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Reducing acetate excretion from *E. coli* K-12 by over-expressing the small RNA *sgrS*

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Abstract

When exposed to the non-metabolized glucose derivative alpha methyl glucoside, both *E. coli* K-12 (JM109 and MG1655) and *E. coli B* (BL21) respond by reducing the concentration of the mRNA of the *ptsG* gene which is responsible for the biosynthesis of the glucose transporter $EIICB^{glu}$. This occurs through the over-expression of the non-coding small RNA SgrS, which interacts specifically with the mRNA of the *ptsG* gene and prevents its translation. However, when these bacteria are exposed to a glucose concentration of 40 g/L, over-expression of SgrS is observed only in *E. coli* B (BL21). Unlike *E. coli* K-12 (JM109 and MG1655), which are affected by high glucose concentration and produce higher levels of acetate, *E. coli* B (BL21) is not affected. Based on this information, it was assumed that over-expression of SgrS enables *E. coli* B (BL21) to reduce its acetate excretion by controlling the glucose transport. When SgrS was over-expressed in both *E. coli* K-12 strains from a multicopy plasmid, it was possible to reduce their acetate excretion levels to those seen in *E. coli* B. This observation opens a new approach towards controlling bacterial metabolism through the use of non-coding RNA.

Keywords

small RNA; SgrS; acetate; *E. coli*; glucose

Introduction

E. coli responds to environmental changes by regulating various functions through complex and interacting networks that affect gene transcription, gene translation and enzyme activity. A class of regulators that currently is of interest is the non-coding small RNAs. More than 70 molecules ranging from 50–250 nucleotides have been identified [1–3]; these molecules can repress or activate the expression of specific genes. A subset of these small RNAs regulates gene translation through base pairing with their target mRNAs and has been investigated intensively. This group of small RNAs interacts with Hfq, an RNA chaperone, to form the base pairing which leads to change in the mRNA translation and stability [2, 3]. The small RNA SgrS, was identified in 2004 [4] as a member of this group. SgrS expression is induced when *E. coli* K-12 (MG1655) is either exposed to the non-metabolizable glucose

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derivative αMG [4] or when a mutation is introduced to block the glycolytic pathway [5, 6]; both cases are considered to be stress conditions. It was established that SgrS prevents the translation of the mRNA of the glucose transporter gene *ptsG* and consequently lowers the concentration of the glucose transporter $EIICB^{glu}$ and the glucose transport into the cell. The proposed trigger for this action is the accumulation of glucose 6 phosphate which causes the induction of *sgrS* expression [5, 6]. In our previous report [7], we showed that *E. coli* B (BL21) responded to the presence of αMG in the same way as *E. coli* K-12 (MG1655 and JM109); both strains over expressed SgrS and lowered the levels of the *ptsG* transcript and glucose transport. However, when cells were exposed to environmental stress caused by high glucose concentration, only *E. coli* B (BL21) over-expressed SgrS, lowering *ptsG* and glucose consumption. Under the same conditions both *E. coli* K-12 strains did not express SgrS and did not affect glucose transport. We suggested that, together with the different operation of the central carbon metabolism [8], this is an additional mechanism that allows *E. coli* B (BL21) to respond better to high glucose concentrations and to lower acetate excretion.

Controlling acetate excretion during high density growth of *E. coli* K-12 is a topic under investigation [9–13] since high concentrations of acetate affect growth and recombinant protein production. Various methodologies were developed to reduce acetate accumulation, among them different growth strategies [9, 10], introducing changes in the central carbon metabolism [11–13] and in the glucose transport process [14, 15] by modifying gene expression and transcription. Based on our finding that SgrS is expressed differently in *E. coli* K and B, we suggested that perhaps it will be possible to reduce acetate excretion in *E. coli* K-12 by manipulating the expression of the small RNA SgrS and affecting the translation of the glucose transporter $EIICB^{glu}$. The effect of over expressing SgrS on acetate excretion in *E. coli* K-12 is reported in this manuscript.

Material and methods

E.coli strains

- **i.** The two parental strains used in this study were *E. coli* K-12 (JM109) (DE3) (*endA1, recA1, gyrA96, thi, hsdR17* (*rk-, mk+*), *relA1, supE44*, λ-, Δ(*lac-proAB*), [F', *traD36, proAB, laclqZΔM15*], DE3) (Promega Corp, Madison, WI), and *E. coli* K-12 (MG1655) (F-, λ-, *ilvG-, rfb-50, rph-1*).
- **ii.** Strains over expressing SgrS were prepared by transforming *E. coli* K-12 (JM109) and *E. coli* NM525, an *E. coli* K-12 (MG1655) containing the $lacI^q$ mutation (MG1655 $lacI^q$) that improved transcriptional repression of the pLCV1 (P_{LlacO}-*sgrS*) plasmid until induction with IPTG [4].
- **iii.** Transformation procedure: The *E. coli* K-12 JM109 and *E.* coli K-12 MG1655 $lacI^q$ strains were transformed with the plasmid pLCV1 (P_{LlacO}-*sgrS*) by electroporation using an electrocell manipulator (BTX Harvard Apparatus, Holliston, MA). The electroporation was performed according to Sambrook and Russell, 2001 [17]. Competent cells were grown at 37°C in LB medium to an OD_{600}~ 0.4. Forty μl cells (8×10^8) were washed twice with cold water and once with 10% cold glycerol, and suspended in cold GYT medium (10% v/v glycerol, 1.125% w/v yeast extract, 0.25% w/v tryptone). The conditions used in the 2 mm electroporation cuvette, were electrical pulse of 50 μF capacitance, 2.45 kV, and 125 ohm resistance. Cells were recovered in S.O.C. medium (Invitrogen, Carlsbad, CA) and incubated for 1h at 37°C. Cells were plated in LB agar plates containing 100μg/mL ampicillin to select for transformants.

Cell growth

Cells were grown at 37°C in modified LB medium containing 10 g/L tryptone, 15 g/L yeast extract, 5 g/L NaCl and 5 g/L K_2HPO_4. After sterilization, 10 mM $MgSO_4$ 1 ml/L trace elements solution were added, and glucose concentration was adjusted to 40 g/L, 100μg/mL ampicillin were added to the SgrS over-expressing stain. pH was controlled at 7.0 by addition of 50 % (v/v) NH_4OH, and dissolved oxygen (DO) was controlled at 30% air saturation. A 5 liter bioreactor (Sartorious) was inoculated with an overnight culture to an OD_{600}= 0.3. Cell density was determined by measuring OD_{600} with a Pharmacia Biotech Ultrospec 3000 UV/Visible spectrophotometer, when the culture reached an OD_{600} of 1.0, it was induced with IPTG; 100μM for *E. coli* K-12 (JM109) and 50μM for *E. coli* K-12 (MG1655). Samples were collected at specific times and centrifuged at 13,000 g for 5 min; the cell pellet and the supernatant for RNA extraction and metabolites analysis were maintained at −80°C. (Growth experiments were replicated 3 times).

Metabolite analysis

Glucose was determined by YSI 2700 SELECT Biochemistry Analyzer. Acetate was analyzed by HPLC, Hewlett Packard 1100 Series using Aminex resin-based HPX-87H column (Bio-Rad). Separation conditions were as follows: wavelength 210 nm; mobile phase 0.008 N H_2SO_4, flow rate 0.6 mL/min, temperature 35°C retention time was 14 min.

RNA extraction

The hot phenol method was used: cell pellets were resuspended in 0.5 % SDS, 20 mM NaAc, and 10 mM EDTA and extracted twice with hot acid phenol:chloroform (5:1 pH 4.5) followed by two extractions with phenol:chlorform isoamyl alcohol (25:24:1). Ethanol was added to the extract and the mixture kept at −80°C for 15min. After centrifugation at 14,000 g for 15 min, the pellets were washed in 70% ethanol, air dried and resuspended in ultrapure water (KD medical USA). RNA was quantified using NanoDrop 1000 spectrophotometer (Thermofisher Scientific).

Northern blot analysis

Northern blot analyses to detect sRNA SgrS were performed as described previously [4]. 5 μg of total RNA was separated on a TBE 10 % urea polyacrylamide gel and transferred to a positively charged nylon membrane. A 5’ biotinylated SgrS-specific probe and the Bright-Star Biodetect non-isotopic kit (Ambion, Inc.) were used for probing and detection. The detection of the *ptsG* mRNA was performed as follows: 5 μg of total RNA was separated on a 1.2 % denaturing agarose gel and transferred to a positively charged nylon membrane [18]. The membranes were probed, washed and conjugated with streptavidin-alkaline phosphatase using the BrightStar Biodetect Kit (Ambion). Chemiluminscent signals were detected using the Fujifilm LAS-4000 imaging system. The image before the saturation point was recorded. The internal controls *ssrA* and *ompA* were detected by probing membranes that were stripped with boiling in 0.5 % SDS. The 5’ to 3’ sequences of the probes used were:

sgrS (Bio)-GCAACCAGCACAACTTCGCTGTCGCGGTAAAATAGTG

ptsG (Bio)-CAGCCAGCTGAAATTCGCGGAACCGACGCCCAGCAG

ssrA (Bio)-CGCCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCA

ompA (Bio)-CCATTGTTGTTGATGAAACCAGTGTCATGGTACTGGGACCAGC

Results

1. Effect of over expression of SgrS in *E. coli* K-12 (JM 109) on growth, glucose consumption and acetate excretion

Growth and glucose consumption of *E. coli* K-12 (JM109) and *E. coli* K-12 (JM109) over-expressing SgrS are shown in Figure 1A. The patterns of the bacterial growth and the residual glucose concentration of the two strains are similar, but significant difference has been observed in the acetate excretion pattern (Fig 1B); 10 g/L acetate were accumulated in the parent *E. coli* K-12(JM109) while less than 2 g/L were accumulated in the strain over expressing SgrS.

2. Transcription of SgrS and *ptsG* in *E. coli K-12* (JM 109) and in *E. coli* K-12 (JM109) overexpressing SgrS

Time course transcription measurements of SgrS and *ptsG* in *E. coli* K12 (JM109) and in *E. coli* K-12 (JM109) over-expressing SgrS are shown in Figure 2. No transcription of SgrS and high transcription of *ptsG* were detected in the parental *E. coli* K-12 (JM109) while the opposite was seen in *E. coli* K-12 (JM109) over-expressing SgrS. The constitutive transcription of *ssrA* and *ompA* was used as an internal control.

3. Effect of over-expression of SgrS in *E. coli* K-12 (MG1655) on growth, glucose consumption and acetate excretion

Growth and glucose consumption of parental *E. coli* K-12 (MG1655) and *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS are shown in Figure 3A. The growth pattern of the two strains is similar but the glucose consumption of the strain over-expressing SgrS is slightly slower. Significant difference was observed in the acetate excretion pattern (Fig 3B); there was almost no accumulation of acetate in the strain over-expressing SgrS.

4. Transcription of SgrS and *ptsG* in *E. coli* K-12 (MG1655) and in *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS

Time course transcription measurements of SgrS and *ptsG* in *E. coli* K-12 (MG1655) and *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS are shown in Figure 4. No transcription of SgrS and high transcription of *ptsG* were detected in *E. coli* K-12 (MG1655). However, high transcription of SgrS and low transcription of *ptsG* were observed in *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS. The constitutive transcription of *ssrA* and *ompA* was used as an internal control.

Discussion

Controlling glucose transport is one of the means used by *E. coli* when responding to stress conditions such as the presence of non-metabolizable glucose derivatives or high glucose concentrations [14, 15]. Glucose transport into cells is mediated by the PTS (phosphoenolpyruvate-dependent sugar phosphotransferase) system that contains several components, of which the EIICB^{glu} is the specific glucose transporter encoded by the *ptsG* gene [19, 20]. Concentration of EIICB^{glu} is adjusted by the global regulator Mlc that inhibits the *ptsG* transcription [19–21], and by the small RNA SgrS that inactivates *ptsG* translation by interacting with the *ptsG* mRNA [4, 22, 23]. SgrS is expressed in *E. coli* K-12 (JM109 and MG1655) and *E. coli* B (BL21) when the cells are exposed to the non-metabolizable glucose derivative αMG; however, when cells are exposed to high glucose concentrations, only *E. coli* B (BL21) expresses SgrS [7]. The expression of SgrS and the inactivation of the *ptsG* mRNA is likely responsible for the reduced acetate excretion in the *E. coli* B (BL21) compared to the *E. coli* K-12 strain where SgrS is not expressed and there is no reduction in acetate excretion. This difference in response to high glucose concentrations is in addition to

the previously identified difference in activity of the central carbon metabolism at high glucose concentrations [8].

Acetate excretion by *E. coli* K-12 affects both growth and protein production. As a result, efforts are being devoted to lowering acetate excretion from this strain especially when cells are exposed to high glucose [24]. Currently two main strategies are implemented; one involves the development of different growth methods [9, 10] and the other is associated with the manipulation of genes and enzymes related to the central carbon metabolism [11–13] and to glucose transport into cells [14, 15]. In this report, we demonstrate that it is possible to decrease acetate excretion in *E. coli* K-12 by manipulating the expression of the SgrS sRNA. Over-expression of SgrS in both *E. coli* K-12 strains (JM109 and MG1655) reduced acetate excretion to a similar level observed in *E. coli* B (BL21).

Ample information is available on small RNA properties and mechanisms of operation [3], but so far no attempt has been made to implement this information for modifying bacterial growth and metabolism. The lowering of acetate excretion in the *E. coli* K-12 strains by over-expressing SgrS is a preliminary step in the attempt to regulate bacterial metabolism by using small RNAs. Over-expression of SgrS was achieved by inducing the gene from an inserted plasmid that is not subjected to the internal control mechanism of the bacteria and is not expressed in response to specific growth properties or media composition. It is not known how the bacteria controlled the expression of SgrS and why over-expression by IPTG induction from a plasmid did not eliminate entirely the *ptsG* transcription as was the case when the cells were exposed to αMG [7]. Clearly, further work is needed to incorporate SgrS expression into the bacterial chromosome and to establish a mechanism to regulate its expression based on the growth properties and media composition.

Conclusions

When growing at high glucose concentrations, *E. coli* K-12 (JM109 and MG1165) produce high concentrations of acetate. We assumed that this is, in addition to inefficient central carbon metabolism, the result of the inability of this strain to express the small RNA SgrS which inhibits the biosynthesis of the glucose transporter $EIICB^{glu}$. By over-expressing SgrS in these strains, it was possible to reduce the acetate excretion when the bacteria were exposed to high glucose concentration. This finding suggests that external control of small RNA can potentially be used to regulate bacterial growth and metabolism.

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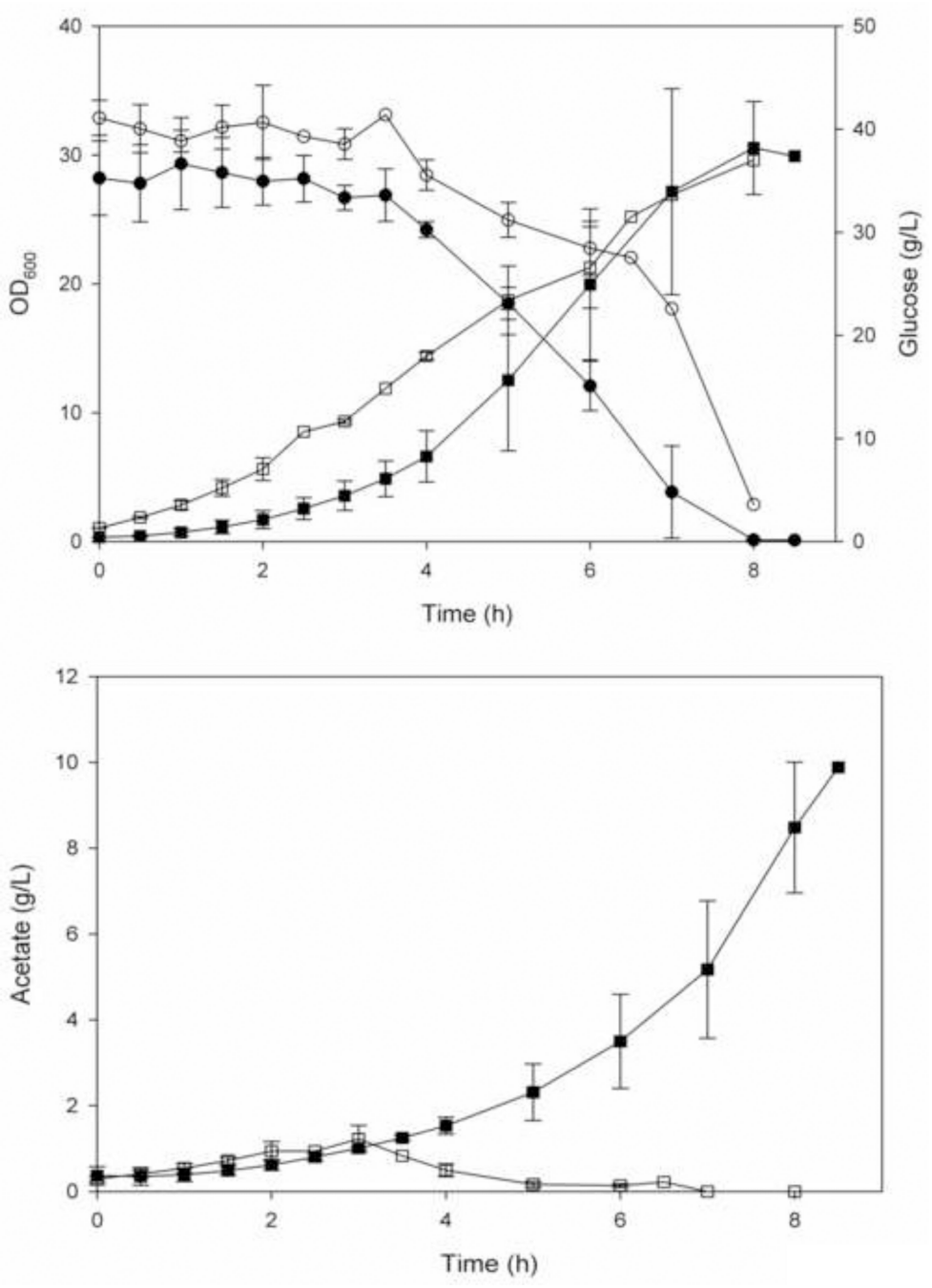

Figure 1.

Growth, glucose consumption and acetate production in *E. coli* K-12 (JM109) and *E. coli* K-12 (JM109) containing plasmid pLCV1 over-expressing SgrS. (a) Growth and glucose consumption (solid and dashed lines, respectively). (b) Acetate excretion. (■, ●) *E. coli* K-12 (JM109). (□, ○) *E. coli* K-12 (JM109) over expressing SgrS

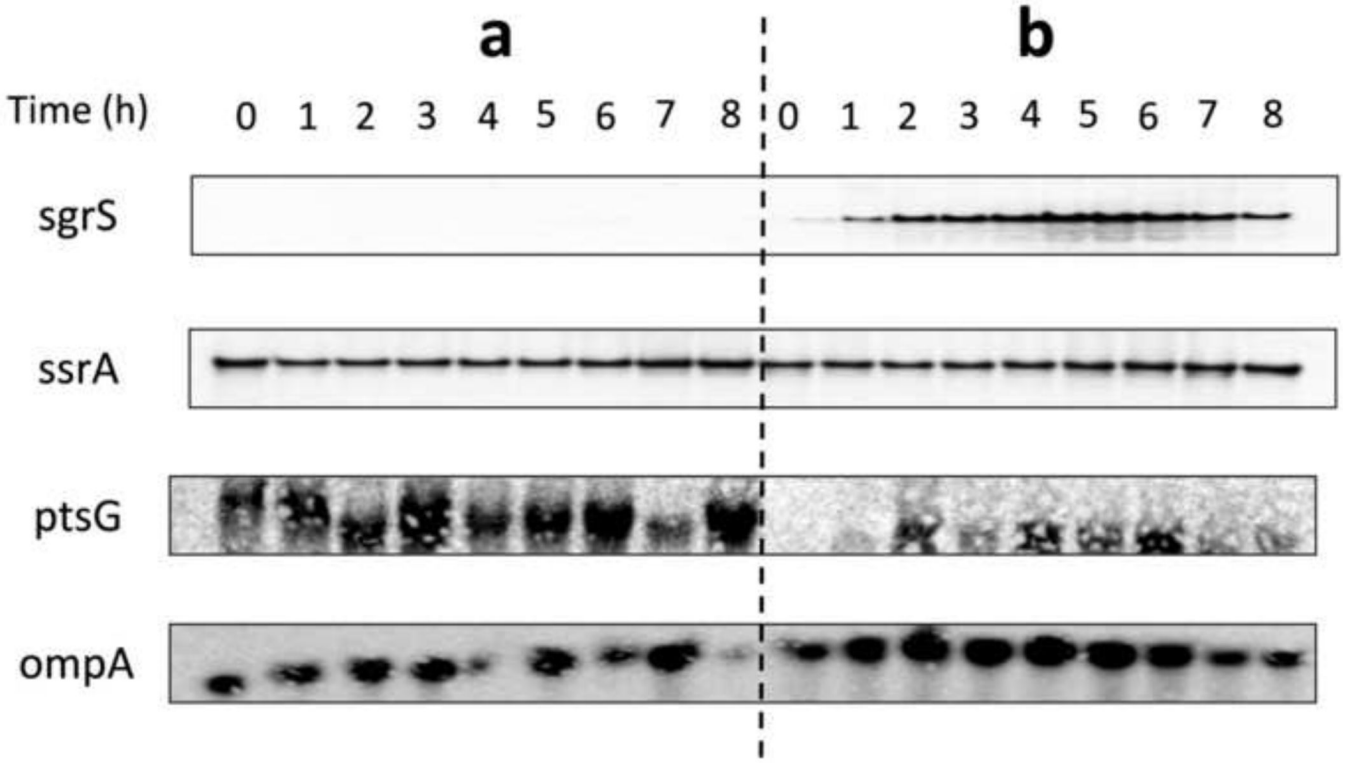

Figure 2.
Transcription analysis of SgrS and ptsG in *E. coli* K12 (JM109) and *E. coli* K-12 (JM109) containing plasmid pLCV1-over expressing SgrS. (a) *E. coli* K12 (JM109). (b) *E. coli* K-12 (JM109) over expressing SgrS

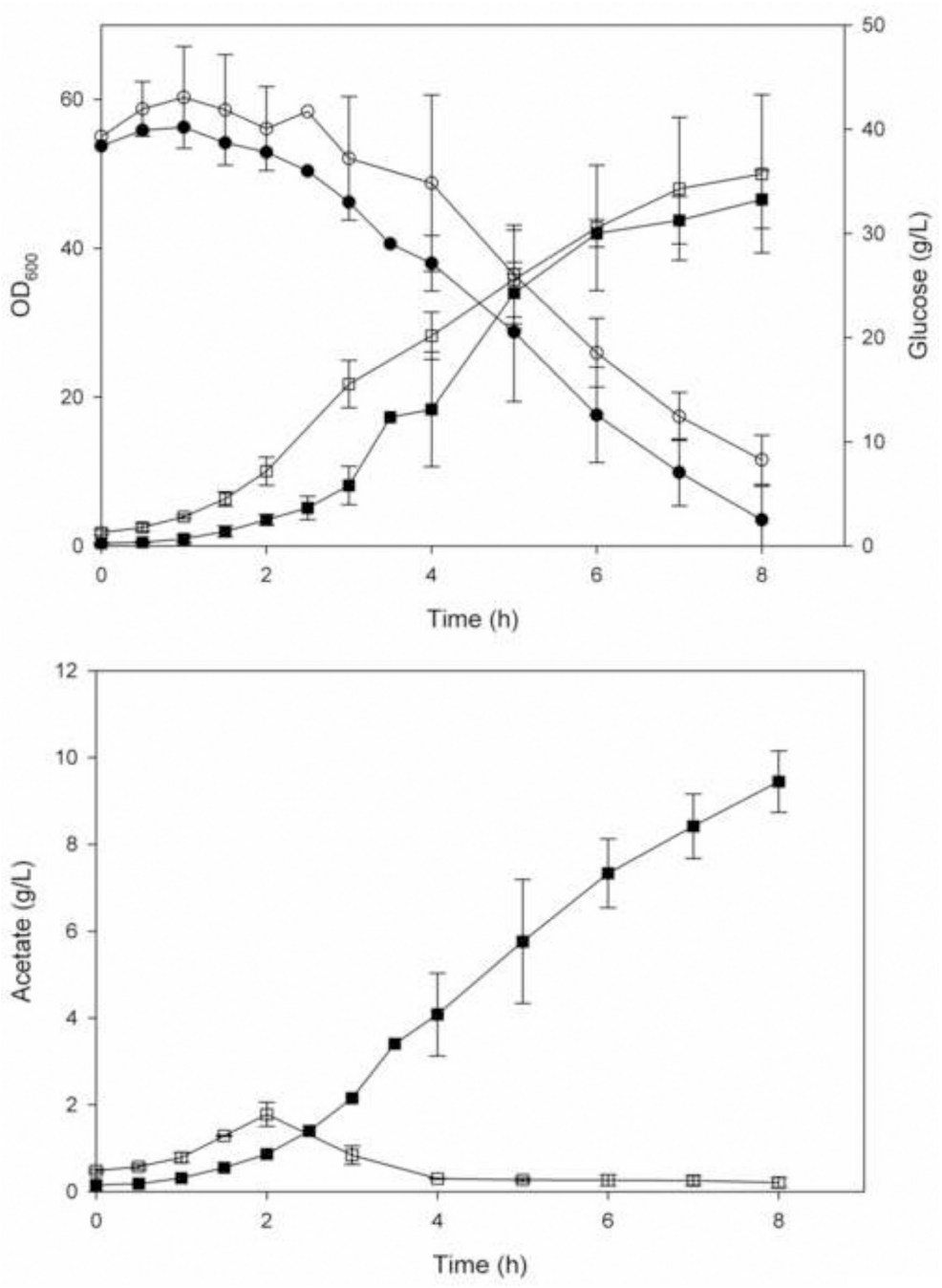

Figure 3.

Growth, glucose consumption and acetate production in *E. coli* K12 (MG1655) and *E. coli* K-12 (MG1655 *lacI*^{q}) containing plasmid pLCV1 over expressing SgrS. (a) Growth and glucose consumption (solid and dashed lines, respectively). (b) Acetate excretion. (■, ●) *E. coli* K12 (MG1655). (□, ○) *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS

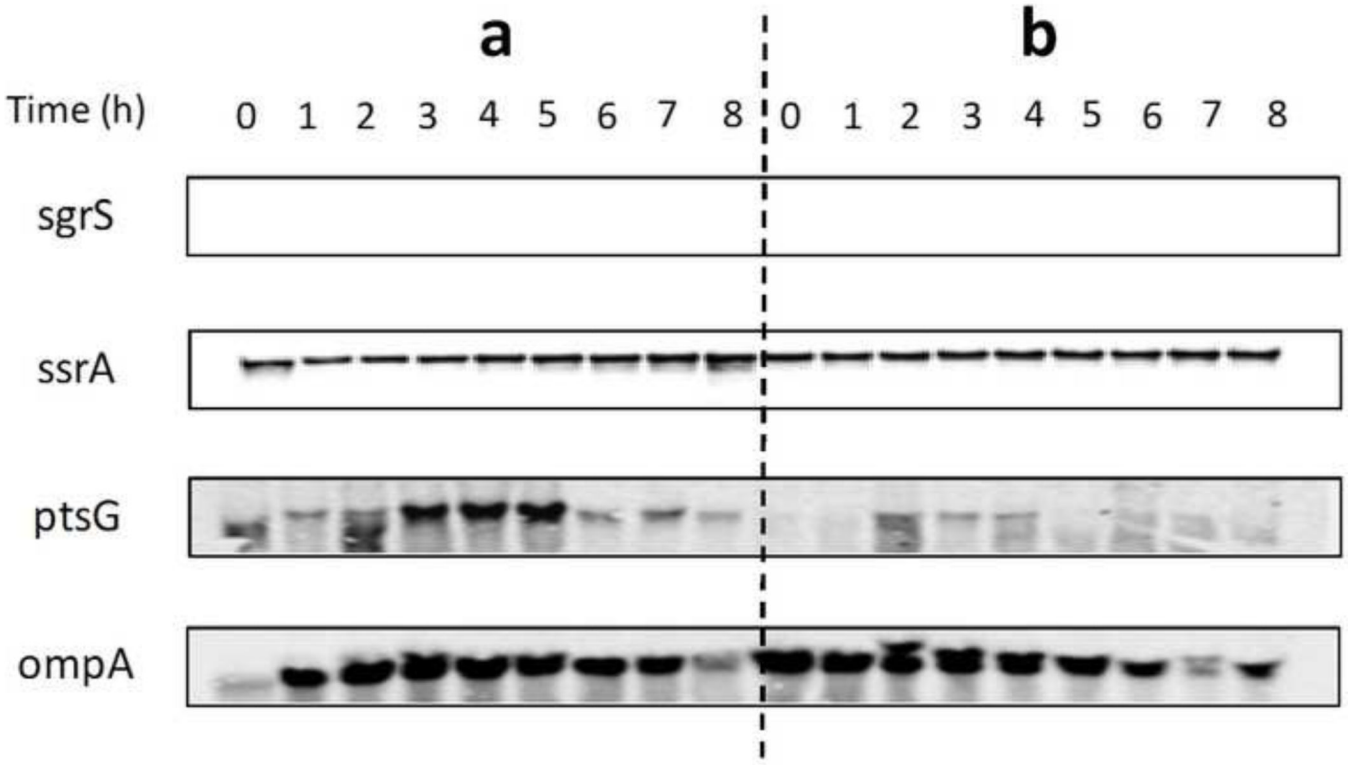

Figure 4.
Transcription analysis of SgrS and ptsG in *E. coli* K-12 (MG1655) and *E. coli* K-12 (MG1655 *lacI*^{q}) containing plasmid pLCV1 over expressing SgrS. (a) *E. coli* K12 (MG1655). (b) *E. coli* K-12 (MG1655 *lacI*^{q}) over-expressing SgrS