ISSN 0006-2979, Biochemistry (Moscow), 2018, Vol. 83, No. 3, pp. 270-280. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © O. V. Sergeeva, D. O. Bredikhin, M. V. Nesterchuk, M. V. Serebryakova, P. V. Sergiev, O. A. Dontsova, 2018, published in Biokhimiya, 2018, Vol. 83, No. 3, pp. 391-403.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM17-414, January 15, 2018.

Possible Role of *Escherichia coli* Protein YbgI

O. V. Sergeeva[1,2]*, D. O. Bredikhin[2], M. V. Nesterchuk[1], M. V. Serebryakova[2], P. V. Sergiev[1,2], and O. A. Dontsova[1,2]

[1]*Skolkovo Institute of Science and Technology, 143026 Skolkovo, Moscow Region, Russia; E-mail: o.sergeeva@skoltech.ru*
[2]*Lomonosov Moscow State University, 119991 Moscow, Russia*

Received September 5, 2017
Revision received December 5, 2017

Abstract—Proteins containing the NIF3 domain are highly conserved and are found in bacteria, eukaryotes, and archaea. YbgI is an *Escherichia coli* protein whose gene is conserved among bacteria. The structure of YbgI is known; however, the function of this protein in cells remains obscure. Our studies of *E. coli* cells with deleted *ybgI* gene suggest that YbgI is involved in formation of the bacterial cell wall.

DOI: 10.1134/S0006297918030070

Keywords: *Escherichia coli*, cell stress, bacterial cell wall

The genome of one important model organism, the eubacterium *Escherichia coli*, contains numerous genes whose functions are still unknown. One of them is *ybgI*, which belongs to an operon that also includes poorly studied genes: *ybgJ* (predicted hydrolase), *ybgK* (predicted carboxyl transferase), *ybgL* (predicted component of lactam metabolism), and *nei* (endonuclease VIII) genes. Endonuclease VIII is involved in base excision repair (BER) [1]; it can also act as a lyase in the removal of apurinic/apyrimidinic sites from DNA [2].

YbgI protein encoded by the *ybgI* gene consists of 247 amino acids and has a molecular weight of 27 kDa. It belongs to the DUF34 protein family with unknown function [3]. Proteins of this family contain the highly conserved NIF3 domain that has been found in eukaryotes, bacteria, and archaea [4]. The best-studied representative of this family is the yeast protein NIF3; its amino acid sequence is 22% identical to the sequence of *E. coli* YbgI [5]. NIF3 interacts with the *N*-terminal domain of the NGG1p transcription coactivator in yeast [6]. NIF3L1 from *Homo sapiens* and Nif3l1 from *Mus musculus* are 22 and 37% identical, respectfully, to yeast NIF3. These proteins are expressed in stem cells, but their role in cells remains obscure. Note that both NIF3L1 and Nif3l1 have highly conserved *N*- and *C*-terminal domains connected by a sequence that is absent in yeast NIF3 and most bacterial proteins of the NIF3 family [7].

Studies of the cell stress response revealed that mitomycin C-induced genotoxic stress in *E. coli* is accompanied by a 3.5-fold upregulation of the expression of *ybgI*. However, after some time, the level of *ybgI* expression diminishes despite the presence of mitomycin C [8]. Also, deletion of the *ybgI* gene decreases by 2 to 3 orders of magnitude the survival of bacterial cells subjected to ionizing radiation (2000 Gy) [9].

According to X-ray crystallography, three dimeric YbgI molecules form a toroid in which each subunit has a metal-binding site at the inner surface. The identity of divalent metal ligands in the YbgI molecule is unknown; it was suggested that they might be iron, zinc, manganese, or magnesium ions [5]. Based on previously obtained data and colocalization of the *ybgI* and endonuclease VIII genes in the same operon, it was suggested that YbgI is involved in BER under stress conditions. The goal of this work was to investigate the role of YbgI in *E. coli* cells.

MATERIALS AND METHODS

Analysis of the surface charge of YbgI. To identify and analyze equipotential surfaces in the YbgI molecule, we used the APBS (Adaptive Poisson–Boltzmann Solver) program package [10] for the PyMol molecular graphics system [11], which estimates the electrostatic properties of biomolecules by solving the Poisson–Boltzmann equation.

Affinity chromatography. *Escherichia coli* cells transformed with the pCA24 plasmid carrying the recombinant

* To whom correspondence should be addressed.

His_6-YbgI protein gene [12] were grown in Petri dishes on LB agar medium (1% bactotryptone, 0.5% yeast extract, and 171 mM NaCl) containing chloramphenicol. Single colonies were selected for growth in liquid LB medium. The cells were incubated overnight in the presence of chloramphenicol and then used for inoculation of chloramphenicol-containing LB medium. The cells were grown at 37°C on a shaker (180 rpm) to optical density $A_{600} = 0.3$. Protein biosynthesis was induced by adding 1 mM IPTG, and the cells were incubated for another 2 h. The cells were cooled, pelleted by centrifugation at 9000 rpm at 4°C for 15 min, and washed with lysis buffer (20 mM HEPES-KOH, pH 7.5, 200 mM NH_4OAc, 10 mM imidazole, 0.1% Triton X-100, and 1 mg/ml lysozyme). The pellet was then resuspended in the lysis buffer, and the cells were disintegrated by sonication (10 cycles for 15 s with a 45-s pause between the cycles). The supernatant was mixed with 50% suspension of Ni-NTA-agarose (Qiagen, USA) and incubated on a rotator mixer at 200 rpm for 1 h at 4°C. The resin was separated by centrifugation at 3000 rpm for 1 min, washed with buffer P (20 mM HEPES-KOH, pH 7.5, 200 mM NH_4OAc, 40 mM imidazole, and 0.1% Triton X-100) for 15 min, and separated from the buffer by centrifugation at 3000 rpm for 1 min. The protein was eluted with a step-wise gradient of imidazole (40, 60, 80, 100, 150, 200, 250, 300, and 600 mM) in buffer L (20 mM HEPES-KOH, pH 7.5, 200 mM NH_4OAc, and 0.1% Triton X-100). At each imidazole concentration, the resin was incubated with the eluent for 15 min with gentle shaking at 4°C and then centrifuged at 3000 rpm for 1 min to collect the supernatant.

The resulting protein solutions were dialyzed against buffer D (20 mM HEPES-KOH, pH 7.5, 1 mM $Mg(OAc)_2$, 200 mM NH_4OAc, 10% glycerol, and 0.1% Triton X-100) for 2 h at room temperature and analyzed by electrophoresis in denaturing polyacrylamide gel. Protein aliquots were mixed with sample buffer (50 mM Tris-HCl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% Bromophenol Blue, 10% glycerol) and fractionated by 8-12% SDS-PAGE to determine protein purity and concentration. Lysates of wild-type (wt) *E. coli* cells and cell overexpressing His_6-tagged YhhF protein (molecular mass ~22 kDa) were used as controls. The protein was further identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) of tryptic hydrolysates of the protein.

Cell growth in the presence of various nitrogen sources. *Escherichia coli* wt (BW25141) and *ybgI*-deficient cells (Δ*ybgI*; JW0700 from the Keio collection [13]) were grown in minimal M9 medium without ammonium chloride. The following compounds were used as nitrogen sources (20 mM): adenine, NH_4Cl, urea, uridine, and α-amino acids (L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine).

The optical density of the cell suspension was determined every 40 min for 20 h, i.e., before the cells entered the stationary phase. The number of cells after time Δt was calculated using the formula:

$$N_2 = 2^{\Delta t/T} \times N_1.$$

The doubling time was calculated using the formula:

$$T = \Delta t \cdot \ln(2)/[\ln(N_2) - \ln(N_1)],$$

where T is the doubling time, N_1 and N_2 are the values of cell suspension optical density that corresponded to the cell number in a culture, and Δt is the time between measurements N_1 and N_2.

RT-qPCR for mRNA quantification. *Escherichia coli* wt and Δ*ybgI* cells were grown in the minimal M9 medium to the logarithmic and stationary phases. Total RNA was isolated with an RNeasy Mini Kit (Qiagen) and used as a template in the reaction of cDNA synthesis with a Maxima Universal First Strand cDNA Synthesis Kit (Thermo Scientific, USA) and random primers.

qRCR was performed using the SYBR Green dye. Based on the results of earlier studies, we amplified the *dps*, *gadA*, *glmS*, *pheA*, *prsA*, *rsuA*, *tnaA*, *ygaU*, and *ygiW* genes from *E. coli* using primers given in Table 1. 16S rRNA was used as a reference molecule because its con-

Table 1. Primers used in the study

Target	Primer sequence, 5′-3′	
	forward	reverse
glmS	GTACTTCTTATAACTCCG	GTATCCGCGGTTTCGC
rsuA	GCAGTTTCCACGCTAC	CCTGAACATGATGTCGC
prsA	GTAGCCCGATCCTGC	GATGATATGCATCACCTG
pheA	CCATTGTTTGTTGGTCTC	GGGTAACAGCCCAATAC
tnaA	CTGAAAACGCCTATTTCA	GTCGTCTTTCATGCACA
ygiW	CTGCGCGGCAATATC	GAATTCCAGTCTTTATCG
dps	GGTCCCCAGAGCTAC	GATTACCAAACAAGCGC
ygaU	GCGAAGGAGAAAATCCT	CGAAGATTTTATTGTACAG
16S rRNA	TCGTCAGCTCGTGTTGTG	GCTTCTCTTTGTATGCGCCATT

centration in bacterial cells varies much less that the concentrations of other RNAs. Two-fold (or higher) change in the mRNA content was considered significant. All experiments were performed in triplicate.

Differential two-dimensional (2D) electrophoresis. Cells were centrifuged at 4000 rpm; the pellets were washed twice with TBS (50 mM Tris-HCl, pH 7.6, 150 mM NaCl), centrifuged again at 4000 rpm, resuspended in 40% CHAPS/NP-40, incubated with 0.08 mg/ml RNase A and 1 U/μl DNase I, and lysed by subjecting them to 5 cycles of freezing/thawing (liquid nitrogen/37°C). The lysates were incubated on ice for 30 min, mixed with sample loading buffer (30 mM Tris-HCl, pH 8.5, 7 M urea, 2 M thiourea, 3% CHAPS, 1% Nonidet P-40), and centrifuged at 12,000 rpm. Protein concentration in the supernatants was determined by the Bradford method [14].

Aliquots containing 100 μg protein were mixed with 400 pmol of fluorescent dyes Cy3 (wt cells) or Cy5 (Δ*ybgI* cells) and incubated for 30 min on ice; 10 mM lysine was then added, and the mixtures were incubated on ice for another 15 min.

Gels for isoelectric focusing were cast in glass tubes (diameter, 1 mm). Protein samples from the wt and Δ*ybgI* cells were mixed with each other; 1% ampholytes (BioRad, USA), and 1% dithiothreitol were added. The samples were loaded onto the gels, and isoelectrofocusing was performed in the following regime: linear voltage gradient of 50-600 V for 6 h, 700 V for 10 h, and 900 V for 2 h.

After isofocusing, the gels were extracted from the tubes, incubated for 30 min in the equilibration buffer (150 mM Tris-HCl, pH 6.8, 8 M urea, 2.5% SDS, and 50% glycerol), and placed on 8-16% gradient polyacrylamide gels. Electrophoresis in the second direction was performed at 250 V for 5 h. After the electrophoresis, the gels were fixed in 20% ethanol, 10% acetic acid for 3 h, washed three times with deionized water, and scanned with a Typhoon fluorescence scanner (GE Healthcare Life Sciences, USA) to identify protein zones that differed in the compared samples. The gels were then stained with silver nitrate to cut out the protein spots for further identification. The gels were washed with 0.03% sodium thiosulfate, rinsed three times with water, stained with 0.1% silver nitrate in 0.04% formaldehyde for 15-20 min, washed with water, and incubated with developing solution (4% Na_2CO_3, 0.0006% $Na_2S_2O_3$, 0.04% formaldehyde) until full development. The staining was stopped with 10% acetic acid.

Identification of PAGE-separated proteins. Protein zones from the silver-stained gels were cut out, rinsed twice with washing buffer (50 mM NH_4HCO_3, 40% acetonitrile) for 20 min, dehydrated in acetonitrile for 5 min, and dried in the open air. Dried pieces of the gel were incubated with 10 ng/μl trypsin solution in 100 mM NH_4HCO_3 for 4 h at 37°C. After incubation, an equal volume of 0.5% trifluoroacetic acid was added; the samples were loaded on a support, dried in the open air, and analyzed by MALDI-MS using an Ultraflex II mass spectrometer (Bruker, USA). The mass spectra were analyzed with the flexAnalysis 3.2 program; the proteins were identified using the Mascot 2.4.2 program and the NCBI database (see Supplement to this article on the journal website http://protein.bio.msu.ru/biokhimiya and Springer site Link.springer.com).

Intracellular localization of YbgI. *Escherichia coli* cells expressing chimeric GFP-modified PstB, TktA, CheW, and DnaT proteins from the ASKA *E. coli* cells' library [12] were used for preparing competent cells. These proteins were chosen based on information from the MicrobesOnline database and published data on their cell localization. The competent cells were transformed with the plasmid coding for the chimeric mCherry-YbgI protein (obtained in our laboratory). The cells were grown to the logarithmic phase, placed on microscope slides in Mowiol mounting medium (Sigma, USA), covered with coverslips, and analyzed under a Nikon C2 fluorescence microscope (Nikon, Japan) for colocalization of the green and red fluorescence.

Cell response to UV-induced DNA damage. *Escherichia coli* wt, Δ*ybgI*, and *recA*-deficient cells were grown in LB medium to the logarithmic phase and the same optical density. Cell aliquots (~10 μl) were placed on a piece of Parafilm, subjected to UV irradiation at 254 nm (dose, 50 mJ/cm^2) for different time intervals (from 5 s to 10 min), plated on Petri dishes with LB agar medium, and incubated overnight at 37°C. The grown colonies were counted the next day.

RESULTS

Role of YbgI in cell response to UV-induced DNA damage. Based on the YbgI crystal structure, Ladner et al. [5] suggested that YbgI interacts with DNA. YbgI consists of three domains that form a toroidal structure with an inner diameter of 30 Å. It can be assumed that nucleic acids can bind in the center of the protein complex, because the diameter of the B-DNA double helix is 20 Å [15].

To test this hypothesis, we calculated the distribution of the surface charge on the YbgI molecule using the PyMol program with the APBS plug-in (Fig. 1). As seen in Fig. 1, the protein has positively and negatively charged areas, although most of its surface around the central hole of the toroid is negatively charged, which suggests against nucleic acid binding to YbgI.

Nevertheless, the location of the *ybgI* gene in the same operon with the *nei* gene for endonuclease VIII might indicate a possible role of YbgI in DNA repair. To see if this is the case, we UV-irradiated wt and Δ*ybgI* cells at 254 nm (intensity, 50 mJ) for time intervals of 5 s to 10 min. As a positive control, we used cells deficient in

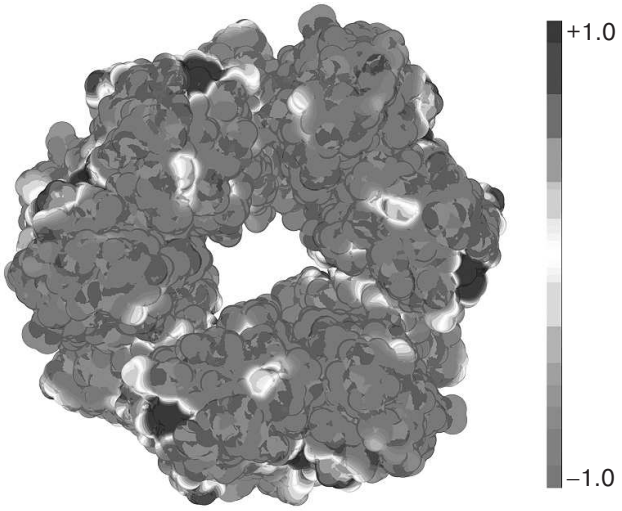

Fig. 1. Distribution of electrostatic charge on the surface of YbgI protein (PDB ID: 1NMP). Gray and black colors designate negative and positive surface charges, respectively.

the *recA* gene required for recombinational DNA repair and SOS response in bacteria [16].

As expected, *E. coli recA*-deficient cells were more sensitive to DNA damage and started to die already after 4 min of irradiation. At the same time, survival of the Δ*ybgI* cells was not affected even by irradiation for 10 min (Fig. 2). Therefore, it is unlikely that YbgI contributes to the repair of the UV-induced DNA damage to the same extent as RecA.

Cellular localization of YbgI. Cellular localization of YbgI might help in understanding the function of this protein. We expressed the chimeric protein YbgI protein *C*-terminally fused with the red fluorescent protein mCherry in *E. coli* AG1 cells. Unexpectedly, YbgI localized to the cell poles (Fig. 3a).

Next, we studied YbgI-mCherry colocalization with some recombinant *E. coli* proteins modified with GFP at their *C*-termini. The proteins were chosen based on published data on their cellular localization and analysis of the MicrobesOnline database for potential protein–protein interactions. The plasmid coding for YbgI-mCherry was used for transformation of *E. coil* AG1 cells expressing these GFP-containing proteins [12]. We found that YbgI colocalized only with two proteins: PstB and TktA (Fig. 3, b and c). TktA is a transketolase and a key enzyme in glycolysis and pentose phosphate pathway [17]; PstB is the ATP-binding subunit of the cell protein complex responsible for phosphate transport across the cell membrane [18].

Search for YbgI-interacting proteins. Recombinant YbgI with the *C*-terminal His_6-tag (pCA24YbgI plasmid) was expressed in *E. coli* AG1 cells and purified [12] by

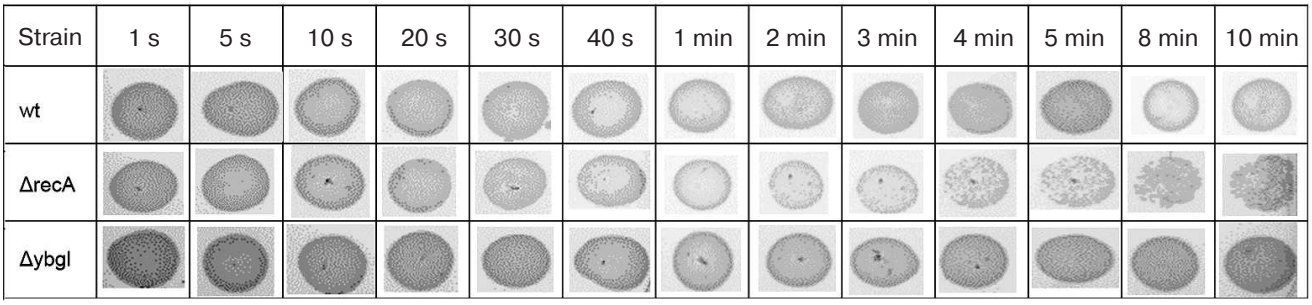

Fig. 2. Cell survival (number of colonies grown after plating 10 μl of bacterial culture from the exponential growth phase) after UV-irradiation for indicated times.

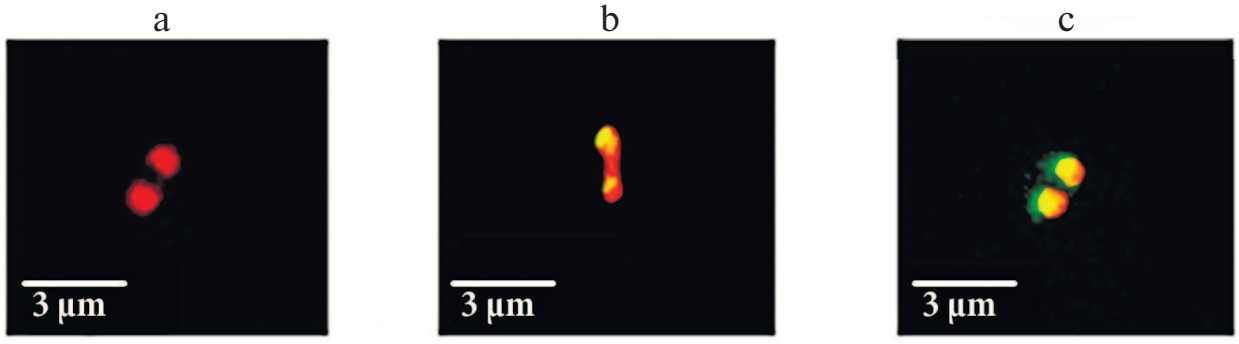

Fig. 3. Localization of YbgI in bacterial cells. a) YbgI-mCherry chimeric protein. b) Colocalization of YbgI-mCherry with recombinant PstB-GFP protein. c) Colocalization of YbgI-mCherry with recombinant TktA-GFP protein. Each picture shows a single bacterial cell.

affinity chromatography with the goal of identifying potential YbgI interaction partners. Bacterial cell lysate containing YbgI-His_6 was incubated with Ni-NTA-Sepharose; the bound proteins were eluted with a gradient of imidazole concentration, fractionated in denaturing polyacrylamide gel, and analyzed by MALDI mass spectrometry. We suggested that proteins that copurify with YbgI might interact with it in the cell. As a control, we used *N*-terminally His_6-tagged bacterial methyltransferase YhhF.

Four proteins copurifying with YbgI were identified by mass spectrometry of the tryptic hydrolysate of the eluted protein (Fig. 4): prephenate dehydratase PheA (*1*), L-glutamine:D-fructose-6-phosphate aminotransferase GlmS (*2*), ribose-phosphate pyrophosphokinase PrsA (*3*), and 16S rRNA pseudouridylate synthase RsuA (*4*) (see Supplement). GlmS was the only protein found in the fraction with the maximum YbgI content after elution with high imidazole concentration (600 mM); therefore, GlmS might be an interaction partner of YbgI in the cell.

GlmS catalyzes conversion of fructose-6-phosphate into glucosamine-6-phosphate utilizing glutamine as a nitrogen source; one of the reaction products is L-glutamate [19]. This reaction is one of the stages in the synthesis of UDP-N-acetylglucosamine, which is among major components of the cell wall peptidoglycan in bacteria [20].

Characterization of Δ*ybgI* cells. GlmS uses glutamine as a nitrogen source and copurifies with YbgI. We compared the growth rates of *E. coli* Δ*ybgI* [13] and wt cells in minimal medium containing various nitrogen sources (Fig. 5). We found that deletion of the *ybgI* gene did not affect the growth of cells for any of the nitrogen sources used.

Comparative proteomic analysis of the wt and Δ*ybgI* cells might help to elucidate the role of YbgI protein in the cell. The bacteria were grown in the LB and M9 minimal media. Proteins from the wt cells were labeled with green fluorescent Cy3 dye; proteins from the Δ*ybgI* cells – with red fluorescent Cy5 dye. Both dyes covalently bind to the side chain amino groups in proteins. The samples were fractionated by isoelectric focusing followed by electrophoresis in denaturing polyacrylamide gel. If the content of the protein remained the same, the corresponding zone in the gel was identified by its yellow color; if the level of protein expression changed, the color of the corresponding spot was either green or red (Fig. 6).

No differences were found between wt and Δ*ybgI* cells grown in the LB medium under normal conditions. However, in the M9 minimal medium, the expression levels of eight proteins changed in the Δ*ybgI* cells (Table 2). The expression of TnaA and AphA was upregulated, while expression of YgiW, YgaU, Dps, and OsmY was downregulated.

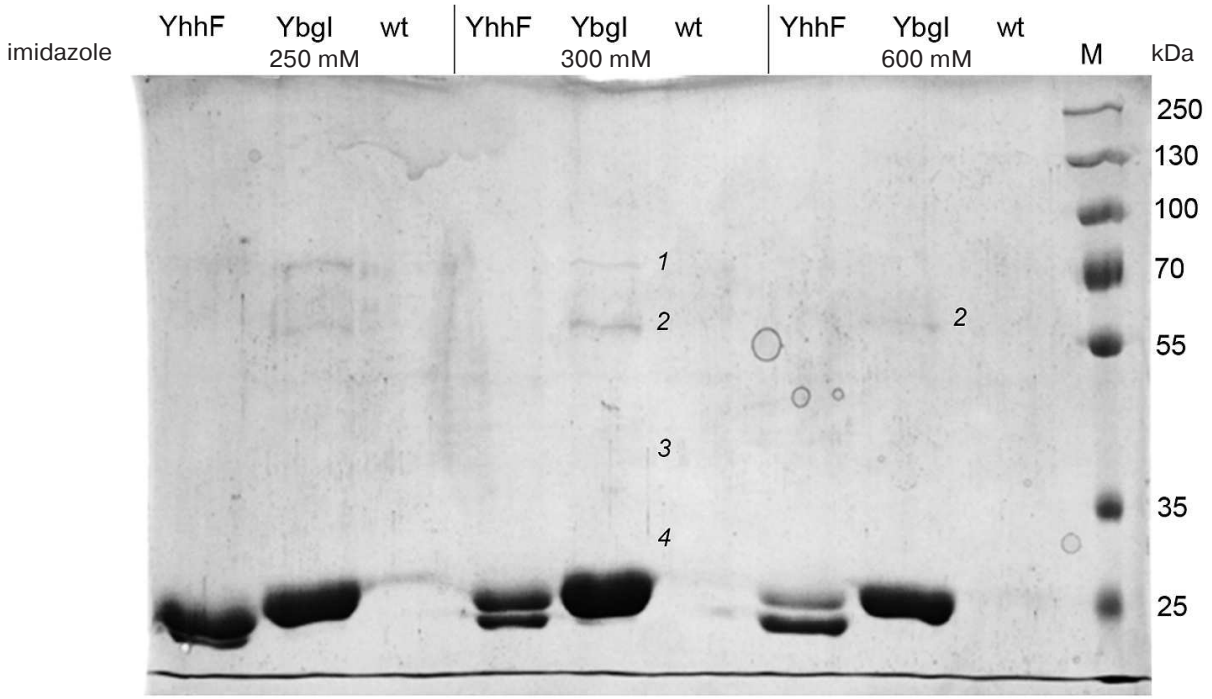

Fig. 4. Affinity purification of YbgI-His6; YhhF and wt are used as controls; 250, 300, and 600, imidazole concentration in the eluent (mM); M, PageRuler Plus Prestained Protein Ladder (ThermoScientific, USA); *1-4*) fractions analyzed by MALDI mass spectrometry (see description in the text).

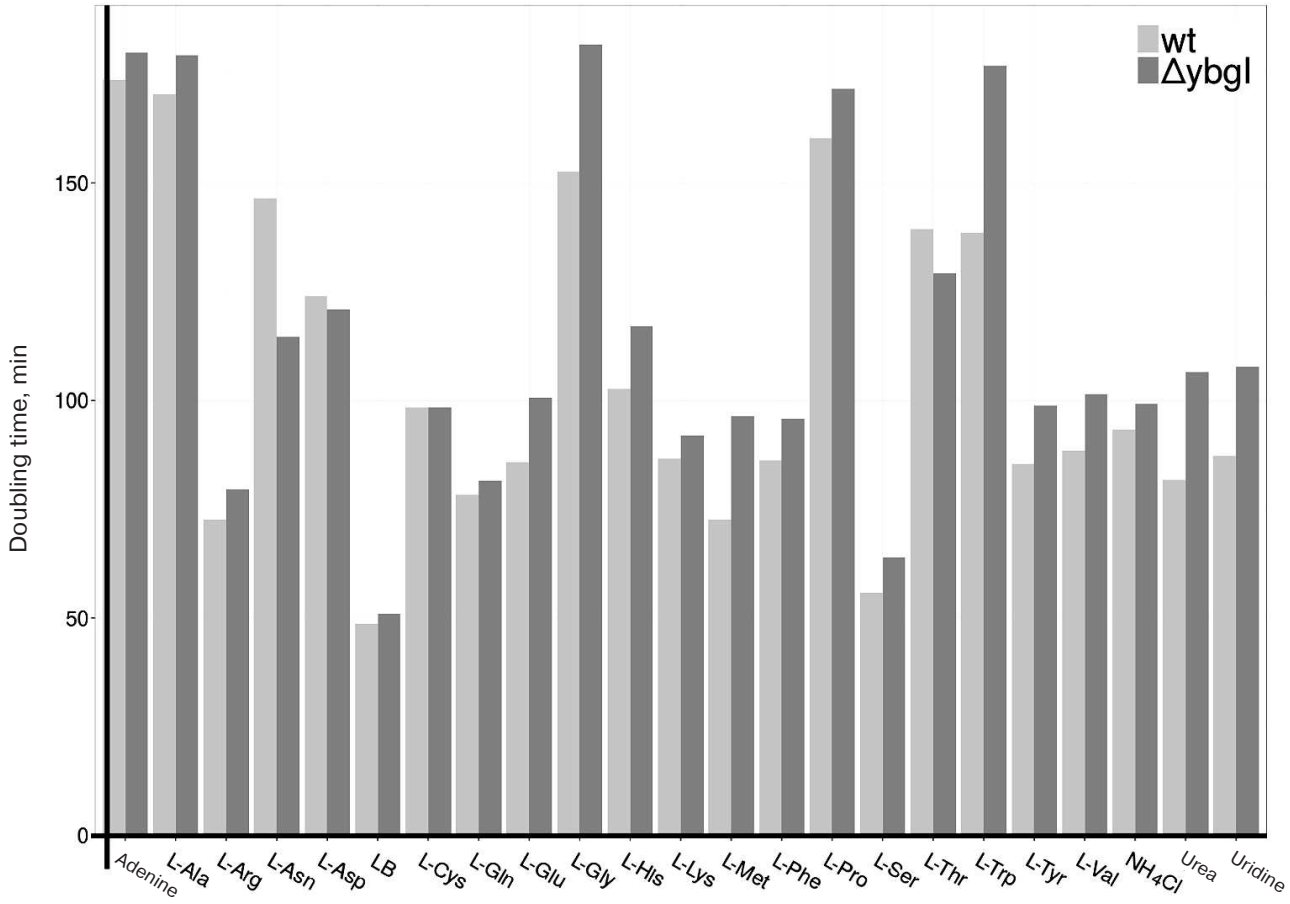

Fig. 5. Doubling times (min) for wt and Δ*ybgI* cells in the presence of different nitrogen sources.

To evaluate the role of *ybgI* in cell signaling pathways, we used several reporter plasmids encoding the fluorescent proteins CER and RFP [27]. In all the plasmid, the *RFP* gene was under control of the same promoter and had identical ribosome-binding sites, so it was used as an internal control. The *CER* gene was placed under control of genetic regulatory elements modulated by the cell signaling pathways (Table 3). *Escherichia coli* wt and Δ*ybgI* cells were transformed with the reporter plasmids (Fig. 7) and grown in LB and M9 media.

In the Δ*ybgI* cells grown in LB medium, the expression of only two reporter genes was altered; when the same cells were grown in M9 medium, the number of affected genes was higher (Table 4).

To confirm the results of affinity chromatography, proteomic analysis, and differential expression of the reporter constructs, we analyzed the content of certain mRNAs in Δ*ybgI* cells grown to the logarithmic phase in the minimal M9 medium, since these were the conditions under which the most pronounced changes were observed. The data were normalized to the content of 16S rRNA (Fig. 8).

We found that in Δ*ybgI* cells, expression of the *tnaA* and *glmS* genes was upregulated, while expression of *ygaU*, *ygiW*, and *dps* was downregulated. The amount of *rsuA* mRNA did not change. These data correlate well with the results of proteomic analysis and reporter gene expression.

Finally, we analyzed the resistance of Δ*ybgI* cells to various antibiotics: carbenicillin, roxithromycin, levofloxacin, fosfomycin, vancomycin, novobiocin, nalidixic acid, and amoxicillin (Fig. 9, a and b). The choice of the tested antibiotics was based on published data on the sensitivity of Δ*ybgI* cells to various factors [38].

We found that deletion of the *ybgI* gene resulted in increase in the zone of inhibition for carbenicillin, fosfomycin, vancomycin, and amoxicillin (Fig. 9c). All these antibiotics affect the synthesis of bacterial cell wall [39].

DISCUSSION

There is very little published information on the function of YbgI protein. It was found that YbgI molecules have a toroidal structure, which led to the suggestion that the protein interacts with nucleic acids [5]. To test this hypothesis, we modeled the electrostatic charge distribution on the YbgI protein molecule surface and

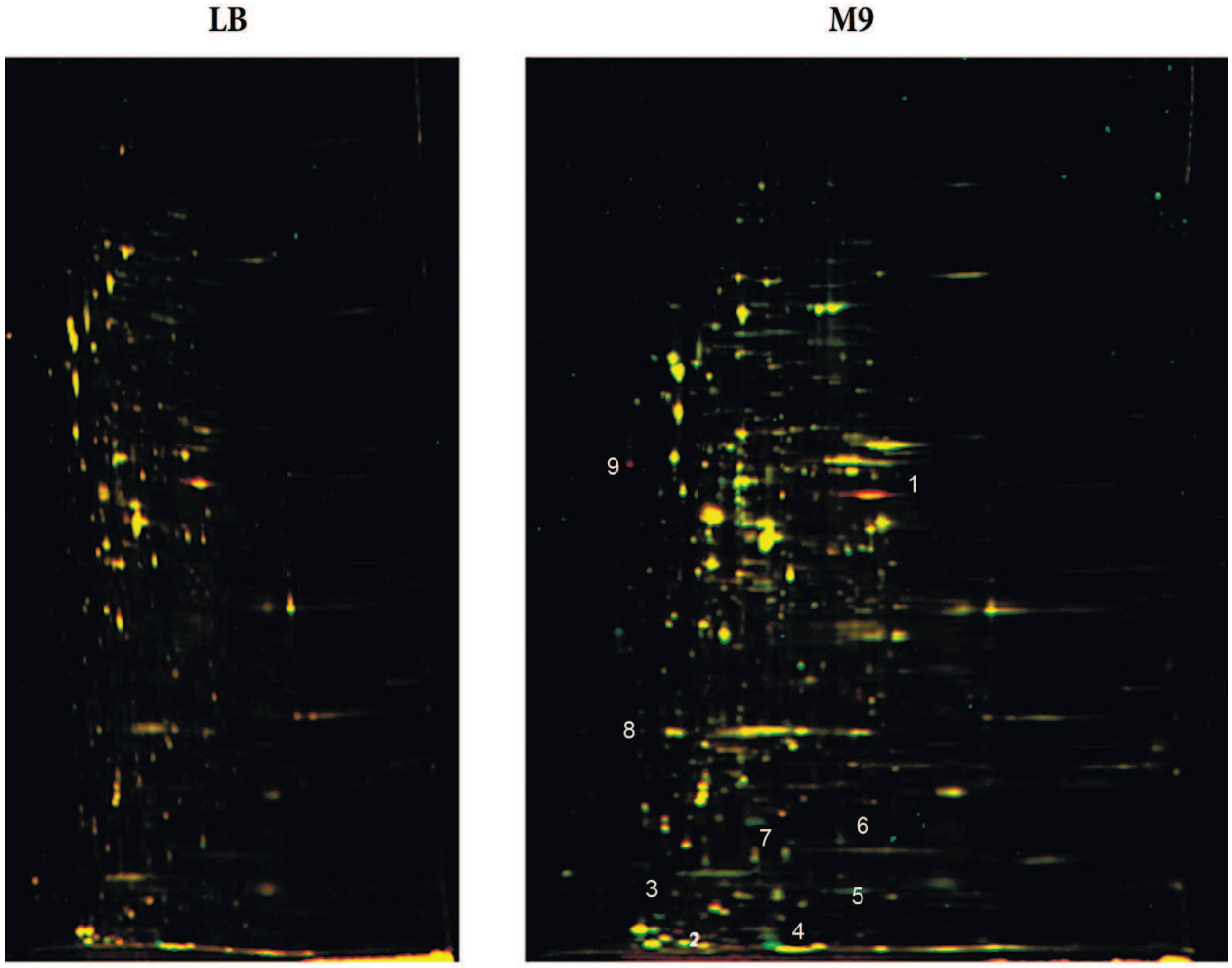

Fig. 6. Proteome of Δ*ybgI* cells grown in LB and M9 media. Protein samples were prepared by mixing cell lysates of wt (Cy3-labeled, green fluorescence) and Δ*ybgI* (Cy5-labeled, red fluorescence) cells and then fractionated by 2D-electrophoresis. Numbers indicate the proteins whose expression changes in Δ*ybgI* cells (see also Table 2).

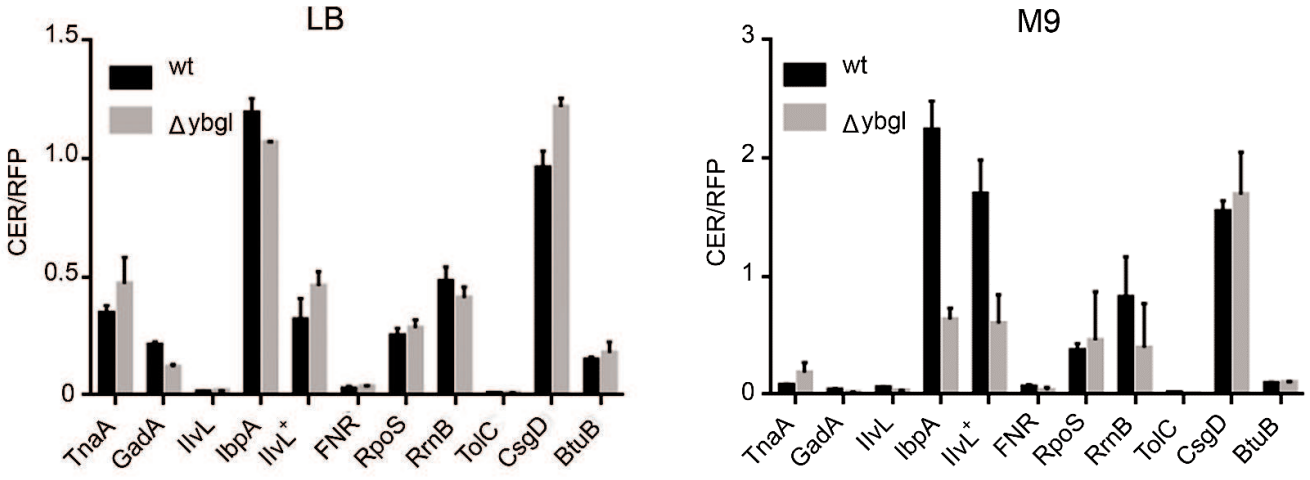

Fig. 7. Fluorescence intensity of reporter genes *CER/RFP* in wt (black) and Δ*ybgI* (grey) cells in LB and M9 media.

Table 2. Proteins whose expression is affected by the *ybgI* gene deletion

Protein No.	Protein	Function
1	TnaA	tryptophanase [21]
3	YgiW	hypothetical stress-induced protein [22]
2, 4, 9		not determined
5	YgaU	K^+-binding protein [23]
6	Dps	component of the stationary phase nucleoid [24]
7	OsmY	hypothetical protein activated by the increase in the medium ionic strength [25]
8	AphA	phosphotransferase [26]

Note: Numbers correspond to protein zones on Fig. 6.

Table 3. Reporter plasmids for expression of fluorescent proteins CER and RFP

Regulatory element	Role in the cell
TnaA	promoter is controlled by the stop peptide, whose translation is regulated by tryptophan concentration [28]
GadA	promoter is activated by acidic stress [29]
IlvL и $IlvL^+$	attenuator and promoter of the hydrophobic acid operon activate transcription of downstream genes upon deficit of isoleucine and valine [30]
IbpA	regulatory element of the heat-shock gene activated by heat shock [31]
Fnr	promoter is activated under anaerobic conditions [32]
RpoS	promoter is activated at stationary growth phase [33]
RrnB	promoter of ribosomal operon that is activated at exponential growth phase and inhibited by induction of stringent response upon amino acid starvation [34]
TolC	promoter is activated by toxin-induced stress [35]
CsgD	promoter is activated by curli formation [36]
BtuB	riboswitch activates expression of downstream gene under vitamin B12 deficit [37]

Table 4. Reporter constructs whose expression was affected by *ybgI* gene deletion

Gene	Description
	LB
GadA	promoter of glutamate decarboxylase gene
CsgD	promoter of gene regulating curli formation
	M9
GadA	promoter of glutamate decarboxylase gene
IbpA	regulatory element in untranslated 5′-region of heat-shock protein mRNA
IlvL, $IlvL^+$	attenuator and promoter of ilvGEDA operon leader peptide
TolC	promoter of membrane porin gene
FNR	promoter of transcription regulator activated under anaerobic conditions

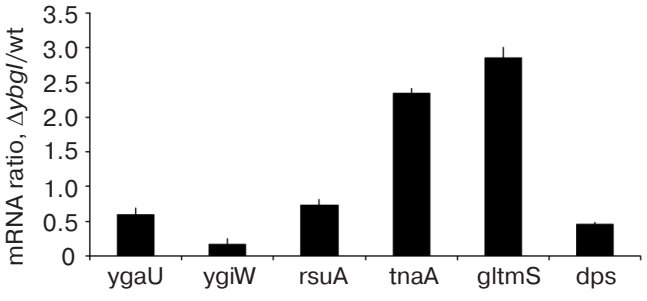

Fig. 8. Ratio between mRNA contents in Δ*ybgI* and wt cells.

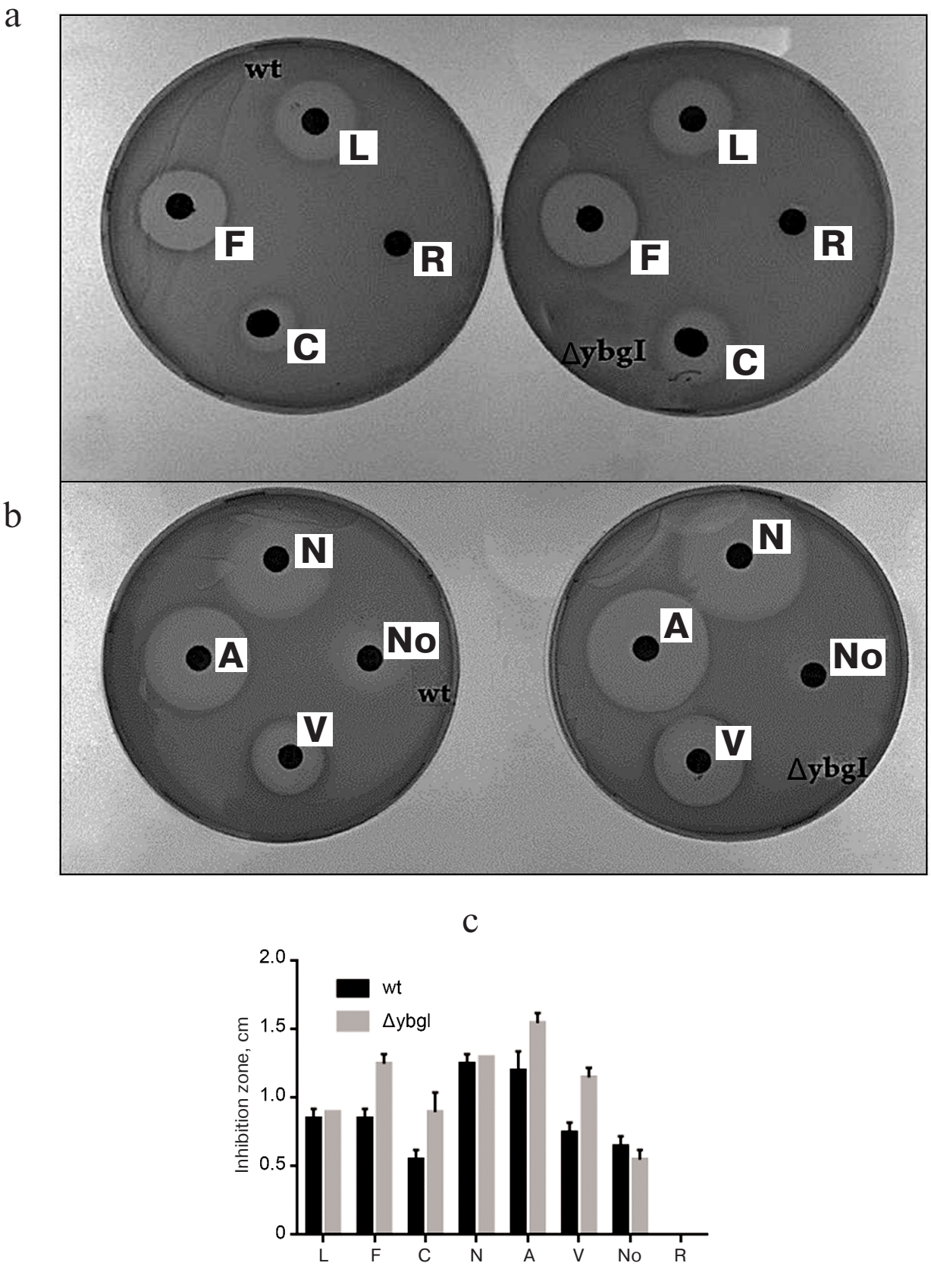

Fig. 9. Zones of inhibition of growth of wt and Δ*ybgI* cells by antibiotics: a) levofloxacin (L); fosfomycin (F); carbenicillin (C); roxithromycin (R); b) amoxicillin (A); vancomycin (V); nalidixic acid (N); novobiocin (No); c) quantification of the zone of antibiotics inhibition.

found that the surface is mostly negatively charged, which would prevent binding of nucleic acids. Another suggested role of YbgI is its involvement in cell response to DNA damage. However, we found that deletion of the *ybgI* gene did not affect cell survival after UV-induced DNA damage. Nevertheless, data were published on cell response to radiation-induced damage that demonstrated that YbgI might be involved in this process [8].

To elucidate the role of YbgI in cells, we used different approaches. First, we identified proteins that copurify with YbgI and found that the protein that most reliably interacts with YbgI is GlmS. Second, we analyzed the proteome of Δ*ybgI* cells and showed that the amount of YgiW, OsmY, YgaU, and Dps proteins decreased, while amount of TnaA and AphA proteins increased compared to the wt cells. Analysis of the gene expression by RT-PCR confirmed upregulation of *tnaA* and *glmS* gene expression and downregulation of *ygaU*, *ygiW*, and *dps* gene expression in Δ*ybgI* cells. Let us consider these changes in more detail. GlmS is an enzyme that catalyzes the first stage of biosynthesis of hexosamine, which is an essential precursor of cell wall peptidoglycan [40]. TnaA is a component of the system for tryptophan decomposition into indole, an important signaling molecule in bacteria [41]. GadA is a glutamate decarboxylase involved in the glutamate-dependent mechanism of bacterial cell resistance to acidification of the medium [42]. YgiW is a component of the cell response to peroxide stress [22, 43]. A homolog of this protein binds to the cell wall peptidoglycan [44]. YgaU is a potassium-binding protein; its deletion affects the structure of the cell wall peptidoglycans [45]. OsmY and Dps are involved in cell stress response; AphA and PheA participate in the biosynthesis of amino acids involving L-glutamate. We found that Δ*ybgI* cells are especially sensitive to the antibiotics that affect the cell wall biosynthesis: carbenicillin (interferes with peptidoglycan binding), fosfomycin (suppresses the first stage of cell wall biosynthesis), amoxicillin (inhibits transpeptidase needed for peptidoglycan biosynthesis), and vancomycin (changes permeability of the cell wall) [39].

Peptidoglycans of bacterial cell wall are composed of N-acetylglucosamine and N-acetylmuramic acid connected with short peptide chains via N-acetylmuramic acid lactate residues [46]. The peptides are composed mainly of alanine, glutamine, and lysine residues. This three-dimensional structure makes the bacterial cell wall rigid and protects the cell from osmotic stress. Our results demonstrate that the loss of the Δ*ybgI* gene leads to the changes in the translation and transcription of genes involved in the biosynthesis and function of bacterial cell wall peptidoglycans. The observed effects of various antibiotics on Δ*ybgI* cells confirmed the suggestion of the involvement of YbgI in the formation of bacterial cell wall in *E. coli*. It is also possible that YbgI is an additional, rather than essential protein, since its deletion is not lethal, but it causes very slight changes in the cell functioning. It is possible that YbgI has several functions that are manifested under normal and stress conditions [5].

Acknowledgments

This work was supported by the Russian Science Foundation (project 14-14-00072) and the Russian Foundation for Fundamental Research (projects 16-04-01100 and 17-00-00366).

REFERENCES

1. Jiang, D., Hatahet, Z., Blaisdell, J. O., Melamede, R. J., and Wallace, S. S. (1997) *Escherichia coli* endonuclease VIII: cloning, sequencing, and overexpression of the *nei* structural gene and characterization of *nei* and *nei* nth mutants, *J. Bacteriol.*, **179**, 3773-3782.
2. Jiang, D., Hatahet, Z., Melamede, R. J., Kow, Y. W., and Wallace, S. S. (1997) Characterization of *Escherichia coli* endonuclease VIII, *J. Biol. Chem.*, **272**, 32230-32239.
3. Pfam: Protein families database of alignments and HMMs, http://www.sanger.ac.uk/Software/Pfam/.
4. Galperin, M. Y., and Koonin, E. V. (2004) Conserved hypothetical proteins: prioritization of targets for experimental study, *Nucleic Acids Res.*, **32**, 5452-5463.
5. Ladner, J. E., Obmolova, G., Teplyakov, A., Howard, A. J., Khil, P. P., Camerini-Otero, R. D., and Gilliland, G. L. (2003) Crystal structure of *Escherichia coli* protein ybgI, a toroidal structure with a dinuclear metal site, *BMC Struct. Biol.*, **3**, 7.
6. Martens, J. A., Genereaux, J., Saleh, A., and Brandl, C. J. (1996) Transcriptional activation by yeast PDR1p is inhibited by its association with NGG1p/ADA3p, *J. Biol. Chem.*, **271**, 15884-15890.
7. Tascou, S., Uedelhoven, J., Dixkens, C., Nayernia, K., Engel, W., and Burfeind, P. (2000) Isolation and characterization of a novel human gene, *NIF3L1*, and its mouse ortholog, *Nif3l1*, highly conserved from bacteria to mammals, *Cytogen. Genome Res.*, **90**, 330-336.
8. Khil, P. P., and Camerini-Otero, R. D. (2002) Over 1000 genes are involved in the DNA damage response of *Escherichia coli*, *Mol. Microbiol.*, **44**, 89-105.
9. Byrne, R. T., Chen, S. H., Wood, E. A., Cabot, E. L., and Cox, M. M. (2014) *Escherichia coli* genes and pathways involved in surviving extreme exposure to ionizing radiation, *J. Bacteriol.*, **196**, 3534-3545.
10. Baker, N. A., Sept, D., Simpson, J., Holst, M. J., and McCammon, A. J. (2001) Electrostatics of nanosystems: application to microtubules and the ribosome, *Proc. Natl. Acad. Sci. USA*, **98**, 10037-10041.
11. DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, USA.
12. Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H., and Mori, H. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E. coli* K-12 ORF archive): unique resources for biological research, *DNA Res.*, **12**, 291-299.
13. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection, *Mol. Syst. Biol.*, **2**, 2006.0008; Epub 2006 Feb 21.

14. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, *Anal. Biochem.*, **72**, 248-254.
15. Ghosh, A., and Bansal, M. (2003) A glossary of DNA structures from A to Z, *Acta Crystallogr. D Biol. Crystallogr.*, **59**, 620-626.
16. Gascon, J., Oubina, A., Perez-Lezaun, A., and Urmeneta, J. (1995) Sensitivity of selected bacterial species to UV radiation, *Curr. Microbiol.*, **30**, 177-182.
17. Iida, A., Teshiba, S., and Mizobuchi, K. (1993) Identification and characterization of the *tktB* gene encoding a second transketolase in *Escherichia coli* K-12, *J. Bacteriol.*, **175**, 5375-5783.
18. Gardner, S. G., Johns, K. D., Tanner, R., and McCleary, W. R. (2012) The PhoU protein from *Escherichia coli* interacts with PhoR, PstB, and metals to form a phosphate-signaling complex at the membrane, *J. Bacteriol.*, **196**, 1741-1752.
19. Brooks, K. M., and Hampel, K. J. (2011) Rapid steps in the glmS ribozyme catalytic pathway: cation and ligand requirements, *Biochemistry*, **50**, 2424-2433.
20. Dutka-Malen, S., Mazodier, P., and Badet, B. (1988) Molecular cloning and overexpression of the glucosamine synthetase gene from *Escherichia coli*, *Biochimie*, **70**, 287-290.
21. Watanabe, T., and Snell, E. E. (1977) The interaction of *Escherichia coli* tryptophanase with various amino and their analogs. Active site mapping, *J. Biochem.*, **82**, 733-745.
22. Lee, J., Hiibel, S. R., Reardon, K. F., and Wood, T. K. (2010) Identification of stress-related proteins in *Escherichia coli* using the pollutant *cis*-dichloroethylene, *J. Appl. Microbiol.*, **108**, 2088-2102.
23. Ashraf, K. U., Josts, I., Mosbahi, K., Kelly, S. M., Byron, O., Smith, B. O., and Walker, D. (2016) The potassium binding protein Kbp is a cytoplasmic potassium sensor, *Structure*, **24**, 741-749.
24. Almiron, M., Link, A. J., Furlong, D., and Kolter, R. (1992) A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*, *Genes Dev.*, **6**, 2646-2654.
25. Yim, H. H., and Villarejo, M. (1992) *OsmY*, a new hyperosmotically inducible gene, encodes a periplasmic protein in *Escherichia coli*, *J. Bacteriol.*, **174**, 3637-3644.
26. Thaller, M. C., Schippa, S., Bonci, A., Cresti, S., and Rossolini, G. M. (1997) Identification of the gene (*aphA*) encoding the class B acid phosphatase/phosphotransferase of *Escherichia coli* MG1655 and characterization of its product, *FEMS Microbiol. Lett.*, **146**, 191-198.
27. Osterman, I. A., Prokhorova, I. V., Sysoev, V. O., Boykova, Y. V., Efremenkova, O. V., Svetlov, M. S., Kolb, V. A., Bogdanov, A. A., Sergiev, P. V., and Dontsova, O. A. (2012) Attenuation-based dual-fluorescent-protein reporter for screening translation inhibitors, *Antimicrob. Agents Chemother.*, **56**, 1774-1783.
28. Li, G., and Young, K. D. (2015) A new suite of tnaA mutants suggests that *Escherichia coli* tryptophanase is regulated by intracellular sequestration and by occlusion of its active site, *BMC Microbiol.*, **15**, 14.
29. Moreau, P. L. (2007) The lysine decarboxylase CadA protects *Escherichia coli* starved of phosphate against fermentation acids, *J. Bacteriol.*, **189**, 2249-2261.
30. Vitreschak, A. G., Lyubetskaya, E. V., Shirshin, M. A., Gelfand, M. S., and Lyubetsky, V. A. (2004) Attenuation regulation of amino acid biosynthetic operons in proteobacteria: comparative genomics analysis, *FEMS Microbiol. Lett.*, **234**, 357-370.
31. Laskowska, E., Wawrzynow, A., and Taylor, A. (1996) IbpA and IbpB, the new heat-shock proteins, bind to endogenous *Escherichia coli* proteins aggregated intracellularly by heat shock, *Biochimie*, **78**, 117-122.
32. Unden, G., and Schirawski, J. (1997) The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions, *Mol. Microbiol.*, **25**, 205-210.
33. Maciag, A., Peano, C., Pietrelli, A., Egli, T., De Bellis, G., and Landini, P. (2011) *In vitro* transcription profiling of the σS subunit of bacterial RNA polymerase: re-definition of the σS regulon and identification of σS-specific promoter sequence elements, *Nucleic Acids Res.*, **39**, 5338-5355.
34. Gralla, J. D. (2005) *Escherichia coli* ribosomal RNA transcription: regulatory roles for ppGpp, NTPs, architectural proteins and a polymerase-binding protein, *Mol. Microbiol.*, **55**, 973-977.
35. Wiriyathanawudhiwong, N., Ohtsu, I., Li, Z. D., Mori, H., and Takagi, H. (2009) The outer membrane TolC is involved in cysteine tolerance and overproduction in *Escherichia coli*, *Appl. Microbiol. Biotech.*, **81**, 903-913.
36. Hammar, M., Arnqvist, A., Bian, Z., Olsen, A., and Normark, S. (1995) Expression of two *csg* operons is required for production of fibronectin- and Congo Red-binding curli polymers in *Escherichia coli* K-12, *Mol. Microbiol.*, **18**, 661-670.
37. Roth, J. R., Lawrence, J. G., and Bobik, T. A. (1996) Cobalamin (coenzyme B12): synthesis and biological significance, *Annu. Rev. Microbiol.*, **50**, 137-181.
38. Liu, A., Tran, L., Becket, E., Lee, K., Chinn, L., Park, E., Tran, K., and Miller, J. H. (2010) Antibiotic sensitivity profiles determined with an *Escherichia coli* gene knockout collection: generating an antibiotic bar code, *Antimicrob. Agents Chemother.*, **54**, 1393-1403.
39. Bush, K. (2012) Antimicrobial agents targeting bacterial cell walls and cell membranes, *Rev. Sci. Tech.*, **31**, 43-56.
40. Barreteau, H., Kovac, A., Boniface, A., Sova, M., Gobec, S., and Blanot, D. (2008) Cytoplasmic steps of peptidoglycan biosynthesis, *FEMS Microbiol. Rev.*, **32**, 168-207.
41. Awano, N., Wada, M., Kohdoh, A., Oikawa, T., Takagi, H., and Nakamori, S. (2005) Effect of cysteine desulfhydrase gene disruption on L-cysteine overproduction in *Escherichia coli*, *Appl. Microbiol. Biotech.*, **62**, 239-243.
42. De Biase, D., Tramonti, A., John, R. A., and Bossa, F. (1996) Isolation, overexpression, and biochemical characterization of the two isoforms of glutamic acid decarboxylase from *Escherichia coli*, *Protein Expr. Purif.*, **8**, 430-438.
43. Juarez-Rodriguez, M. D., Torres-Escobar, A., and Demuth, D. R. (2013) ygiW and qseBC are co-expressed in *Aggregatibacter actinomycetemcomitans* and regulate biofilm growth, *Microbiology*, **159**, 989-1001.
44. Moreira, C. G., Herrera, C. M., Needham, B. D., Parker, C. T., Libby, S. J., Fang, F. C., Trent, M. S., and Sperandio, V. (2013) Virulence and stress-related periplasmic protein (VisP) in bacterial/host associations, *Proc. Natl. Acad. Sci. USA*, **110**, 1470-1475.
45. Bernal-Cabas, M., Ayala, J. A., and Raivio, T. L. (2015) The Cpx envelope stress response modifies peptidoglycan cross-linking via the L,D-transpeptidase LdtD and the novel protein YgaU, *J. Bacteriol.*, **197**, 603-614.
46. Vollmer, W., and Bertsche, U. (2008) Murein (peptidoglycan) structure, architecture and biosynthesis in *Escherichia coli*, *Biochim. Biophys. Acta*, **1778**, 1714-1734.