Acid adaptation of Escherichia coli: a genetic study

V. Deputienė, K. Sušiedėlis, E. Sušiedėlienė*

Department of Biochemistry and Biophysics, Faculty of Natural Sciences of Vilnius University, M. K. Ėiurlionio 21, Vilnius LT-01513, Lithuania.
E-mail: edita.suziedeliene@gf.vu.lt

E. coli log-phase cells are capable of inducing of log-phase acid tolerance response (ATR), but stationary-phase cells do not exhibit this adaptive ability. To study the genetic systems responsible for the induction of log-phase ATR, we investigated E. coli mutants deficient in the known acid resistance systems. Our results show that RpoS-dependent oxidative and arginine decarboxylase systems are not induced during acid adaptation of log-phase cells. Glutamate decarboxylases were found to be induced during adaptation and result in a more efficient acid-protection. However, our study shows that other highly efficient protection mechanisms are induced, operate and play the main role in log-phase ATR.

Key words: E. coli acid tolerance response (ATR), acid resistance systems (AR), RpoS-dependent oxidative system, glutamate and arginine decarboxylase systems

INTRODUCTION

A sublethal environmental acid stress (pH 5.5–4.5) induces an adaptive tolerance response in many bacteria and provides protection against subsequent exposure to a lethal stress (pH < 4.0) by a mechanism known as acid tolerance response (ATR). ATR has been identified and studied in a wide variety of gram-negative and gram-positive bacterial species [1]. ATR includes both log-phase and stationary-phase systems [2], however, stationary-phase ATR varies greatly among species [3] and strains of the same species [4]. The ATR of Salmonella requires a number of acid-shock proteins that are thought to prevent and repair cellular damage caused by acid stress [5]. The main defense strategies protecting the cell from acid are changes in membrane composition [6, 7], homeostasis systems for internal pH [2, 8] and pathways to repair or protect the essential cellular components [9, 10].

Although acid tolerance response was observed in E. coli first [1], it was not studied in detail. It has been shown that E. coli log-phase cells are capable of inducing log-phase ATR [3]. Paul and Hirsfield have found that pre-exposure of E. coli log-phase cells to pH 5.5 and pH 4.3 induces the synthesis of acid shock proteins, which most likely are responsible for the induction of ATR [11], but largely are not defined yet.

In contrast, the E. coli acid resistance (AR) phenomenon, which is based on three complex cellular systems, has been studied extensively [3]. These systems are RpoS-dependent oxidative system and the inducible amino acid decarboxylases – arginine decarboxylase and glutamate decarboxylase. The latter two are presumed to consume intracellular protons at low pH via decarboxylation reaction, after which a membrane antiporter exchanges the reaction product for more of the amino acid substrate [8, 12]. The protection mechanism of the oxidative system remains to be a mystery. All three systems were found to function effectively in stationary phase cells [8].

The aim of the present study was to investigate the induction of ATR in E. coli and to determine if acid resistance systems could be involved in cell protection during log-phase ATR.

MATERIALS AND METHODS

Bacterial strains, culture media and acid survival experiments. The E. coli K-12 strain used was MG1655. The derivatives of MG1655 generated and used in this study are listed in Table. Unless otherwise indicated, E. coli cells were grown in Luria-Bertani (LB) medium [13]. When necessary, antibiotics were added at the following concentrations: kanamycin 60 µg ml⁻¹, tetracycline 10 µg ml⁻¹.

For ATR analysis, cultures were grown in LB to an A₆₀₀ of 0.35 for the log phase ATR assay and to A₆₀₀ of 1.5 for the stationary phase ATR assay. To induce acid adaptation, the cells were resuspended in fresh LB adjusted by HCl to pH 4.45. Control (unadapted) and adapted cell cultures were inoculated into a medium acidified with HCl to pH 2.0 to approximately 1 × 10⁶–3 × 10⁸ CFU ml⁻¹. Viable cells
were determined by plating aliquots of serially diluted cultures.

AR assays were performed as described [8]. Briefly, for the analysis of RpoS-dependent oxidative system, cells were grown overnight in LB-MES (pH 5.5) and diluted in M9 (pH 2.0) [13] to 1 × 10^8 CFU ml\(^{-1}\). For the analysis of the glutamate-dependent AR system, cells were grown overnight in LBG (LB 0.4% glucose, 0.1M MOPS, pH 7.4) and diluted in M9 (pH 2.0) supplemented with glutamate (1.5 mM). The arginine-dependent AR system was tested by growing cells in LBG overnight and diluting the culture into M9 (pH 2.0) supplemented with arginine (0.6 mM). Viable-cell counts were determined at 0 and 2 h after the acid challenge by plating serially diluted (in PBS [12]) cell suspensions.

**Construction of gadC, adiA and rpoS mutants.** The gadC and adiA gene knockouts were constructed according to the method of Datsenko [14]. The PCR products needed to create targeted deletions in gadC and adiA genes were made from oligo-GadC1 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-GadC2 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-AdiA1 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-AdiA2 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), respectively. Verification of the mutations was made by PCR using locus-specific primers: oligo-GadC3 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-GadC4 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), respectively. We have shown earlier that the gadC and adiA gene knockouts were constructed according to the method of Datsenko [14]. The PCR products needed to create targeted deletions in gadC and adiA genes were made from oligo-GadC1 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-GadC2 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-AdiA1 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-AdiA2 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), respectively. Verification of the mutations was made by PCR using locus-specific primers: oligo-GadC3 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), oligo-GadC4 (5′-ttccgacaggaatacgcgttgtgcgtgtagcttgactgcc), respectively. We have shown earlier that the gadC and adiA gene knockouts were constructed according to the method of Datsenko [14].

**RESULTS AND DISCUSSION**

**ATR in E. coli cells.** Our results have demonstrated that when the E. coli K-12 strain M.G.1655 is cultured in a rich medium, protection against lethal acid challenge of pH 2.0 is provided by pretreatment of the exponentially growing cells at pH 4.5. During this experiment cells were grown to exponential phase, the exponential growing cells at pH 4.5. During the adaptation process, the cells showed a high survival. There was only a 50% loss of viability upon 80 min exposure to pH 2.0 (Fig. 1). Adaptation at pH 4.5 did not improve the acid-survival. Most likely the high survival was conferred by acid resistance systems such as the RpoS-dependent oxidative system, glutamate decarboxylase and arginine decarboxylase systems, which have been shown to function in stationary phase cells [8].

Our results confirm that E. coli log-phase cells are capable of inducing log-phase ATR, but cells in stationary phase do not exhibit this response. To elucidate the genetic systems responsible for the induction of log-phase ATR, we decided to investigate ATR in E. coli mutants deficient in acid resistant systems, listed above. It has been shown earlier that log-phase cells do not show glutamate decarboxylase activity [8], but it is possible that AR systems are induced during the adaptation process, since the ex-
Acid adaptation of Escherichia coli: a genetic study

Expression of genes encoding glutamate decarboxylases was significant in the cells grown at pH 4.5 [18].

Generation of mutants defective in AR. It has been reported previously that individual acid resistance systems are completely abolished in the following bacterial mutants: RpoS-dependent oxidative system in rpoS (encoding alternative RNA polymerase sigma factor) mutant, glutamate decarboxylase system in gadC (encoding glutamate:GABA antiporter) mutant and arginine decarboxylase system in adiA (encoding arginine decarboxylase) mutant [8]. The gadC, adiA, rpoS mutants in MG1655 strain were constructed as described in Materials and Methods. First, we have tested the effects of the mutations on the functionality of the three AR systems. The mutants JE100, JE101, JE102 and parental strains were grown overnight in an appropriate medium as described in Materials and Methods according to the Castanie–Cornet protocol [8]. To test the efficiency of the oxidative system (Oxi), cell survival was monitored in M9 medium, pH 2.0. The arginine decarboxylase (Adi) and glutamate decarboxylase (Gad) systems were tested in M9 medium, pH 2.0, supplemented either with arginine or glutamate. As shown in Fig. 3, the rpoS mutant lacked oxidative system; the activity of Adi system was reduced 1000-fold in the adiA mutant as compared to the parental strain, and the activity of the Gad system was reduced 10000-fold in the gadC mutant. These results confirm that the created mutants are defective in respective acid resistance systems. Interestingly, rpoS mutant showed a reduced glutamate and arginine-dependent AR. This can be explained partly by the fact that the expression of glutamate decarboxylases is dependent on RpoS [8]. However, there are no data to show that RpoS is invol-

Fig. 1. Acid tolerance response of Escherichia coli strain MG1655. Culture was grown in LB medium pH 7.0. Exponential phase (A600 = 0.35) and stationary phase (A600 = 1.5) cells of E. coli were either unadapted (log phase - ■, stationary phase - ○) or adapted at pH 4.5 for 2 h (log phase - □, stationary phase - •) prior exposure to acidified broth at pH 2.0. The percentage of survival at indicated time points was calculated using CFU. 100% viability equals to the viable counts obtained immediately after exposure to pH 2.0. The values are the means of at least three independent experiments. Bars indicate SD

Fig. 2. Effect of adaptation time on acid tolerance response induction. Culture was grown in LB medium pH 7.0. Exponential phase (A600 = 0.35) cells of E. coli were adapted at pH 4.5 for indicated time period prior exposure to acidified broth at pH 2.0. The percentage of survival after 2 h of incubation at pH 2.0 was calculated using CFU. 100% viability equals the viable counts obtained immediately after exposure to pH 2.0. The values are from one representative experiment. Asterisks indicate survival below detection limits

![Graph](image-url)
in the regulation of expression of the Adi system components.

Analysis of log-phase ATR in the rpoS, adiA and gadC mutants. rpoS, adiA and gadC mutants were grown to exponential phase in LBG medium and were acid-adapted at pH 4.5 or unadapted as described in Materials and Methods. To further test the efficiency of the oxidative system (Oxi), cell survival was monitored upon exposure to M9 medium, pH 2.0. To test the arginine decarboxylase system (Adi), pH 4.5 adapted and unadapted cells were shifted to an M9 medium, pH 2.0, supplemented with arginine, and to test glutamate decarboxylase system (Gad) the cells were exposed to M9 medium, pH 2.0, supplemented with glutamate. As shown in Fig. 4, the rpoS mutant, deficient in oxidative system, survived acid stress similarly as did the parental strain. These results indicate that RpoS is not involved in triggering log-phase ATR and suggest that other protective mechanisms are induced during adaptation in log-phase cells. However, the survival of acid-adapted gadC mutant in M9 medium supplemented with glutamate was reduced 10-fold as compared to the parental strain, indicating that the gadC mutant was more susceptible to acid stress and that the GAD system was induced during adaptation. The activity of the glutamate decarboxylase system was reduced in the rpoS mutant as a result of a reduced expression of the Gad system components [8] as reported before. The acid-adapted adiA mutant did not show a reduced acid survival as compared to the wt, indicating that the arginine decarboxylase system is not involved in log-phase ATR.

We can conclude that E. coli possesses different low-pH inducible acid survival systems depending upon whether the cells are in the exponential or in stationa-
ry-phase. Studies of acid survival in S. typhimurium demonstrated that different types of ATR systems exist in log-phase and stationary-phase cells. RpoS, PhoP and Fur proteins have all been shown to be needed for the log-phase ATR [5, 19, 20] and OmpR has been reported to be required for an efficient induction of the stationary-phase ATR of S. typhimurium [21]. Our results show that RpoS-dependent oxidative and arginine decarboxylase systems are not induced during acid adaptation of log-phase E. coli cells. Glutamate decarboxylases seem to be induced during adaptation and result in a more efficient protection. However, it is clear that other highly efficient protection mechanisms are induced, operate and play the main role during log-phase ATR. They need further investigation.

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