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The Effect of High Hydrostatic Pressure on the Viability and Mutagenesis of *Salmonella typhimurium*

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Abstract—This work is devoted to the study of the influence of high hydrostatic pressure (HHP) on the viability and level of mutagenesis of *Salmonella typhimurium*. It was established that the viability of bacteria significantly decreases under hydrostatic pressure of 200 MPa or higher. In addition, the viability index of the bacteria is six orders of magnitude lower with respect to the number of colony-forming units (CFUs) compared to the data of the flow cytofluorometry analysis. This is probably due to the transition of some part of the bacterial population to a viable but nonculturable state (VBNC). HHP of 50 MPa caused a 1.9-fold increase in the number of His^+ revertants of the *S. typhimurium* strain TA98, which indicates the potential of the induction of gene mutations under these conditions. The mechanisms to reduce the viability and genetic changes in bacterial cells under HHP conditions are discussed.

Keywords: high hydrostatic pressure, viability, nonculturable state, mutagenesis, mutations, *Salmonella typhimurium*, flow cytometry, Ames test

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INTRODUCTION

Microorganisms are the oldest inhabitants on Earth; they have adapted to living on different substrates under constantly changing environmental conditions in the process of long-term evolution (Wachtershauser, 2006; Zeng et al., 2009). The basic physicochemical factors that determine the life of microorganisms include the pH, temperature, hydrostatic pressure, and electromagnetic radiation.

Hydrostatic pressure is one of the key physical parameters of the biosphere; its value can vary from 0.1 MPa at sea level (atmospheric pressure) to 110 MPa in the Challenger Abyss, the deepest point of the ocean that is located 11 km below the sea level in the Mariana Trench (Aertsen et al., 2009; Mota et al., 2013).

Many microorganisms successfully grow and reproduce under the normal atmospheric pressure and stop growing when they are exposed to the effect of hydrostatic pressure of over 50 MPa (Mota et al., 2013). At the same time, the optimal hydrostatic pressure for the growth of piezophilic microorganisms living in the deep biosphere (the deep ocean floor , deep-sea hydrothermal vents, and deep water oil wells) is 40–60 MPa (Jannasch and Taylor, 1984; Meersman and McMillan, 2014; Oger and Jebbar, 2010). Obligate piezophiles, e.g., *Moritella yayanosii*, actively grow at a pressure of 80 MPa.

High hydrostatic pressure (HHP) ranging from 200 to 800 MPa has been used to preserve food products since the 1990s. It is believed that HHP not only kills pathogenic microorganisms but also leave the taste and most of nutritional properties of the products, in contrast to thermal treatment (High-Pressure Microbiology, 2008; Aersten et al., 2009). At the same time, it has been found that different species of microorganisms and even different strains of the same species may differ in their sensitivity to the HHP effect (Alpas et al., 1999). Moreover, some studies presented the data on the isolation of the piezoresistant mutants of mesophilic bacteria, *Escherichia coli* (Hauben et al., 1997; Gao et al., 2001) and *Listeria monocytogenes* (Karatzas and Bennik, 2002), surviving at pressure values that are lethal for mesophilic microorganisms (up to 800 MPa). According to the data of Vanlint et al. (2011), piezoresistant *Escherichia coli* mutants isolated by the authors were able to survive even at a pressure of 1.2–2 GPa. Hauben et al. (1997) isolated barotolerant *E. coli* strains after the multiple exposure to HHP. According to the opinion of the authors, the piezoresistant cells of *E. coli* were not initially present in the population of these bacteria and the high level of barotolerance of isolated strains is the result of the accumulation of multiple mutations (Hauben et al., 1997). In some cases, the piezoresistant strains of mesophilic