ORIGINAL ARTICLE

The effects of kanamycin concentration on gene transcription levels in *Escherichia coli*

Xiaozhen Liu[1] · Na Li[2] · Minjun Jia[1] · Shuangli Zhang[1] · Hao Niu[1] · Qiang Li[1] · Pengfei Gu[1]

Abstract

In the area of microbial production of valuable chemicals, plasmids have been widely applied for overexpressing rate-limiting enzymes with high yields. However, the effect of antibiotic concentrations on the transcription of target genes in *E. coli* is less involved in previous reports. In this study, we constructed *E. coli* strains expressing the reporter gene and the kanamycin resistant gene in an operon, and analyzed the transcription levels of the reporter gene and the fluorescent intensity of the recombinant *E. coli* under different kanamycin concentrations. We found that the growth and gene transcription of the recombinant strain were affected obviously by the concentration of kanamycin, indicating the importance of fine-tuning of antibiotic concentrations in microbial fermentation.

Keywords *E. coli* · Plasmids · Kanamycin · Transcription level

Introduction

As possessing the advantages of fast growth, cheap culture medium, and relatively convenient metabolic engineering tools, various microorganisms have been used for bioproduction of valuable chemicals, such as amino acids (Li et al. 2017a; Oldiges et al. 2014; Trondle et al. 2018), succinate (Li et al. 2016; Zhu and Tang 2017), polyhydroxyalkanoates (Chen et al. 2018; Li et al. 2017b), and so on. To overexpress the rate-limiting enzymes with high yields, recombinant DNA technology is widely employed in *Escherichia coli*, a model microorganism which can easily achieve high cell density from less expensive substrates in a short cultivation time.

With the development of molecular biology, plasmids become an important tool for genetic engineering in microorganisms and plant cells. To a certain extent, notable development would not have occurred without plasmids. In addition, industrial production of high value proteins in microorganisms is also dependent on plasmids.

Although plasmids are easy to manipulate, their utilization suffers from genetic instability resulting from segregational instability, structural instability, and allele segregation (Friehs 2004). Since engineered plasmids are often lost during cultivation, it is essential to provide a selective pressure to ensure plasmids' stability, which is often realized by antibiotics. However, metabolic burden will be also generated due to plasmid replication and consumption of precursor metabolites and energy (Bentley et al. 1990; Glick 1995).

Traditionally, antibiotics are supplemented into the medium at a recommended concentration, which depended on whether the plasmids are stringent or relaxed. However, the effect of antibiotic concentration on the transcription of target genes in *E. coli* has not been explored. Kanamycin, an aminoglycoside antibiotic, can inhibit the peptide synthesis of *E. coli* by blocking the translocation process (Semenkov et al. 1982). In this work, green fluorescent protein was selected as a reporter, and kanamycin was selected as a representative for antibiotics. By varying the concentration of kanamycin, transcription level and fluorescent intensity of recombinant *E. coli* were investigated.

✉ Pengfei Gu
  bio_gupf@ujn.edu.cn

1   School of Biological Science and Technology, University of Jinan, Jinan 250022, People's Republic of China

2   Jinan Zhangqiu District People's Hospital, Jinan 250200, People's Republic of China

Materials and methods

Bacterial strains

All strains, plasmids and oligonucleotides used in this study are listed in Tables 1 and 2. *E. coli* W3110 was selected as a host strain. *E. coli* strain DH5α was used as the host of recombinant DNA manipulation.

Growth conditions

Strains for cloning and inoculation were grown in Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) at 37 °C for 8–12 h. Ampicillin (100 mg/L), kanamycin (25 mg/L), and spectinomycin (50 mg/L) were incorporated into the medium when necessary. IPTG was added at a final concentration of 0.2 mM.

Construction of recombinant plasmids

The pTrc99a and pCL1920 plasmids were selected to overexpress *kan* and *gfp* genes encoding the kanamycin resistant protein and green fluorescent protein, respectively. The *kan* and *gfp* genes were amplified by PCR using pKD4 and pG-1 as templates, and kan-AF/kan-AR and gfp-AF/gfp-AR were used as primers. The ribosome binding site of the plasmid pET-28a was designed in primers kan-AF and gfp-AF, respectively. Then, these two fragments were ligated into pTrc99a by restriction endonucleases *EcoR*I, *Sal*I and *Hin*dIII, and T4 DNA ligase. Similarly, to ligate the *kan* and *gfp* fragments into pCL1920, pKD4 and pG-1 were selected as templates, and kan-CF/kan-CR and gfp-CF/gfp-CR were used as primers. The ribosome binding site of the plasmid pET-28a was also designed in primers kan-CF and gfp-CF, respectively. And then, these two fragments were ligated into pCL1920 by restriction

Table 1 Strains and plasmids used in this study

Strains	Genotype	References
W3110	*F-, l-, rph-1, IN (rrnD, rrnE)*	Lab stock
DH5a	*F⁻, endA1, hsdR17 (rK⁻, mK⁺), supE44, thi-l, l⁻,*	Lab stock
JZ-1	*recA1, gyrA96, ΔlacU169 (F80lacZ ΔM15)*	This study
JZ-2	W3110/ pJZ-1	This study
	W3110/ pJZ-2	
Plasmids		
pTrc99a	*oripBR322, bla*(ApR)	Lab stock
pCL1920	*oripSC101, spc/str*	Lerner and Inouye (1990)
pKD4	*oriR6Kγ, bla*(ApR), *kan, rgnB(Ter)*	Datsenko and Wanner (2000)
pJZ-1	pCL1920-*kan-gfp*	This study
pJZ-2	pTrc99a-*kan-gfp*	This study

Table 2 Primers used in this study

Primers	Sequences
kan-CF	5′-CCCAAGCTTAAGGAGATATACATATGATTGAACAAGATGGATTGC-3′
kan-CR	
gfp-CF	5′-ACGCGTCGACTCAGAAGAACTCGTCAAGAAGGCGATAGA-3′
gfp-CR	5′-ACGCGTCGACAAGGAGATATACATATGCGTAAAGGCGAAGAGCTG-3′
kan-AF	5′-CGCGGATCCTCATTTGTACAGTTCATCCATACCAT-3′
kan-AR	5′-CCGGAATTCAAGGAGCATCTATGATTGAACAAGATGGATTGCACGCAGGT-3′
	5′-CGCGGATCCTCAGAAGAACTCGTCAAGAAGGCGATAGAA-3′
gfp-AF	5′-CGCGGATCCAAGGAGCATCTATGCGTAAAGGAGAAGAACT-3′
gfp-AR	5′-CGCGGATCCTCATTTGTACAGTTCATCCATACCAT-3′
Primers for qRT-PCR	
rrsG-QF	GGTGTAGCGGTGAAATGCGTAG
rrsG-QR	TCAAGGGCACAACCTCCAAGTC
kanr-F	CTGCTATTGGGCGAAGTG
kanr-R	GATGTTTCGCTTGGTGGTC
gfp-QF	TCACTACTTTCGGTTATGG
gfp-QF	AAACTTGACTTCAGCACG

endonucleases *Hin*dIII, *Sal*I and *Bam*HI, and T4 DNA ligase. As a result, recombinant plasmids, pJZ-1 and pJZ-2 were obtained, respectively. At last, these two plasmids were transformed into wild type W3110 separately to generate strains, JZ-1 and JZ-2.

qRT-PCR

Samples for mRNA preparation were cultivated 6 h after the addition of inducer. Total mRNA of samples was extracted by an RNA Simple Total RNA kit (Tiangen, Beijing, China). Reverse transcription was performed using random 6-mers and oligo dT primers with a PrimeScript RT reagent kit (Takara, Dalian, China). RT-PCR was conducted by SYBR Premix Ex Taq II (Takara) following the LightCycler 480 RT-PCR System protocol (Roche, Basel, Switzerland). The qRT-PCR primers are listed in Table 2.
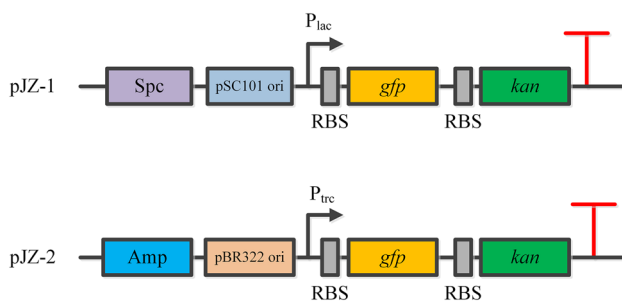
Analytical methods

Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}) with a UV5100H spectrophotometer (METASH, Shanghai China). The fluorescence of recombinant strains was determined as described previously (Li et al. 2012).

Results and discussion

To explore the relationship between the concentration of kanamycin and the expression level of report genes, the *kan* and *gfp* genes were assembled into an operon and ligated into pCL1920 and pTrc99a to generate pJZ-1 and pJZ-2, respectively (Fig. 1). And then, batch fermentation with different concentrations of kanamycin was first performed for the recombinant strain JZ-1, and the results are shown in Fig. 2. When 0, 5, 10 or 25 mg/L kanamycin was used, the recombinant strain exhibited similar growth curve. However, 50 or 100 mg/L of kanamycin interfered with the normal growth of JZ-1 seriously, indicated by the maximum OD_{600} decrease to 4.68 or 4.02 separately. This result was consistent with our expectations, as the normal concentration of kanamycin for *E. coli* was 25 mg/L. In a previous study, Marini et al. explored the effects of kanamycin concentrations on cell growth (Marini et al. 2014). Different from our results, they found that varying the concentration of kanamycin at 10, 30 or 50 mg/L did not interfere with the growth of *E. coli*. Similar results were also obtained from another report (Einsfeldt et al. 2011). The maximum applied concentration of kanamycin in these two reports was only 50 mg/L, while 100 mg/L of kanamycin was used in this

Fig. 1 The structure of recombinant plasmids pJZ-1 and pJZ-2

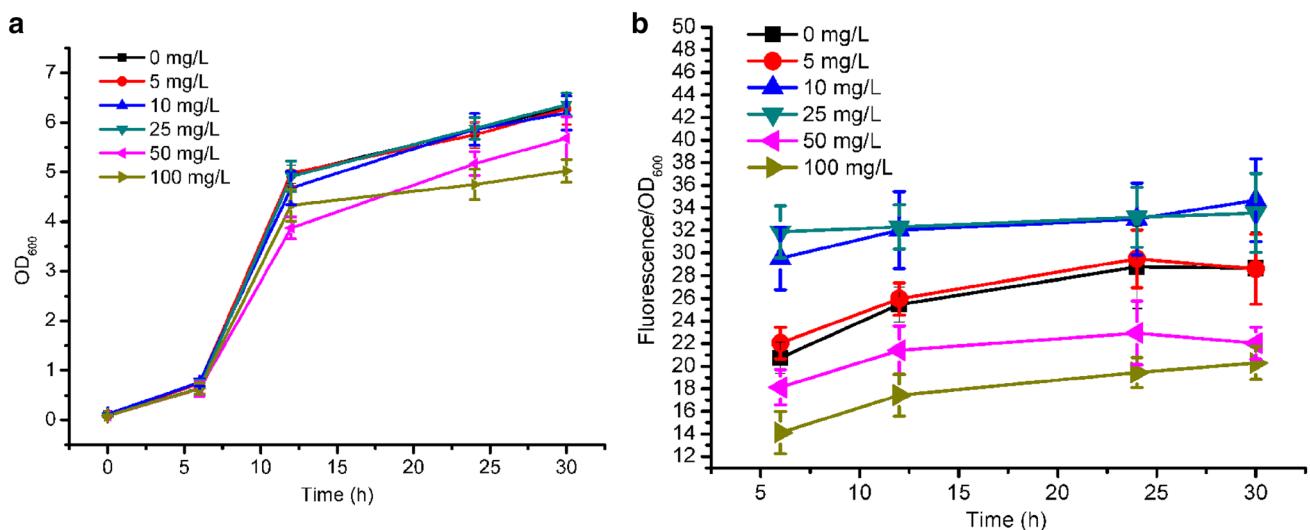Fig. 2 The OD_{600} (**a**) and fluorescence (**b**) in JZ-1 with different concentrations of kanamycin. The error bars represent standard deviations from three replicate fermentations. The fluorescence intensity of *gfp* was normalized by OD_{600}

study. On the other hand, different plasmids with different genetic backgrounds were employed in this work compared to other reports. Maybe these discrepancies led to different effects of kanamycin on cell growth of *E. coli*.

And then, the fluorescence of JZ-1 was also examined. When no kanamycin or only 5 mg/L of kanamycin was added into the media, the expression level of GFP was relatively low, probably due to partial loss of plasmids. In contrast, 10 or 25 mg/L of kanamycin caused the fluorescence of GFP increase to 34.68 ± 3.67 and 33.55 ± 3.50, respectively. This indicated that a lower concentration of kanamycin
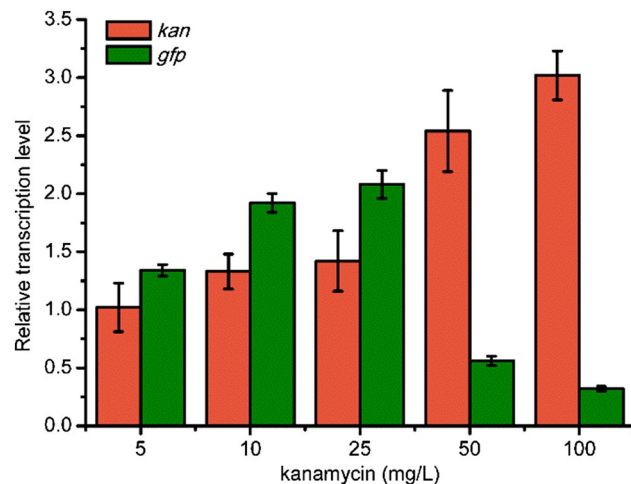

Fig. 3 Relative transcription level of *gfp* and *kan* in *E. coli* JZ-1 with different concentrations of kanamycin. *E. coli* JZ-1 with no kanamycin added was selected as the control. The *rrsG* transcripts were selected as the standard and each measurement was repeated three times. The error bars indicate standard deviations from three replicate samples

(10 mg/L) could also achieve similar expression level of proteins compared to that of the recommended concentration. Surprisingly, JZ-1 with 50 or 100 mg/L kanamycin exhibited even weaker fluorescence than the control with no kanamycin added. Maybe the transcription level of *kan* increased with the increased concentration of kanamycin, while the expression of *gfp* was severely harmed. To test our suspect, qRT-PCR was performed for JZ-1, with different kanamycin concentrations added to the medium. As shown in Fig. 3, when 50 or 100 mg/L kanamycin was supplemented into the medium, the transcription level of *kan* increased over 2.5-fold compared to the same strain with no kanamycin supplement. In contrast, the transcription level of *gfp* decreased by over 50% when the transcription of *kan* increased. In a previous study, different genes in the same operon could exhibit different transcription levels, and the gene expression in the operon has linear relationship with the transcription distance (Lim et al. 2011). In this study, we also found different transcription levels of the genes in an operon, but this difference was induced by improving the concentration of kanamycin. In 2011, the relationship between the expression of protease ClpP and kanamycin concentration was investigated by Karen Einsfeldt and co-researchers (Einsfeldt et al. 2011). Compared to recombinant *E. coli* with 0 or 50 mg/L kanamycin, supplement of 25 mg/L of kanamycin into the medium led to higher expression of ClpP, which also indicates the effect of kanamycin concentration on gene expression (Fig. 4).

To examine whether different plasmid copy-number leads to similar results, pTrc99a with medium copies in a cell was selected to construct pJZ-2, and the *kan* and *gfp* genes are in the same operon as well. In batch fermentation, strain JZ-2 containing pJZ-2 with different
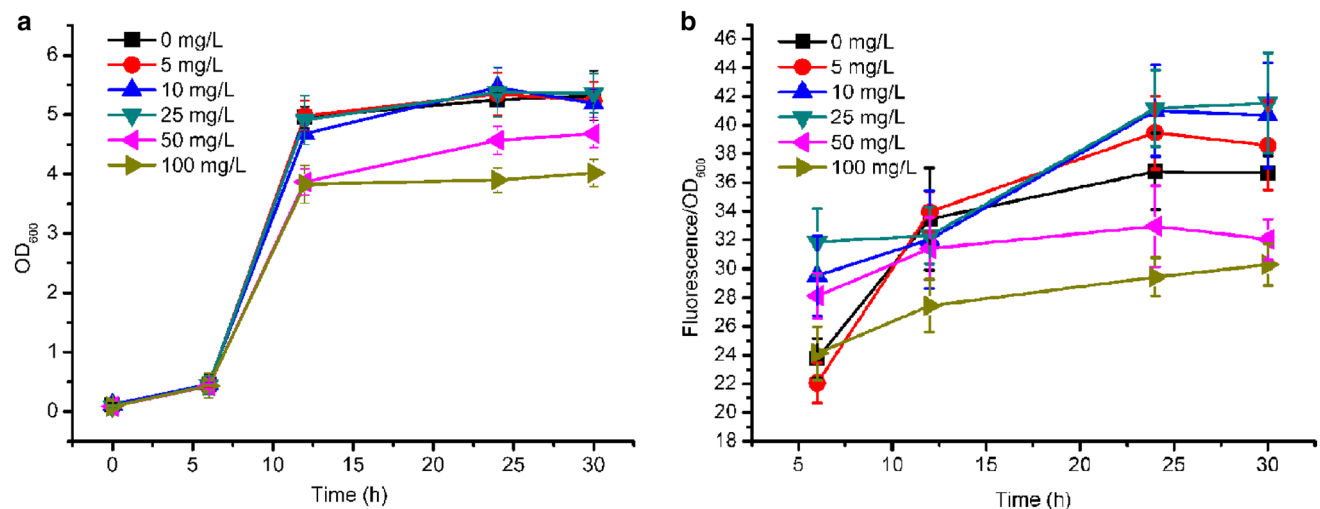

Fig. 4 The OD_{600} (**a**) and fluorescence (**b**) in JZ-2 with different concentrations of kanamycin. The error bars represent standard deviations from three replicate fermentations. The fluorescence intensity of *gfp* was normalized by OD_{600}
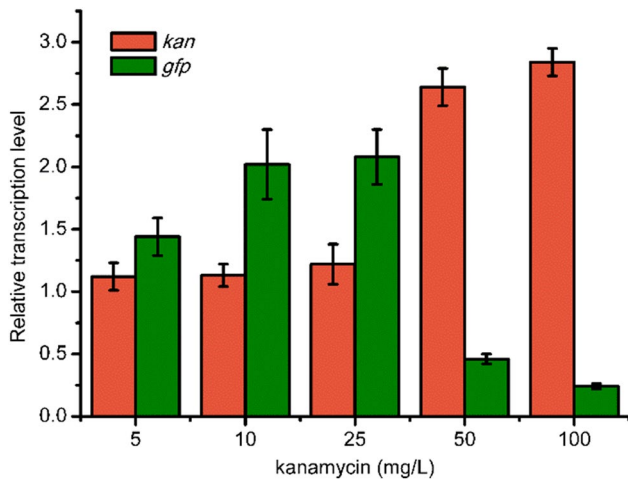
Fig. 5 Relative transcription level of *gfp* and *kan* in *E. coli* JZ-2 with different concentrations of kanamycin. *E. coli* JZ-2 with no kanamycin added was selected as the control. The *rrsG* transcripts were selected as the standard and each measurement was repeated three times. The error bars indicate standard deviations from three replicate samples

concentrations of kanamycin exhibited similar growth curve with JZ-1. When 10 or 25 mg/L kanamycin was supplemented, the expression level of GFP was almost the same. In addition, the fluorescence of JZ-2 was higher than JZ-1, probably because of the higher copy-number of pTrc99a than pCL1920. However, with 50 or 100 mg/L of kanamycin added into the medium, the maximum fluorescence of *gfp* decreased to 32.95 ± 2.83 and 30.29 ± 1.45. This result suggested that the impact of kanamycin on gene transcription in the same operon was not interfered by the copy-number of plasmids. To further verify this speculation, qRT-PCR was also performed for JZ-2. As shown in Fig. 5, the transcription of *kan* increased obviously when the concentration of kanamycin was 50 or 100 mg/L. In contrast, the transcription of *gfp* decreased to only 0.46- and 0.24-fold, respectively, compared to the control. In 2014, Lin et al. found that the expression of LamB and Odp1 complex were crucial for antibiotic resistance in *E. coli*. Lower expression of a LamB–Odp1 complex was a general response of resistance to different classes of antibiotics (Lin et al. 2014). These results indicated that expression of several different genes can be affected by the pressure of antibiotics.

In conclusion, recombinant *E. coli* strains overexpressing GFP and kanamycin resistant genes in an operon with two different plasmids were constructed. We found that the growth and gene transcription of recombinant strains were affected by the concentration of kanamycin. This work provides a useful supplement for the effect of antibiotics on gene expression levels.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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