, according to the manufacturer's guidelines. To evaluate the quality and quantity of the isolated RNA, a NanoDrop spectrophotometer was employed, considering the absorbance ratio (which should be  $1.8 \leq A_{260}/A_{280} \leq 2.0$ ). Moreover, band patterns of total RNA were visualized by electrophoresis. The absence of DNA from RNA samples was controlled by PCR amplification and by real-time PCR amplification (all analyzed genes were tested). RNA preparations were stored at -80°C. cDNA was obtained with Transcriptor Reverse Transcriptase and random hexamer primers (Roche Applied Science), using total RNA samples (1.25  $\mu$ g) as templates. cDNA reaction mixtures were diluted 10-fold for use in real-time PCR.

**2.5. Real-Time PCR Assay and Data Analysis.** The patterns of genes' expression were determined by quantitative real-time reverse transcription-PCR (qRT-PCR), using the LightCycler 480 Real-Time PCR System (Roche Applied Science) and cDNA samples from lysogenic bacteria. Transcripts of tested phage and bacterial genes were compared in parallel to 16S rRNA housekeeping gene (according to a procedure described previously [34]), whose expression was found to be constant. Primers were developed by Primer3web version 4.0.0 and produced by Sigma-Aldrich or GENOMED. The transcriptional analysis of phage and bacterial genes from lysogenic strains was performed with primers presented in Table 3. Real-time PCR amplifications were carried out for 55 cycles in 20  $\mu$ L reaction volume, using LightCycler 480 SYBR Green I Master (Roche Applied Science) as a fluorescent detection dye. Reactions were performed in Roche 96-well plates containing 10  $\mu$ L 2x SYBR Green I

Master Mix, 6.25 ng/ $\mu$ L cDNA, and 200 nM of each gene-specific primer (Table 3). Relative quantification assays were performed with cDNA of 16S rRNA and phage/bacterial genes multiplex assay. All templates were amplified using the following program: incubation at 95°C for 5 min, followed by 55 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 15 s. No template control was included with each run. The specificity of amplified products was examined by melting curve analysis immediately after the final PCR cycle and confirmed by gel electrophoresis. Each experiment was conducted in triplicate.

The relative changes in gene expression revealed by quantitative real-time PCR experiments were analyzed using the calibrator, normalizing relative quantification method with efficiency correction (called the E-Method). This method has been used and described in detail previously [26, 29, 35]. The values obtained at time 0 (representing the conditions of spontaneous prophage induction) were used as calibrators. Thus, the following equation was employed to calculate the final results:

Normalized relative ratio

$$= \frac{E_t^{CT(t) \text{ calibrator} - CT(t) \text{ sample}}}{E_r^{CT(r) \text{ calibrator} - CT(r) \text{ sample}}}, \quad (1)$$

where  $t$  is target and  $r$  is reference.

**2.6. In Silico Analyses.** The multiple sequence alignment was performed using the ClustalW algorithm available at the website <http://www.genome.jp/tools/clustalw/>. The Pfam protein families database [36], available at the website <http://pfam.xfam.org/>, was used to identify protein domains.

### 3. Results and Discussion

**3.1. Deletion of the Exo-Xis Region Impairs  $\Phi24_B$  but Not  $\lambda$  Prophage Induction after Treatment with Hydrogen Peroxide.** Until now, all *in vivo* studies on effects of the *exo-xis* region on host or phage development were performed with the use of strains overexpressing genes from this region [21–24, 26]. In this work, we have constructed a series of bacteriophage  $\lambda$  and  $\Phi24_B$  mutants with deletions of either the whole *exo-xis* region or individual genes or open reading frames (Table 1). When wild-type  $\lambda$  and  $\Phi24_B$  prophages were induced by UV irradiation (employed in this work as positive control conditions causing effective prophage induction) or hydrogen peroxide treatment of the lysogenic cells, efficiencies of induction and further phage lytic development were comparable in both phages, though some differences were observed in the duration of the lag phase of the phage development (Figure 2). Induction of  $\lambda\Delta_{exo-xis}$  mutant with UV irradiation was similar to that observed for the wild-type  $\lambda$ , and treatment with hydrogen peroxide caused only a slight delay in the mutant phage development. The decrease in the phage titer at later times of the experiments is characteristic for  $\lambda$  and most probably arises from adsorption of the progeny virions on fragments of disrupted cell envelopes [15, 24]. However, induction of  $\Phi24_B\Delta_{exo-xis}$  prophage by UV irradiation was less efficient than that of the wild-type  $\Phi24_B$ , and induction

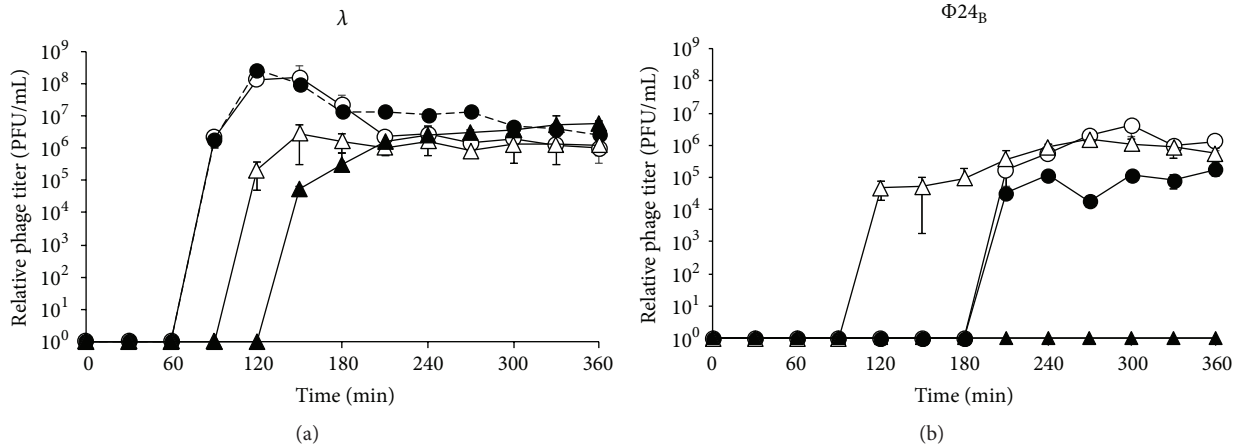


FIGURE 2: Lytic development of bacteriophages  $\lambda$  (a) and  $\Phi 24_B$  (b), either wild-type (open symbols) or  $\Delta exo-xis$  (closed symbols), after induction of lysogenic *E. coli* MG1655 with UV irradiation ( $50 \text{ J/m}^2$ , circles) or hydrogen peroxide (1 mM, triangles). The presented results are mean values from 3 independent experiments (biological samples), with error bars indicating SD. Statistically significant differences ( $p < 0.05$  in *t*-test) between wild-type and  $\Delta exo-xis$  phages were found at times 270, 300, and 360 min of experiments with hydrogen peroxide and at 270, 300, 330, and 360 min of experiments with UV for  $\lambda$  and at times 120, 240, 270, 300, 330, and 360 min of experiments with hydrogen peroxide and at 270, 300, 330, and 360 min of experiments with UV for  $\Phi 24_B$ .

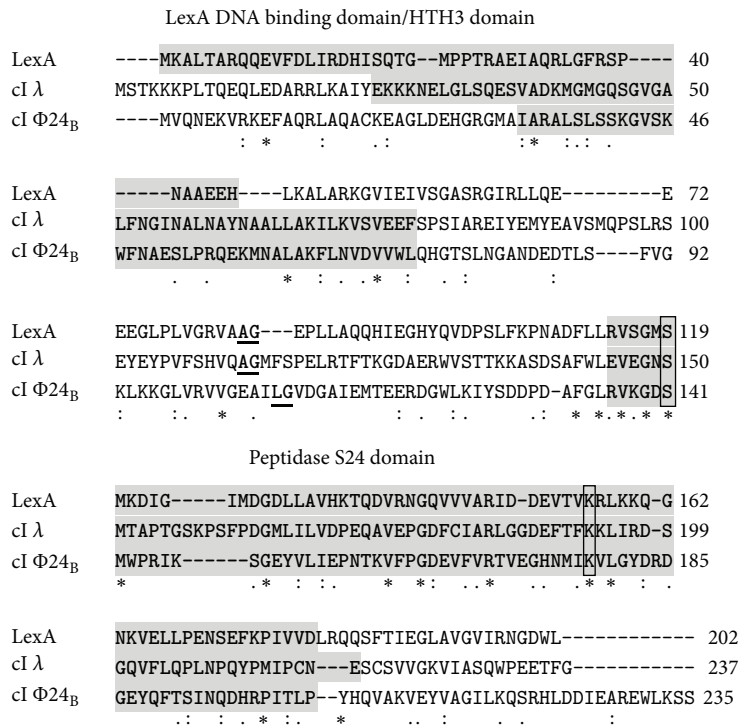


FIGURE 3: Alignment of amino acid sequences of *E. coli* LexA protein and cI repressors of bacteriophages  $\lambda$  and  $\Phi 24_B$ . Specific protein domains are indicated by grey background. Self-cleavage sites are underlined (two amino acid residues between which the cleavage occurs). The active sites of the peptidase domains are framed. Symbols under the sequence alignment indicate conserved sequence (\*), conservative mutations (:), semiconservative mutations (.), and nonconservative mutations (.)

of the mutant by hydrogen peroxide was severely impaired (Figure 2). More detailed analyses, based on the full-plate phage titration method, allowing detection of 10 pfu/mL (see Section 2 for details), indicated that the number of pfu per mL of  $\Phi 24_B \Delta exo-xis$  phage after induction with hydrogen

peroxide was at the same range ( $10^3/\text{mL}$ ) as that measured without specific induction (i.e., representing efficiency of spontaneous prophage induction). Nevertheless, the titer of  $\Phi 24_B \Delta exo-xis$  measured at 240 and 360 min after induction was  $9.0 \pm 0.2 \times 10^3$  and  $7.9 \pm 0.9 \times 10^3$ , respectively, that

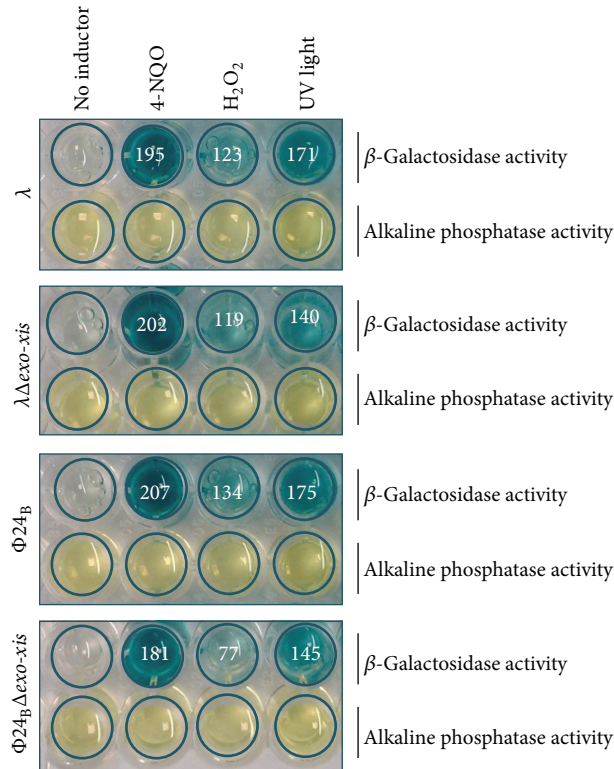


FIGURE 4: Induction of the S.O.S. response in *E. coli* PQ37 lysogenic with  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi 24_B$ , or  $\Phi 24_B\Delta_{exo-xis}$ , treated with 4-NQO (4-nitro-quinoline oxide, positive control),  $H_2O_2$  (1 mM), or UV (50 J/m<sup>2</sup>), using the SOS ChromoTest.  $\beta$ -Galactosidase activity (identified by the blue spots) represents induction of the S.O.S. regulon. Alkaline phosphatase activity (identified by yellow spots) evaluates viability of tested bacteria. Quantification of  $\beta$ -galactosidase activity was performed by densitometry, using the ImageJ software (available at <http://imagej.nih.gov/ij/index.html>). The results (in arbitrary units reflecting value = 1 ascribed to samples with no inductor), presented as numbers inside the corresponding spots, are mean values from three measurements (with SD < 10% in each case). All these values were significantly ( $p < 0.001$  in  $t$ -test) higher than that in the control experiments with no inductor. When  $\Delta_{exo-xis}$  mutants were compared to wild-type phages, the only significant difference ( $p < 0.05$  in  $t$ -test) occurred between  $\Phi 24_B$  and  $\Phi 24_B\Delta_{exo-xis}$  lysogens induced with hydrogen peroxide.

is, still 3–4 times higher than that without induction, which was  $2.1 \pm 0.3 \times 10^3$  and  $2.6 \pm 0.4 \times 10^3$ , respectively (note that the titer of the wild-type  $\Phi 24_B$  after prophage induction was several orders of magnitude higher than that without induction, Figure 2).

Deletions of individual genes and open reading frames from the *exo-xis* region in  $\Phi 24_B$  did not affect significantly the phage titer. However, such deletions resulted in delays in prophage induction by hydrogen peroxide (Table 4). Interestingly, when prophage induction was stimulated by UV irradiation, such effect was not observed, and in some cases even more rapid induction of the mutant prophages occurred. In bacteriophage  $\lambda$ , only slight effects of deletions of individual genes and open reading frames were detected (Table 4).

We conclude that the genes and open reading frames from the *exo-xis* region play important roles in the regulation of lambdoid prophage induction, as deletions of the whole region or single *loci* caused significant changes in efficiency and timing of this process. The effects of mutations are more pronounced in Shiga toxin-converting phage  $\Phi 24_B$  than in  $\lambda$  and in lysogenic *E. coli* cells treated with hydrogen peroxide than in UV-irradiated ones. Thus, the *exo-xis* region seems to be particularly important for  $\Phi 24_B$  phage under conditions of

the oxidative stress, the most likely conditions causing Shiga toxin-converting prophage induction during infection with EHEC.

**3.2. Hydrogen Peroxide-Mediated Prophage Induction Is a RecA-Dependent Process.** Efficient induction of lambdoid prophages is a RecA-dependent process due to a molecular mimicry between the phage cI repressor and the host-encoded LexA repressor which is self-cleaved after stimulation by the activated form of RecA protein under the S.O.S. response conditions [12–15]. Such a mimicry is well known for bacteriophage  $\lambda$  cI protein and LexA [12, 13], and we found that both domain structure and amino acid residues crucial for the self-cleavage are also conserved in cI repressor of phage  $24_B$  (Figure 3) (note that cI sequence of  $24_B$  is identical to that of another Shiga toxin-converting bacteriophage, 933 W [37]). Nevertheless, since RecA-independent induction of Shiga toxin-converting prophages has also been reported [16], we asked whether hydrogen peroxide-caused prophage induction depends on the activation of the S.O.S. response.

When testing  $H_2O_2$ - or UV-dependent induction of prophages  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi 24_B$ , and  $\Phi 24_B\Delta_{exo-xis}$  in *recA13*



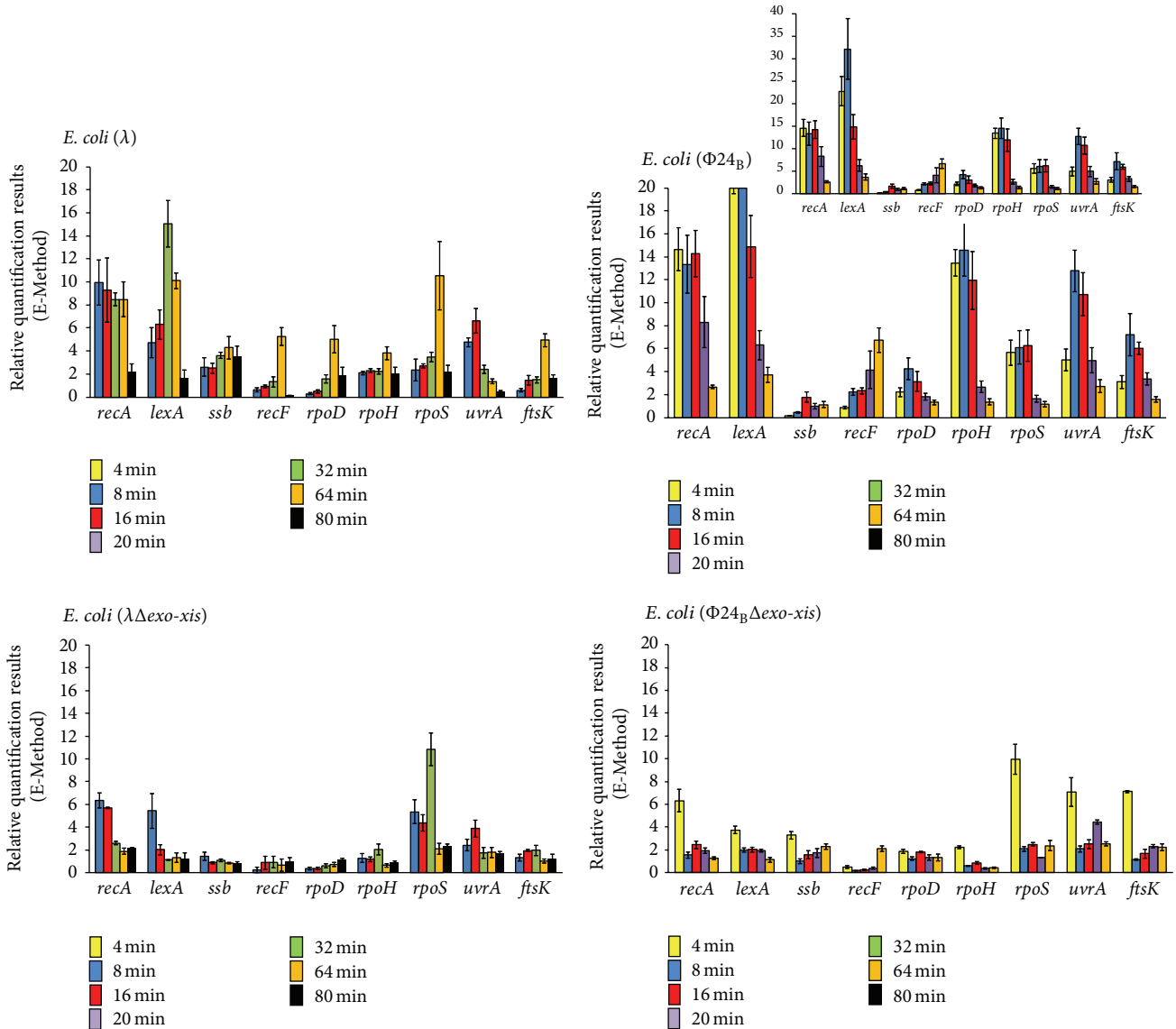


FIGURE 5: Expression of genes from the S.O.S. regulon in *E. coli* MG1655 lysogenic with  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi_{24B}$ , or  $\Phi_{24B}\Delta_{exo-xis}$ , at indicated times after treatment with 1 mM  $H_2O_2$ , as estimated by reverse transcription quantitative real-time PCR. The values obtained with untreated cells were used as calibrators and were subtracted from the values determined at particular time points; thus, the presented values indicate the induction of expression of tested genes. The presented results are mean values from 3 independent experiments (biological samples), with error bars indicating SD. The additional panel for *E. coli* ( $\Phi_{24B}$ ) represents the results with different scale.

mutant host, in the assays analogous to those presented in Figure 2, pfu/mL values were at the levels of those estimated for the uninduced controls ( $1.8 \pm 0.1 \times 10^2$ ,  $4.1 \pm 1.4 \times 10^2$ ,  $1.3 \pm 0.1 \times 10^3$ , and  $2.8 \pm 0.3 \times 10^3$  pfu/mL for  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi_{24B}$ , and  $\Phi_{24B}\Delta_{exo-xis}$ , resp.). Therefore, we conclude that induction of the investigated prophages under conditions of the oxidative stress (treatment with hydrogen peroxide) strongly depends on RecA function. Indeed, in cells lysogenic for  $\lambda$  or  $\Phi_{24B}$  and treated with UV light or hydrogen peroxide, efficient induction of the S.O.S. response was evident, as estimated with the SOS ChromoTest (Figure 4). Intriguingly, while induction of the S.O.S. response by hydrogen peroxide in  $\lambda\Delta_{exo-xis}$  lysogen was comparable to that in  $\lambda$  lysogen, the signal in the SOS ChromoTest in  $\Phi_{24B}\Delta_{exo-xis}$  lysogen

was considerably weaker than in the analogous experiment with  $\Phi_{24B}$  lysogen (Figure 4). No such difference could be observed in UV-irradiated bacteria (Figure 4).

**3.3. Deletion of the Exo-Xis Region Negatively Influences Expression of Genes from the S.O.S. Regulon in Hydrogen Peroxide-Treated Lysogenic Bacteria.** Since unexpected results were obtained in experiments with hydrogen peroxide-treated  $\Phi_{24B}\Delta_{exo-xis}$  lysogenic cells (Figure 4), we aimed to investigate the phenomenon of a less efficient induction of the S.O.S. response in more detail. Thus, expression of genes from the S.O.S. regulon was tested by reverse transcription quantitative real-time PCR in *E. coli* cells lysogenic for  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi_{24B}$ , and  $\Phi_{24B}\Delta_{exo-xis}$

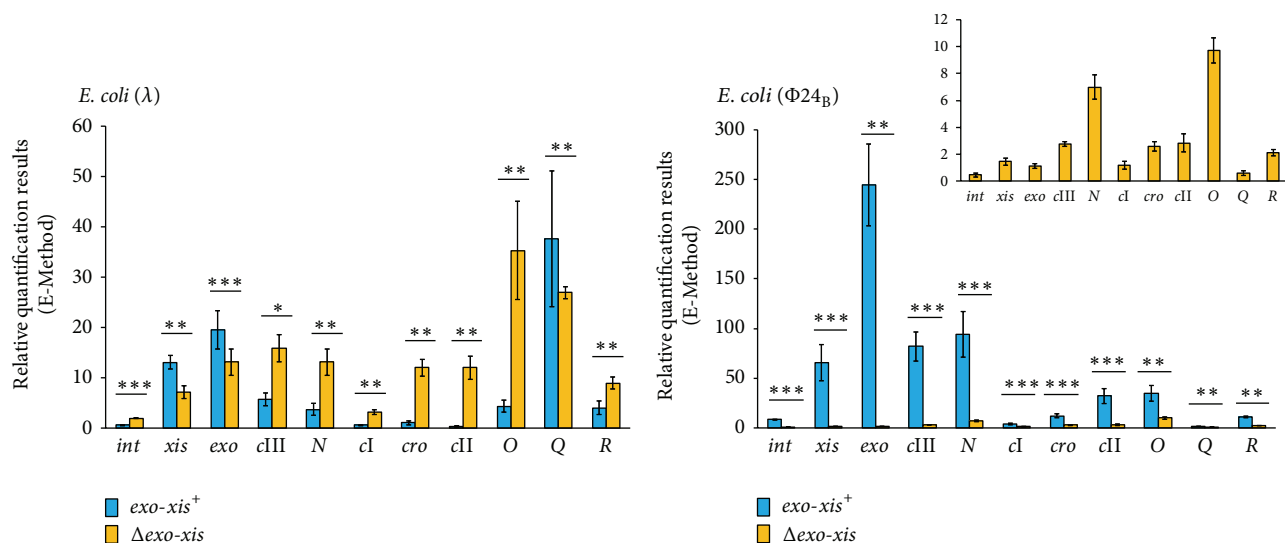


FIGURE 6: Expression of selected bacteriophage genes in *E. coli* MG1655 lysogenic with  $\lambda$  or  $\Phi 24_B$ , either wild-type (blue columns) or  $\Delta$ *exo-xis* (yellow columns) at 160 min after treatment with 1 mM  $H_2O_2$ , as estimated by reverse transcription quantitative real-time PCR. The values obtained with untreated cells were used as calibrators and were subtracted from the values determined at particular time points; thus, the presented values indicate the induction of expression of tested genes. The presented results are mean values from 3 independent experiments (biological samples), with error bars indicating SD. The additional panel for *E. coli* ( $24_B$ ) represents the results of  $\Delta$ *exo-xis* variant with different scale, due to very small values measured. Statistically significant differences (in *t*-test) are marked as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

TABLE 4: Duration of the lag phase of the phage lytic development after prophage induction with either hydrogen peroxide (1 mM) or UV irradiation ( $50 J/m^2$ ).

Strain	The time range of the switch from lag to log phase	
	$H_2O_2$ (1 mM)	UV ( $50 J/m^2$ )
MG1655 ( $\lambda$ )	60–90 min	60 min
MG1655 ( $\lambda\Delta$ <i>exo-xis</i> )	90–120 min	30–60 min
MG1655 ( $\lambda\Delta$ <i>orf5</i> )	60–90 min	30–60 min
MG1655 ( $\lambda\Delta$ <i>orf60a</i> )	60–90 min	30–60 min
MG1655 ( $\lambda\Delta$ <i>orf63</i> )	60–90 min	30–90 min
MG1655 ( $\lambda\Delta$ <i>orf61</i> )	60–90 min	30–60 min
MG1655 ( $\lambda\Delta$ <i>orf73</i> )	30–60 min	0–30 min
MG1655 ( $\lambda\Delta$ <i>ea22</i> )	60–90 min	30–60 min
MG1655 ( $\lambda\Delta$ <i>ea8.5</i> )	60–90 min	30–60 min
MG1655 ( $\Phi 24_B$ )	60–90 min	150–180 min
MG1655 ( $\Phi 24_B\Delta$ <i>exo-xis</i> )	<sup>a</sup>	150–180 min
MG1655 ( $\Phi 24_B\Delta$ <i>orf5</i> )	150–180 min	150–180 min
MG1655 ( $\Phi 24_B\Delta$ <i>orf60a</i> )	120–150 min	90–120 min
MG1655 ( $\Phi 24_B\Delta$ <i>orf63</i> )	120–150 min	30–60 min
MG1655 ( $\Phi 24_B\Delta$ <i>orf61</i> )	90–120 min	30–60 min
MG1655 ( $\Phi 24_B\Delta$ <i>orf73</i> )	90–120 min	120–150 min
MG1655 ( $\Phi 24_B\Delta$ <i>ea22</i> )	120–150 min	30–60 min

<sup>a</sup>The value was not determined due to a very low efficiency of prophage induction under these conditions (as shown in Figure 2).

and treated with hydrogen peroxide. In both  $\lambda$  and  $\Phi 24_B$ , deletion of the *exo-xis* region caused a significant reduction in the mRNA levels of most of the S.O.S. regulon genes relative to wild-type prophages, with exceptions of *rpoS*

gene in both phages and *ssb*, *uvrA*, and *ftsK* genes in  $\Phi 24_B$ , especially at later times after the treatment (Figure 5). Interestingly, in the case of wild-type  $\Phi 24_B$  lysogenic cells, the enhanced expression of particular genes from the S.O.S. regulon persisted longer, in most cases until 16 min after induction, whereas in the deletion mutant it decreases after 4 min (Figure 5). The impairment in expression of genes from the S.O.S. regulon (in particular *recA* and *lexA* genes, encoding the main regulators of the S.O.S. response) in the absence of the *exo-xis* region was more pronounced in  $\Phi 24_B$  than in  $\lambda$ . Moreover, induction of the S.O.S. regulon occurred significantly earlier in  $\Phi 24_B$  and  $\Phi 24_B\Delta$ *exo-xis* lysogens than in cells bearing  $\lambda$  and  $\lambda\Delta$ *exo-xis* prophages (Figure 5). These results might explain, at least partially, effects of deletions of *exo-xis* genes on prophage induction, demonstrated in Figure 2 and Table 4, particularly delayed induction of  $\Phi 24_B$  prophage devoid of certain genes and open reading frames, and less pronounced effects of their lack in  $\lambda$  than in  $\Phi 24_B$ .

Indications that overexpression of some genes from the *exo-xis* region of  $\lambda$  can influence host cell cycle and DNA replication have been reported previously [21, 22]. Suggestions that some genes of  $\Phi 24_B$  prophage may affect host growth were also published [38]. However, the results described in this subsection demonstrate for the first time that the *exo-xis* region can significantly modulate one of global cellular responses, the S.O.S. response, after treatment with hydrogen peroxide.

3.4. Expression of Crucial Phage Genes Is Dramatically Decreased after Treatment of Lysogenic Cells with Hydrogen Peroxide in the Absence of the Exo-Xis Region in  $\Phi 24_B$  Prophage. Expression of phage genes, crucial for the

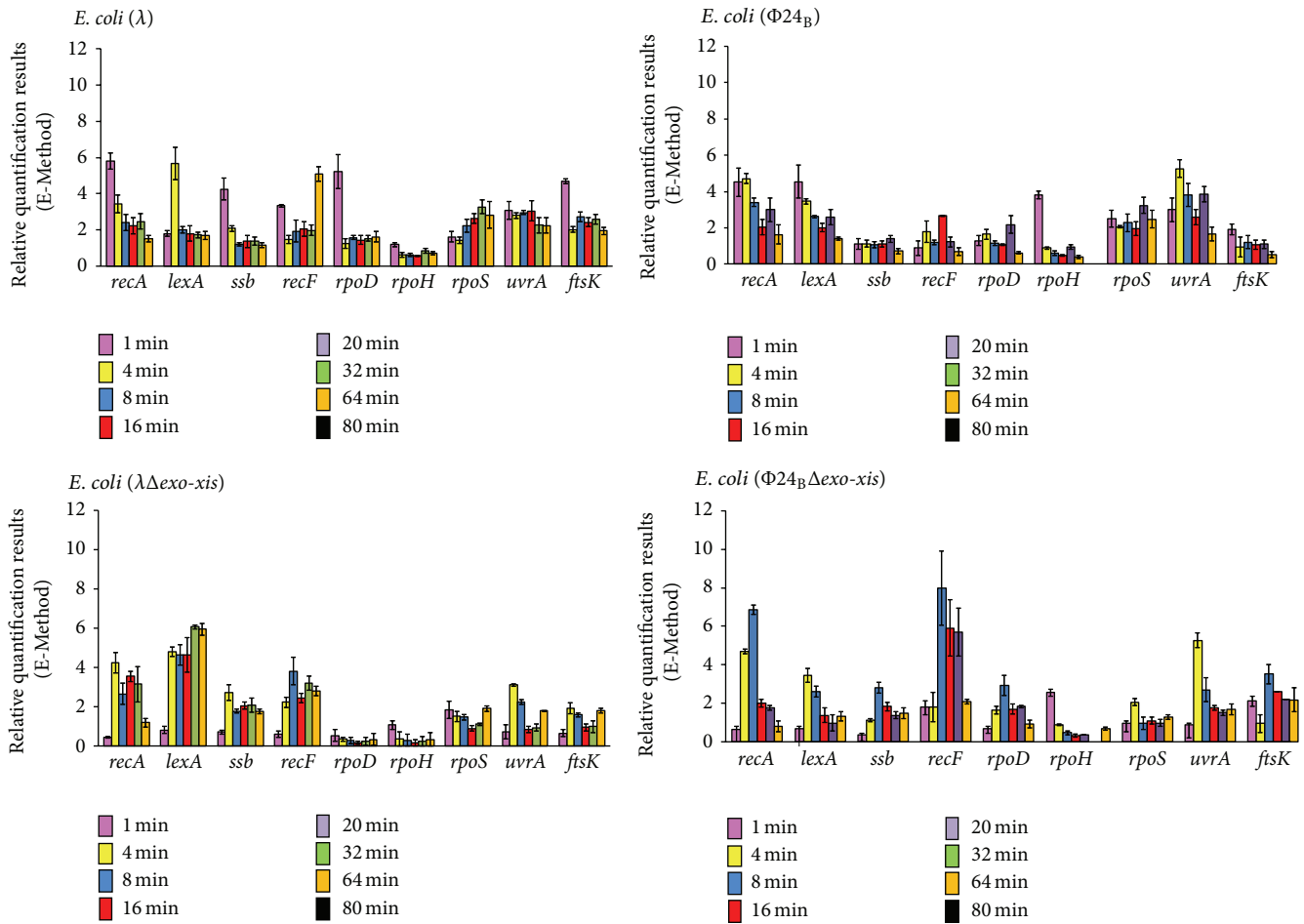


FIGURE 7: Expression of genes from the S.O.S. regulon in *E. coli* MG1655 lysogenic with  $\lambda$ ,  $\lambda\Delta_{exo-xis}$ ,  $\Phi24_B$ , or  $\Phi24_B\Delta_{exo-xis}$ , at indicated times after UV irradiation ( $50 \text{ J/m}^2$ ), as estimated by reverse transcription quantitative real-time PCR. The values obtained with untreated cells were used as calibrators and were subtracted from the values determined at particular time points; thus, the presented values indicate the induction of expression of tested genes. The presented results are mean values from 3 independent experiments (biological samples), with error bars indicating SD.

regulatory processes and lytic development, has been tested under the same conditions as described in the preceding subsection. The specific conditions and time after addition of hydrogen peroxide into the cell culture at which samples were withdrawn were chosen on the basis of similar experiments reported previously [26]. Interestingly, different effects of the deletion of the *exo-xis* region were observed for phages  $\lambda$  and  $24_B$ . In  $\lambda$ , deletion of genes and open reading frames located between *exo* and *xis* genes did not cause considerable effects on mRNA levels for *xis*, *exo*, and *Q*, whereas expression of *int*, *cIII*, *N*, *cI*, *cro*, *cII*, *O*, and *R* was enhanced upon treatment with hydrogen peroxide (Figure 6). Completely different results were obtained when  $\Phi24_B$  and  $\Phi24_B\Delta_{exo-xis}$  lysogens were studied; namely, expression of all tested genes was drastically impaired in hydrogen peroxide-treated bacteria in the absence of the *exo-xis* region on the prophage (Figure 6).

While negative regulation of transcription from *cII*-dependent promoters by overexpression of the *exo-xis* region has been reported previously in phage  $\lambda$  [23], this study demonstrated for the first time significant effects of this

region on expression of a battery of phage genes under conditions of the oxidative stress. The results presented in Figure 6 for phage  $\lambda$  are compatible with those published previously (though obtained with different methods) [23], as overexpression of the *exo-xis* region had opposite effects to those observed in its absence. On the other hand, severely impaired expression of all tested phage genes in  $\Phi24_B\Delta_{exo-xis}$  was unexpected. However, these results (Figure 6) can explain a strong defect in the induction of  $\Phi24_B\Delta_{exo-xis}$  prophage (and perhaps further lytic development) by hydrogen peroxide (Figure 2). Similarly, drastic differences between effects of  $\Delta_{exo-xis}$  mutations on hydrogen peroxide-mediated prophage induction between  $\lambda$  and  $24_B$  (Figure 2) can be ascribed to opposite regulation of expression of phage genes in the absence of the *exo-xis* region.

**3.5. Effects of the Exo-Xis Region on Expression of Host and Phage Gene in UV-Irradiated Lysogenic Cells.** Experiments analogous to those described in two preceding subsections were performed with lysogenic cells irradiated with UV.

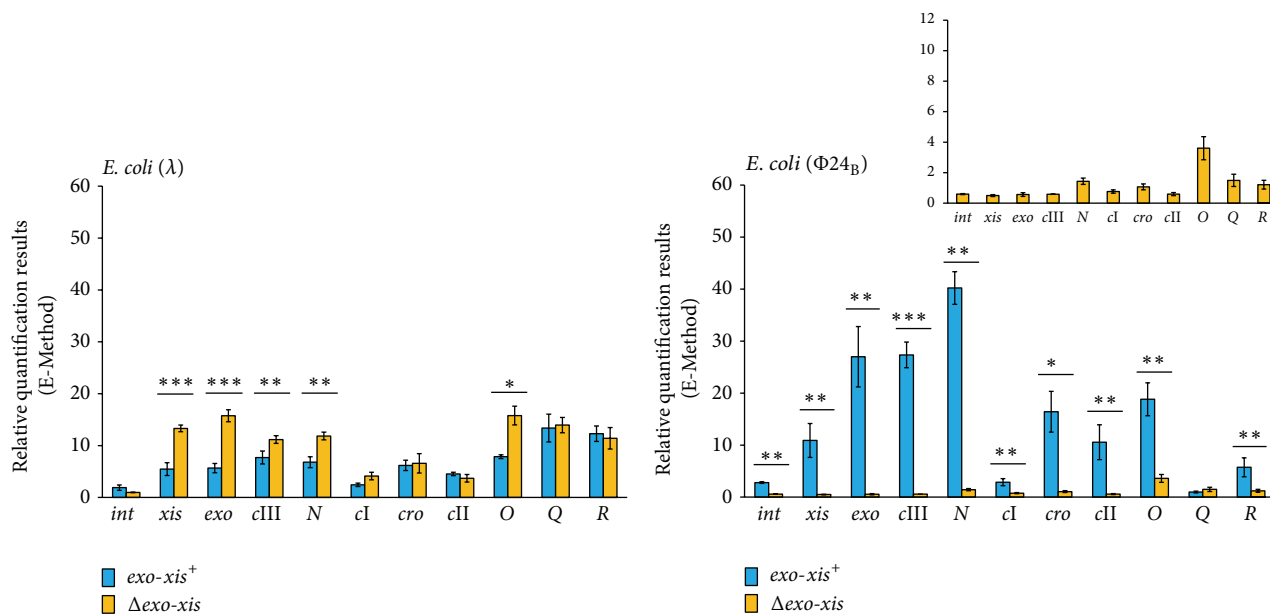


FIGURE 8: Expression of selected bacteriophage genes in *E. coli* MG1655 lysogenic with  $\lambda$  or  $\Phi$ 24<sub>B</sub>, either wild-type (blue columns) or  $\Delta$ *exo-xis* (yellow columns) at 160 min after UV irradiation (50 J/m<sup>2</sup>), as estimated by reverse transcription quantitative real-time PCR. The values obtained with untreated cells were used as calibrators and were subtracted from the values determined at particular time points; thus, the presented values indicate the induction of expression of tested genes. The presented results are mean values from 3 independent experiments (biological samples), with error bars indicating SD. The additional panel for *E. coli* (24<sub>B</sub>) represents the results of  $\Delta$ *exo-xis* variant with different scale, due to very small values measured. Statistically significant differences (in *t*-test) are marked as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

Interestingly, in both  $\lambda$  and 24<sub>B</sub> phages, deletion of the *exo-xis* region caused only moderate effects on expression of most genes from the S.O.S. regulon (Figure 7), contrary to hydrogen peroxide-treated bacteria where the differences were significantly higher (compare Figures 5 and 7). The exceptions in UV-irradiated cells were *rpoD*, *rpoH*, and *rpoS* genes in  $\lambda$  and *rpoH* and *rpoS* in 24<sub>B</sub>, whose expressions were at considerably lower level in the absence of the *exo-xis* region (Figure 7). One should also note that the induction of the S.O.S. regulon with UV irradiation was quicker than that with hydrogen peroxide. These results indicate that the influence of the *exo-xis* region on the S.O.S. response is particularly well pronounced under conditions of the oxidative stress.

Unlike the S.O.S. regulon expression, levels of mRNAs of bacteriophage genes in UV-irradiated cells were affected similarly to those in hydrogen peroxide-treated lysogenic bacteria by the absence of the *exo-xis* region (Figure 8). Again, although some differences were observed between  $\lambda$  and  $\lambda\Delta$ *exo-xis*, the differences between  $\Phi$ 24<sub>B</sub> and  $\Phi$ 24<sub>B $\Delta$ *exo-xis* were dramatic. This indicates that the influence of the *exo-xis* region on expression of phage genes after prophage induction does not depend on the induction agent.</sub>

#### 4. Conclusions

The *exo-xis* region is necessary for effective, RecA-dependent induction of Shiga toxin-converting bacteriophage  $\Phi$ 24<sub>B</sub> under conditions of the oxidative stress. In hydrogen peroxide-treated *E. coli*, this region positively influences expression of the S.O.S. regulon in both  $\Phi$ 24<sub>B</sub> and  $\lambda$  lysogens

and expression of phage genes crucial for lytic development (particularly *xis*, *exo*, *N*, *cro*, *O*, *Q*, and *R*) in  $\Phi$ 24<sub>B</sub>, but not in  $\lambda$ . Since the oxidative stress appears to be the major cause of induction of Shiga toxin-converting prophages during infections of human intestine by enterohemorrhagic *E. coli* (EHEC), the *exo-xis* region and/or products of its expression might be considered as potential targets for anti-EHEC drugs.

#### Conflict of Interests

The authors declare no conflict of interests.

#### Authors' Contribution

Katarzyna Licznarska and Aleksandra Dydecka contributed equally to this work.

#### Acknowledgment

This work was supported by the National Science Center (Poland) Grant no. 2013/09/B/NZ2/02366 to Alicja Węgrzyn.

#### References

- [1] C. L. Gyles, "Shiga toxin-producing *Escherichia coli*: an overview," *Journal of Animal Science*, vol. 85, no. 13, supplement, pp. E45–E62, 2007.
- [2] J. M. Hunt, "Shiga toxin-producing *Escherichia coli* (STEC)," *Clinics in Laboratory Medicine*, vol. 30, no. 1, pp. 21–45, 2010.

- [3] S. Razzaq, "Hemolytic uremic syndrome: an emerging health risk," *American Family Physician*, vol. 74, no. 6, pp. 991–998, 2006.
- [4] D. Law, "Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*," *Journal of Applied Microbiology*, vol. 88, no. 5, pp. 729–745, 2000.
- [5] L. Beutin and A. Martin, "Outbreak of shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains," *Journal of Food Protection*, vol. 75, no. 2, pp. 408–418, 2012.
- [6] S. K. Bloch, A. Felczykowska, and B. Nejman-Falenczyk, "*Escherichia coli* O104:H4 outbreak—have we learnt a lesson from it?" *Acta Biochimica Polonica*, vol. 59, no. 4, pp. 483–488, 2012.
- [7] H. Karch, E. Denamur, U. Dobrindt et al., "The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak," *EMBO Molecular Medicine*, vol. 4, no. 9, pp. 841–848, 2012.
- [8] C. R. Laing, Y. Zhang, M. W. Gilmour et al., "A comparison of Shiga-toxin 2 bacteriophage from classical enterohemorrhagic *Escherichia coli* serotypes and the German *E. coli* O104:H4 outbreak strain," *PLoS ONE*, vol. 7, no. 5, Article ID e37362, 2012.
- [9] A. Mellmann, D. Harmsen, C. A. Cummings et al., "Prospective genomic characterization of the german enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology," *PLoS ONE*, vol. 6, no. 7, Article ID e22751, 2011.
- [10] D. Werber, G. Krause, C. Frank et al., "Outbreaks of virulent diarrheagenic *Escherichia coli*—are we in control?" *BMC Medicine*, vol. 10, article 11, 2012.
- [11] H. E. Allison, "Stx-phages: drivers and mediators of the evolution of STEC and STEC-like pathogens," *Future Microbiology*, vol. 2, no. 2, pp. 165–174, 2007.
- [12] J. M. Łoś, M. Łoś, and G. Węgrzyn, "Bacteriophages carrying Shiga toxin genes: genomic variations, detection and potential treatment of pathogenic bacteria," *Future microbiology*, vol. 6, no. 8, pp. 909–924, 2011.
- [13] M. Ptashne, *A Genetic Switch: Phage Lambda Revisited*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 3rd edition, 2004.
- [14] G. Węgrzyn and A. Węgrzyn, "Genetic switches during bacteriophage  $\lambda$  development," *Progress in Nucleic Acid Research and Molecular Biology*, vol. 79, pp. 1–48, 2005.
- [15] G. Węgrzyn, K. Licznarska, and A. Węgrzyn, "Phage  $\lambda$ -new insights into regulatory circuits," *Advances in Virus Research*, vol. 82, pp. 155–178, 2012.
- [16] L. Imamovic and M. Muniesa, "Characterizing RecA-independent induction of Shiga toxin2-encoding phages by EDTA treatment," *PLoS ONE*, vol. 7, no. 2, Article ID e32393, 2012.
- [17] J. M. Łoś, M. Łoś, A. Węgrzyn, and G. Węgrzyn, "Altruism of Shiga toxin-producing *Escherichia coli*: recent hypothesis versus experimental results," *Frontiers in Cellular and Infection Microbiology*, vol. 2, article 166, 2013.
- [18] P. L. Wagner, D. W. K. Acheson, and M. K. Waldor, "Human neutrophils and their products induce Shiga toxin production by enterohemorrhagic *Escherichia coli*," *Infection and Immunity*, vol. 69, no. 3, pp. 1934–1937, 2001.
- [19] J. M. Łoś, M. Łoś, G. Węgrzyn, and A. Węgrzyn, "Differential efficiency of induction of various lambdoid prophages responsible for production of Shiga toxins in response to different induction agents," *Microbial Pathogenesis*, vol. 47, no. 6, pp. 289–298, 2009.
- [20] J. M. Łoś, M. Łoś, A. Węgrzyn, and G. Węgrzyn, "Hydrogen peroxide-mediated induction of the Shiga toxin-converting lambdoid prophage ST2-8624 in *Escherichia coli* O157:H7," *FEMS Immunology and Medical Microbiology*, vol. 58, no. 3, pp. 322–329, 2010.
- [21] P. Kourilsky and A. Knapp, "Lysogenization by bacteriophage lambda. III.—multiplicity dependent phenomena occurring upon infection by lambda," *Biochimie*, vol. 56, no. 11-12, pp. 1517–1523, 1975.
- [22] K. Sergueev, D. Court, L. Reaves, and S. Austin, "*E. coli* cell-cycle regulation by bacteriophage  $\lambda$ ," *Journal of Molecular Biology*, vol. 324, no. 2, pp. 297–307, 2002.
- [23] J. M. Łoś, M. Łoś, A. Węgrzyn, and G. Węgrzyn, "Role of the bacteriophage  $\lambda$  *exo-xis* region in the virus development," *Folia Microbiologica*, vol. 53, no. 5, pp. 443–450, 2008.
- [24] S. Bloch, B. Nejman-Falenczyk, J. M. Łoś et al., "Genes from the *exo-xis* region of  $\lambda$  and Shiga toxin-converting bacteriophages influence lysogenization and prophage induction," *Archives of Microbiology*, vol. 195, no. 10-11, pp. 693–703, 2013.
- [25] J. J. Kwan, E. Smirnova, S. Khazai, F. Evanics, K. L. Maxwell, and L. W. Donaldson, "The solution structures of two prophage homologues of the bacteriophage  $\lambda$  Ea8.5 protein reveal a newly discovered hybrid homeodomain/zinc-finger fold," *Biochemistry*, vol. 52, no. 21, pp. 3612–3614, 2013.
- [26] S. Bloch, B. Nejman-Falenczyk, A. Dydecka et al., "Different expression patterns of genes from the Exo-Xis region of bacteriophage  $\lambda$  and Shiga toxin-converting bacteriophage  $\Phi$ 24<sub>B</sub> following infection or prophage induction in *Escherichia coli*," *PLoS ONE*, vol. 9, no. 10, Article ID e108233, 2014.
- [27] K. F. Jensen, "The *Escherichia coli* K-12 'wild types' W3110 and MG1655 have an *rph* frameshift mutation that leads to pyrimidine starvation due to low *pyrE* expression levels," *Journal of Bacteriology*, vol. 175, no. 11, pp. 3401–3407, 1993.
- [28] H. E. Allison, M. J. Sergeant, C. E. James et al., "Immunity profiles of wild-type and recombinant Shiga-like toxin-encoding bacteriophages and characterization of novel double lysogens," *Infection and Immunity*, vol. 71, no. 6, pp. 3409–3418, 2003.
- [29] B. Nejman-Falenczyk, S. Bloch, K. Licznarska et al., "A small, microRNA-size, ribonucleic acid regulating gene expression and development of Shiga toxin-converting bacteriophage  $\Phi$ 24<sub>B</sub>," *Scientific Reports*, vol. 5, Article ID 10080, 2015.
- [30] J. M. Łoś, P. Golec, G. Węgrzyn, A. Węgrzyn, and M. Łoś, "Simple method for plating *Escherichia coli* bacteriophages forming very small plaques or no plaques under standard conditions," *Applied and Environmental Microbiology*, vol. 74, no. 16, pp. 5113–5120, 2008.
- [31] P. Quillardet, O. Huisman, R. D'Ari, and M. Hofnung, "SOS chromotest, a direct assay of induction of an SOS function in *Escherichia coli* K-12 to measure genotoxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 79, no. 19, pp. 5971–5975, 1982.
- [32] F. Fish, I. Lampert, and A. Halachmi, "The SOS chromotest kit: a rapid method for the detection of genotoxicity," *Toxicity Assessment*, vol. 2, no. 2, pp. 135–147, 1987.
- [33] A. Herman-Antosiewicz and G. Węgrzyn, "Regulation of copy number and stability of phage  $\lambda$  derived pTC $\lambda$ 1 plasmid in the light of the dimer/multimer catastrophe hypothesis," *FEMS Microbiology Letters*, vol. 176, no. 2, pp. 489–493, 1999.
- [34] E. Strauch, J. A. Hammerl, A. Konietzny et al., "Bacteriophage 2851 is a prototype phage for dissemination of the Shiga toxin

- variant gene 2c in *Escherichia coli* O157:H7,” *Infection and Immunity*, vol. 76, no. 12, pp. 5466–5477, 2008.
- [35] D. Nowicki, S. Bloch, B. Nejman-Faleńczyk, A. Szalewska-Pałasz, A. Węgrzyn, and G. Węgrzyn, “Defects in RNA polyadenylation impair both lysogenization by and lytic development of Shiga toxin-converting bacteriophages,” *Journal of General Virology*, vol. 96, no. 7, pp. 1957–1968, 2015.
- [36] R. D. Finn, A. Bateman, J. Clements et al., “Pfam: the protein families database,” *Nucleic Acids Research*, vol. 42, no. 1, pp. D222–D230, 2014.
- [37] A. P. Koudelka, L. A. Hufnagel, and G. B. Koudelka, “Purification and characterization of the repressor of the shiga toxin-encoding bacteriophage 933W: DNA binding, gene regulation, and autocleavage,” *Journal of Bacteriology*, vol. 186, no. 22, pp. 7659–7669, 2004.
- [38] L. M. Riley, M. Veses-Garcia, J. D. Hillman, M. Handfield, A. J. McCarthy, and H. E. Allison, “Identification of genes expressed in cultures of *E. coli* lysogens carrying the Shiga toxin-encoding prophage  $\Phi$ 24B,” *BMC Microbiology*, vol. 12, article 42, 2012.