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Adaptive Mutagenesis Is a Part of the General Response to Stress in *Salmonella typhimurium*

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Abstract: A fluctuation test was done to determine the frequency and the nature mutations in strains SF553 and JF2794 *Salmonella typhimurium*, that different on rpoS-status. Adaptive mutation requires induction of the RpoS-dependent general stress response. The frequency of His + reversion, as well as mutations of resistance to kanamycin in strain SF553 (RpoS +) was 2-8 times higher than that of strain JF2794 (RpoS ¯). To determine the rate of cultures dying in starvation showed that the strain SF553 was more stable than JF2794, however, the difference in the viability of cultures was not more than 30%. The results suggest that differences in the frequency of mutations in these two strains are related to the fact that *rpoS* is directly involved in adaptive mutagenesis. We assume that the RpoS protein plays a regulatory role and is involved in the running stress-induced mutagenesis in bacteria.

Key words: Mutagenesis · Stress · *Salmonella typhimurium* · Adaptation

INTRODUCTION

Enterobacteria like *Salmonella typhimurium* and *Escherichia coli* may inhabit not only host intestine as pathogens or commensals but also invade such media (for example soil or water) where it experience deficiency of nutrients. To survive in unfavorable conditions, a majority of gram-negative bacteria induce specific genes of stress-response that enhance resistance of cells [1].

rpoS gene is a central regulatory gene that controls a complex network of physiological responses to stress. This gene codes sigma-subunit of RNA polymerase known as σ^s or σ^{38}. RpoS protein is activated while transition of bacteria from exponential growth phase to stationary phase that is accompanied by activation of more than 50 RpoS-dependent genes resulted in adaptation event in cells [2].

Phenomenon of adaptive mutagenesis is one of the less investigated bacterial responses to stress [3]. It is a process when mutations in stressed bacteria (for example, in starveling non-dividing cells) result in restoration of normal metabolism [4]. Despite a long history of the phenomenon, its exact nature remains unclear.

Previously, we found that some mutations in *S. typhimurium* may arise owing to adaptive mutagenesis [5-7]. This work was aimed to reveal a role of *rpoS* gene in induction of adaptive His+ reversions and mutations of resistance to kanamycin.

MATERIALS AND METHODS

Organism. *Salmonella typhimurium* was provided by Prof. John Foster (Department of Microbiology and Immunology, University of South Alabama, USA): SF553 (RpoS+) /*rpsL hisG,* JF2794 (RpoS-) /*rpsL hisG xyl rpoS*^{LT2} *zgd-5178*::Tn*10*(dTc).

Fluctuation Test: The test was performed according to standard technique [8]. Single bacterial colony (10^8 cells) grown on full agar medium was diluted in physiological solution till 50-100 cells per 0.1 ml. This suspension was placed to N-broth (Serva). Each volume was separated in aliquots on 1 ml. Series of bacterial cultures was incubated at 37°C for 24-48 h. Aliquots for inoculation onto agar plates were taken by 0.1 ml pipette. Mutants were analyzed after 48 h. Some amounts of mutants were noted 10 days after the beginning of the experiment. To detect a frequency of mutations, the following formula was used: a = h / N, where h = - ln [m0 /m], m0 – culture without mutants, m- total number of cultures, N- total number of cells.

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Detection of Minimal Inhibiting Concentration (MIC): This parameter was detected via inoculation of bacterial culture 10^4 (cells per ml) onto agar plates with various concentrations of antibiotic. MIC was stated as concentration where growth was fully inhibited after 24 h incubation at 37°C.

Detection of viable cells at incubation on agar media. Cells from agar are placed into tubes with 1 ml of physiological solution. Using subsequent dilutions, it is possible to detect a number of cells in each sample. In parallel, it is necessary to detect a number of cells during plating on Petri dishes. It is possible to evaluate changes in size of starveling population via calculation of logarithm of relation of number of cells in the tubes to number of cells in the sample made in a definite period of time.

RESULTS AND DISCUSSION

The fluctuation test was applied to investigate the appearance of kanamycin-resistant mutants as well as to reveal a character of the appearance of His+ revertants of *S. typhimurium*. According to theoretical fundamentals of the method, if mutations are arose preadaptively, than a distribution will be characterized by a sharp fluctuations of a number of mutants from culture to culture [8]. This type of distribution was titled as jackpot distribution by Luria and Delbruck. In turn, if mutants are arose adaptively, than it will be distributed according to Poisson distribution. When mutants are arose preadaptively, dispersion value will exceed on a few times the average number of revertants per one Petri dish. If these two factors will be equal, it will suggest on the adaptive character of the mutations.

It is clear that relation of dispersion is clear to 1: it may suggest that preadaptive mutants do not arise at full medium (Table 1). His^+ revertants arose as response to stress factor, i.e. in conditions of histidine starvation. The adaptive character of His^+ mutations was revealed for both strains. So, mutation in *rpoS* gene in JF2794 strain did not influence character of adaptive mutations. However, frequency of His^+ revertants was 7-17 times higher than in SF553 strain (Table 2).

Data on kanamycin resistance in these strains were also obtained (Table 1, 3). SF553 strains with functional RpoS protein had increased resistance to antibiotic. This may suggest that RpoS protein is needed for mutations not only at conditions of amino acid starvation but also at exposition with amino glycoside antibiotic.

Table 1: Number of His^+ revertants and Kan^R colonies of independent cultures of *S. typhimurium* SF553 and JF2794. Note: MF - mutation frequency

	S. typhimurium strains							
	SF553				JF2794			
	His+		Kan^R		His+		Kan^R	
Number of culture	Independent	Total	Independent	Total	Independent	Total	Independent	Total
1	1	1	1	1	0	0	0	0
2	0	1	0	1	0	0	0	0
3	1	0	4	0	0	0	0	0
4	1	0	5	0	0	0	0	0
5	0	2	0	1	2	0	0	1
6	2	0	1	0	0	0	0	1
7	2	0	0	0	0	0	0	0
8	1	0	0	0	0	0	0	0
9	0	2	1	1	1	0	0	1
10	1	2	1	1	1	0	1	3
11	1	0	1	0	0	0	0	2
12	0	0	0	0	0	1	1	0
13	0	1	0	0	1	0	0	1
14	1	0	2	0	0	0	0	0
15	1	0	0	0	0	0	0	0
16	1	0	0	1	0	2	0	0
17	0	0	1	0	0	0	0	1
18	0	0	1	1	0	0	0	1
19	1	0	0	1	0	0	0	0
20	0	0	0	1	1	0	1	0

Table 2: Continued

Number of cells (N)	$5{,}0\times10^{7}$				$1{,}09\times10^{8}$			
MF (a)	$1{,}83\times10^{-8}$		$1{,}39\times10^{-8}$		$2{,}64\times10^{-9}$		$1{,}49\times10^{-9}$	
Average ($\bar{x}$)	0,7	0,45	0,9	0,45	0,3	0,15	0,15	0,55
Dispersion (σ^2)	0,43	0,58	1,88	0,26	0,33	0,23	0,13	0,68
$\sigma^2/\bar{x}$	0,61	1,29	2,09	0,58	1,1	1,53	0,87	1,24

Table 2: Frequency of His^+ reversions in *S. typhimurium* SF553 and JF2794 strains

No of experiment	SF553	JF2794	a(SF553)/a(JF2794)
1	$1{,}83\times10^{-8}$	$2{,}64\times10^{-9}$	6,93
2	$8{,}47\times10^{-9}$	$1{,}02\times10^{-9}$	8,3
3	$4{,}77\times10^{-9}$	$0{,}27\times10^{-9}$	17,67

Table 3: Frequency of kanamycin-resistant mutants of *S. typhimurium* SF553 and JF2794 strains

No of experiment	SF553	JF2794	a(SF553)/a(JF2794)
1	$2{,}5\times10^{-8}$	$1{,}14\times10^{-8}$	2,19
2	$1{,}39\times10^{-8}$	$1{,}49\times10^{-9}$	9,33
3	$1{,}16\times10^{-8}$	$5{,}13\times10^{-9}$	2,26
4	$1{,}08\times10^{-8}$	$3{,}28\times10^{-9}$	3,29

Previously, a direct participation of *rpoS* gene was stated for *E. coli* [9, 10]. We cannot exclude that differences in the frequency of mutations in SF553 and JF2794 strains may be a result of indirect action of this gene on general resistance of bacteria to negative factors of the environment. For example, *rpoS* mutants of *S. typhimurium* and *E. coli* were loss viability faster than cells of wild type during continuous starvation [11]. It also highly sensitive to many stress factors like ultraviolet light, increased temperature, increased osmolarity, low pH and H_2O_2 [12].

To assess indirect participation of *rpoS* gene on the appearance of mutants, we detect the effect of RpoS on sensitivity of our strains to selective agents. Detection of MIC did not reveal any differences between SF553 and JF2794 strains on sensitivity to kanamycin (MIC 4 mcg/ml). By this reason, it is not possible to explain difference in the frequency of mutants by differences in sensitivity to antibiotic.

Differences of cells to amino acid starvation were assessed on dynamics bacterial dieaway in conditions of histidine starvation. Figure 1 presents results of this experiment. The rate of bacterial dieaway was higher for JF2794 strain. However, differences in the first two days were only by 30% in comparison with SF553 strain while there was 2-fold difference in the frequency. Previously, we reported that 90 % of adaptive His+ revertants are formed during first two days of starvation. Thus, we may conclude that *rpoS* gene directly participates in adaptive mutagenesis.

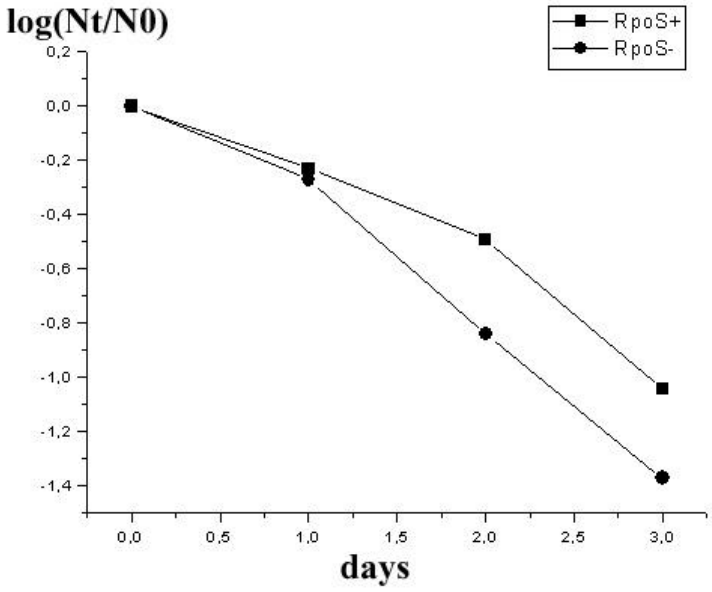

Fig. 1: Dynamics of bacterial dieaway in *S. typhimurium* SF553 and JF2794 strains at conditions of amino acid starvation

The participation of SOS-inducible polymerases in adaptive mutagenesis is an obvious fact [13, 14]. Induction of all three types of SOS-inducible polymerases was found at starvation conditions as well as at stationary phase of bacterial growth [15]. It was presented that Pol IV, one of SOS-inducible polymerases, is induced at stationary phase and that this induction requires RpoS protein [16]. If RpoS is active than the level of Pol IV remains high for 3 days - during a period of the appearance of a majority of adaptive mutations [13]. We suggest that the effect of *rpoS* gene on the appearance of adaptive mutations in *S. typhimurium* is mediated by participation of this gene in induction of SOS-polymerases playing an important role in adaptive mutagenesis.

CONCLUSION

In this study, it was found that RpoS system participates actively in the appearance of His+ revertants and kanamycin-resistant mutants. Besides, this system influences the rate of culture dying. All these findings justify the important role of *rpoS* gene in processes of adaptive mutagenesis and adaptation to stress factors in bacteria.

REFERENCES

1. Hengge-Aronis, R., 2002. Recent insights into the general stress response regulatory network in *Escherichia coli*. Journal of Molecular Microbiology and Biotechnol., 4: 341-346.
2. Lange, R. and R. Hengge-Aronis, 1991. Growth phase-regulated expression of bolA and morphology of stationary-phase Escherichia coli cells are controlled by the novel sigma factor sigma S. Journal of Bacteriol., 173: 4474-4481.
3. Cairns, J., J. Overbaugh and S. Miller, 1988. The origin of mutants. Nature, 335: 142-145.
4. Rosenberg, S.M., 2001. Evolving responsively: adaptive mutation. Nature Reviews Genetics, 2: 504-515.
5. Babynin, E.V., E.V. Gasimova and A. Montano, 2009. Action of adaptive mutagenesis in resistance of *Salmonella typhimurium* to kanamycin. Scientific Letters of KGAVM, 196: 54-60.
6. Babynin, E.V., 2006. The effect of extracellular metabolites on the frequency of Thy+ revertants in *Salmonella typhimurium* populations. Mikrobiologiia, 75: 521-524.
7. Gizatullin, F.S. and E.V. Babynin, 1996. Specificity and time of the appearance of His+ reversions induced by histidine stavation in *Salmonella typhimurium*. Genetika, 32: 1333-4130.
8. Luria, S.E. and M. Delbruck, 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics, 28: 491-511.
9. Bjedov, I., O. Tenaillon, B. Gerard, V. Souza, E. Denamur, M. Radman, F. Taddei and I. Matic, 2003. Stress-induced mutagenesis in bacteria. Sci., 300: 1404-1409.
10. Lombardo, M.J., I. Aponyi and S.M. Rosenberg, 2004. General stress response regulator RpoS in adaptive mutation and amplification in *E. coli*. Genetics, 166: 669-680.
11. Dong T. and H.E. Schellhorn, 2010. Role of RpoS in virulence of pathogens. Infection and Immunity, 78: 887-897.
12. Notley-McRobb, L., T. King and T. Ferenci, 2002. *rpoS* mutations and loss of general stress resistance in *E. coli* populations as a consequence of conflict between competing stress responses. Journal of Bacteriol., 184: 806-811.
13. Gizatullin, F.S. and E.V. Babynin, 1996. The selection-induced His+ reversion in *Salmonella typhimurium*. Mutation Res., 357: 43-56.
14. McKenzie, G.J., P.L. Lee, M.J. Lombardo, P.J. Hastings and S.M. Rosenberg, 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Molecular Cell, 7: 571-579.
15. Yeiser, B., E.D. Pepper, M.F. Goodman and S.E. Finkel, 2002. SOS-induced DNA polymerases enhance long-term survival and evolutionary fitness. Proceedings of the. National Academy of Sciences USA, 99: 8737-8741.
16. Layton, J.C. and P.L. Foster, 2003. Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli.* Molecular Microbiol., 50: 549-561.