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CONFERENCE PROCEEDINGS

The Effect of Extracellular Metabolites on the Frequency of Thy$^+$ Revertants in *Salmonella typhimurium* Populations

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Abstract—There is convincing evidence that adaptation and survival processes in bacterial populations depend on cell-to-cell interactions. Our studies showed that the frequency of stress-induced His$^+$ reversions in an amino-acid- starved *Salmonella typhimurium* culture is inversely proportional to cell density in this culture. The effects of cell density and of different culture liquids prepared from cultures starved for histidine on the frequency of Thy$^+$ revertants were also studied. It was found that the frequency of Thy$^+$ revertants is inversely proportional ($r = –0.74$) to the density of the bacterial culture starved of thymine. The culture liquid prepared from the culture starved of histidine exerted an inhibitory effect on the frequency of Thy$^+$ reversions, indicating that mutations induced by different types of stress have a common mechanism. The study of the effect of the culture liquid prepared from a histidine-starved culture on the frequency of ethyl- methanesulfonate-induced His$^+$ revertants showed that this liquid prevented the induction of His$^+$ reversions.

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Bacterial adaptation to various stresses is typically studied at the intracellular level. Actually, the adaptation of bacteria to unfavorable conditions occurs at the population level. It has been shown that many adaptation and survival processes are controlled by signal molecules involved in intercellular interactions in bacterial populations (the so-called quorum sensing system) [1].

One of the mechanisms that promotes microbial adaptation is adaptive mutation, which consists in microorganisms acquiring a survival mutation under selective conditions when normal cellular metabolism and reproduction are suppressed [2]. The induction of adaptive His$^+$ reversions in *Salmonella thyphimurium* depends on the density of the bacterial culture suffering amino acid starvation [3]. The effect of cell density on induced mutations was found to be due to the intracellular metabolites produced in response to amino acid starvation. This circumstance suggests that the quorum sensing system influences adaptive mutagenesis.

There is a hypothesis that SOS-inducible DNA polymerases are involved in adaptive mutagenesis [4–7]. We attempted to verify this hypothesis by studying the effect of cell density and culture liquids on the frequency of SOS response mutations induced by thymine starvation (more specifically, on the frequency of Thy$^+$ revertants) in *S. typhimurium* strain BA133. A thymine-dependent variant of strain BA133 was derived from strain BA13 (hisG46 araD531 gal-uvrB/pKM101).

Dependence of the frequency of Thy$^+$ revertants on the density of bacterial population. The Thy$^–$ culture of *S. typhimurium* BA133 was grown on nutrient agar for 24 h. Cells were collected with a sterile loop and resuspended in physiological saline solution. The suspension was diluted in different proportions and the dilutions were plated on selective agar. The effect of cell density on the frequency of Thy$^+$ revertants is shown in Fig. 1. As in the case with His$^+$ revertants [3], the frequency of Thy$^+$ revertants was inversely proportional to the density of the starved bacterial culture, the correlation coefficient being statistically significant ($r = –0.74$ at $p < 0.0001$).

The effect of culture liquid on the frequency of His$^+$ and Thy$^+$ revertants. Earlier, we studied the effect of the culture liquid of histidine- starved bacterial culture on the frequency of His$^+$ revertants [3]. In the present study, we investigated the cross-effect of the culture liquid prepared from the histidine-starved bacterial culture on the frequency of Thy$^+$ revertants. The bacterium *S. typhimurium* was grown in a medium of the following composition (g/l): $(NH_4)_2SO_4$, 4; KH_2PO_4, 4; K_2HPO_4, 18; sodium citrate, 1.3; $MgSO_4$, 0.2; glucose, 5; and biotin, 0.03. Strain BA13 was grown in the medium without histidine for 24 h. Cells were collected by centrifugation at 15 000 *g* for 10 min.

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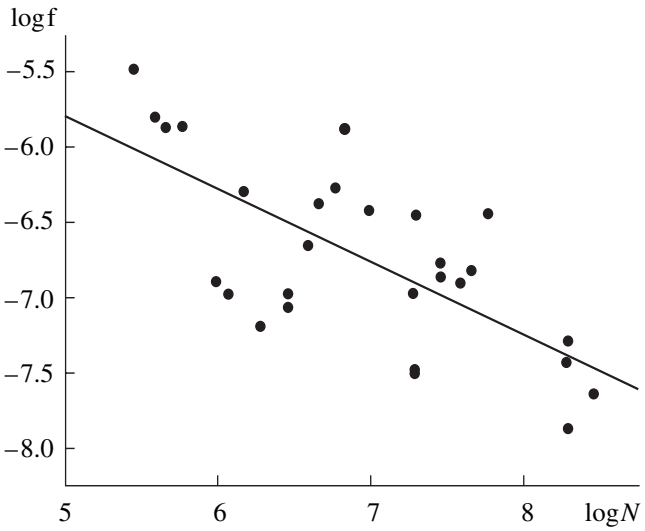

Fig. 1. Dependence of the frequency ofThy$^+$ revertants (log *f*) on cell density in the inoculated suspension of *S. typhimurium* Ba133 Thy$^-$ - cells (log *N*).

The supernatant (i.e., the culture liquid) was sterilized by filtration through a 0.22-μm filter (Millipor).

The culture liquid was found to exert similar antimutagenic effects on both mutations (Fig. 2), the number of both types of revertants decreasing by approximately 30%. These results suggest that the culture liquid exerts a nonspecific effect on the frequency of starvation-induced mutations. Consequently, both mutations may have the same mechanism.

The effect of culture liquid on induced mutagenesis. According to some observations [8], the culture

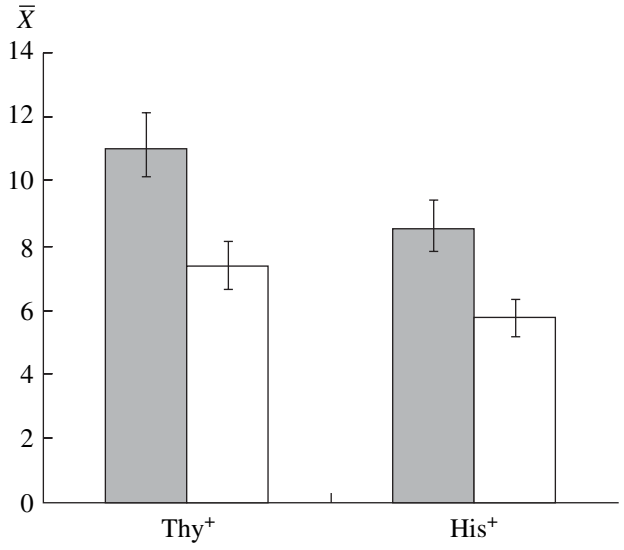

Fig. 2. The effect of culture liquid on the number of Thy$^+$ and His$^+$ revertants. Dark and open bars refer to the control and culture with the added culture liquid, respectively. $\overline{X}$ is the mean number of revertant colonies per plate.

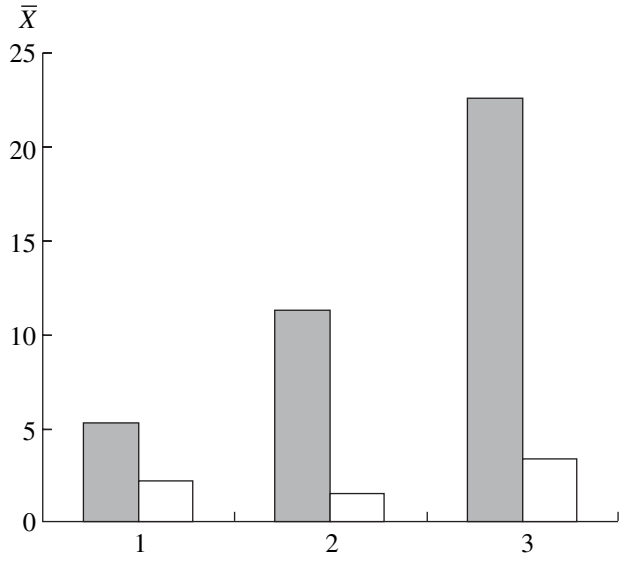

Fig. 3. The effect of culture liquid on the frequency of *S. typhimurium* His$^+$ revertants induced with ethyl methanesulfonate: (1) without mutagen, (2) 0.5 mg/ml EMS, and (3) 0.6 mg/ml EMS. Dark and open bars refer to the control and culture with the added culture liquid, respectively. $\overline{X}$ is the mean number of revertant colonies per plate.

liquids of some bacterial cultures possess antimutagenic activity. For example, the culture liquid of propionic acid bacteria inhibits 4-nitroquinoline-1-oxide–induced mutagenesis in *S. typhimurium*. In the present study, we investigated the effect of the culture liquid of the histidine-starved *S. typhimurium* BA13 strain on the frequency of His$^+$ revertants induced in the same strain with ethyl methanesulfonate (EMS) under the amino acid starvation condition. This culture liquid was plated on minimal agar together with the His$^-$ culture. The experiments showed that the antimutagenic effect of the culture liquid was more profound with respect to EMS-induced mutagenesis than to spontaneous mutagenesis (Fig. 3). The antimutagenic effect was calculated according to the formula:

$$\text{antimutagenic effect (\%)} = \frac{(a-b)100\%}{(a-c)},$$

where *a* is the number of histidine revertants induced with a mutagen, *b* is the number of the revertants induced with the mutagen in the presence of an antimutagen (culture liquid), and *c* is the number of the revertants grown in the presence of the antimutagen.

The antimutagenic effect of the aforementioned culture liquid was found to be 80–90%.

To conclude, the replication of chromosomal DNA in starved cells gives rise to more DNA lesions and, as a consequence, activates SOS response. The SOS response in a His$^-$ or Thy$^-$ cell population may give rise to cells with a necessary mutation. For most cells in the

population, an increased level of mutagenesis is fatal because some mutations are lethal. To avoid this situation, the SOS response in cell populations is accompanied by inhibition of cell division [9], which prevents the formation of new DNA lesions. In other words, the inhibition of DNA synthesis provides for the repair of DNA lesions before the beginning of the next replication cycle.

The inhibitory effect of the SOS response on the replication of chromosomal DNA depends on the activation of *sulA* (*sfiA*), a gene of the SOS regulon [10]. There is evidence that the signal molecules involved in the quorum sensing system induce the SOS response genes *recA*, *uvrA*, and *sulA* [11]. This fact suggests that the exometabolites produced by bacterial cells under stressful conditions induce the *sulA* gene and hence inhibit DNA replication. In turn, the inhibited replication reduces the number of accumulated mutations (in the case under study, reversions) in bacterial chromosomes.

REFERENCES

1. Lazazzera, B.A., Quorum Sensing and Starvation: Signals for Entry into Stationary Phase, *Curr. Opin. Microbiol.*, 2000, vol. 3, pp. 177–182.
2. Cairns, J., Overbaugh, J., and Miller, S., The Origin of Mutants, *Nature*, 1988, vol. 335, pp. 142–145.
3. Babynin, E.V. and Gizatullin, F.Sh., The Dependence of His^+ Reversion Frequency in *Salmonella typhimurium* on the Density of Cell Population, *Genetika*, 1997, vol. 33, no. 9, pp. 1215–1220.
4. Foster, P.L., Adaptive Mutation: Has the Unicorn Landed?, *Genetics*, 1998, vol. 30, pp. 1453–1459.
5. McKenzie, G.J., Harris, R.S., Lee, P.L., and Rosenberg, S.M., The SOS Response Regulates Adaptive Mutation, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, no. 12, pp. 6646–6651.
6. Rosenberg, S.M., Evolving Responsively: Adaptive Mutation, *Nat. Rev. Genet.*, 2001, vol. 2, pp. 504–515.
7. Babynin, E.V., SOS-Inducible DNA Polymerases and Adaptive Mutagenesis, *Genetika*, 2004, vol. 40, no. 5, pp. 581–591.
8. Vorob'eva, L.I., Cherdyntseva, T.A., and Abilev, S.L., The Antimutagenic Effect of Bacteria on Mutagenesis Induced by 4- Nitroquinoline-1-Oxide in *Salmonella typhimurium* TA100, *Mikrobiologiya*, 1993, vol. 62, no. 2, pp. 232–237.
9. Walker, G.C., The SOS Response of *Escherichia coli*, in *Escherichia coli* and *Salmonella typhimurium*, Neidhardt, F.C. et al., Eds., Washington: American Society for Microbiology, 1996, pp. 1400–1416.
10. Huisman, O. and D'Ari, R., An Inducible DNA Replication–Cell Division Coupling Mechanism in *Escherichia coli, Nature*, 1981, vol. 290, pp. 797–799.
11. Sperandio, V., Torres, A.G., Giron, J.A., and Kaper, J.B., Quorum Sensing Is a Global Regulatory Mechanism in Enterohemorrhagic *Escherichia coli* O157:H7, *J. Bacteriol.*, 2001, vol. 183, pp. 5187– 5197.