Mechanisms of Adaptation of Pathogenic Bacteria to Environmental Factors

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Biochemical mechanisms of pathogenic bacteria *Yersinia pseudotuberculosis* and *Listeria monocytogenes* ensuring their survival in the habitat were investigated. Low temperature and poor trophicity induce synthesis of cold isozymes in bacteria and accumulation of coenzymes of NAD and NADP-dependent dehydrogenases and intensify assimilation of hydrogen, oxygen, carbogen, and nitrogen maintaining energy and constructive metabolism under unfavorable environmental conditions.

Key Words: *pathogenic bacteria; ecology; adaptation mechanisms*

Adaptation of pathogenic bacteria and other organisms is the process of accommodation to various environmental conditions.

The problem of adaptation of pathogenic bacteria to environmental conditions assumed great importance in connection with the discussion on principal possibility of reproduction and, hence, the existence in the habitat and the role of the environment as the reservoir of infectious pathogens. In this long-lasting discussion, the prevailing viewpoint assured that the pathogenic microorganisms could not exist in the environment.

In 1958, famous microbiologist V. I. Terskikh criticized the canonized epidemiological thesis on impossibility of the existence of pathogenic bacteria in the environment and substantiated a concept that a number of pathogens of infectious diseases, so-called "sapronoses," could exist outside the organism [12]. He considered these pathogens as free-living species and members of natural biocoenosis. The sapronose pathogens were subclassified into real sapronotic pathogens (truly free-living species known as pathogenic saprophytes, saprophytes of medical importance [1], or accidental parasites [2]) and the saprozoonotic pathogens belonging to facultative parasites more or less dependent on warm-blooded animals and the environment [3] (Fig. 1).

These facultative parasites transit from environment, where they live as saprophytes into the warm-blooded organism, where they demonstrate their parasitic properties, and then again to saprophytic life in the environment, which suggests a dual (saprophytic and parasitic) nature of these infectious pathogens [5].

Recent ecological studies of pathogenic *Yersinia*, *Leptospira*, *Listeria*, *Legionella*, *Bacillus anthracic*, *Vibrio cholerae*, *et al.* [1,3,4,6,9,10] rehabilitated the concept of sapronoses and substantiated possible survival of a number of pathogenic bacteria in the environment. However, the discussion was continued.

To solve this principal problem, it is necessary to reveal genetical and biochemical mechanisms determining the possibility of reproduction of pathogenic bacteria in the environment. Here we discuss the data obtained on the models of pseudotuberculosis (PTM) and listeriosis (LM) microbes.

The study of 279 outbreaks of pseudotuberculosis showed that they were caused by contaminated vegetables and dairy products stored for a long time at a low temperature in refrigerators and store-houses [9]. It should be noted that after many failures, the pseudotuberculosis etiology of the Far-Eastern scarlatinoid fever was revealed with the help of a new

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method: smears from patients were incubated at 4-7°C in a simple buffered saline solution [14] instead of conventional beef-extract broth maintained at 37°C. A hypothesis on psychrophilic nature of pathogenic bacteria and its epidemiological role was proposed [6]. Further studies substantiated this concept.

In accordance with Arrhenius theory, metabolic intensity decreases 2-fold (or by 100%) with every 10°C drop in the environmental temperature. This brings up a question: how the ectothermic organisms can maintain the metabolic rate? In reality, the inhibition of biochemical reactions deviate from the Arrhenius law. Transition from high to low temperatures in ectothermic organisms is associated with activation of enzyme reactions and maintenance of metabolic rate via special adaptive mechanisms. This adaptation of ectothermic organisms was termed as compensation for metabolism intensity.

American biochemists P. Hochachka and J. Somero found that low and high temperatures induce synthesis of cold and heat isozymes in fishes and marine invertebrates, which adjust the intensity of metabolic processes [13].

First on PTM model, and then on other saprozoonotic pathogens we observed synthesis of cold isozymes corresponding to catalase, urease, and hyaluronidase in examined bacteria and activation of corresponding biochemical reactions [8]. This attests to generality of genetic and biochemical mechanisms in the nature and shows that the revealed regularity is only a special case of comprehensive biochemical strategy of adaptation of ectothermic organisms to changes in environmental temperature.

Then we studied energy metabolism in bacterial cells at low temperature.

Gas chromatography revealed that yersinia and listeria could absorb molecular hydrogen (an electron donor in the cellular respiratory chain) from air [7]. Moreover, absorption of hydrogen by these bacteria is more intensive at low (4-6°C) than at high temperatures (37°C). The bacteria cultured at low temperature absorb up to 20% initial hydrogen volume from the gas-air mixture during the first day and only 5% hydrogen volume at 37°C (the corresponding values for day 3 are 25 and 15%). Hydrogen utilization is accompanied by synchronous absorption of oxygen, which attests to activation of biooxidation in bacterial cells (Fig. 2).

Then we studied the first stage of biological oxidation, transport of hydrogen in the electron-proton form from substrates to flavoenzymes and other components of the respiratory chain via NAD- and NADP-coenzymes of pyridine-dependent dehydrogenases. The 1.5-2-fold increase in the content of NAD and NADP observed at low temperatures in comparison

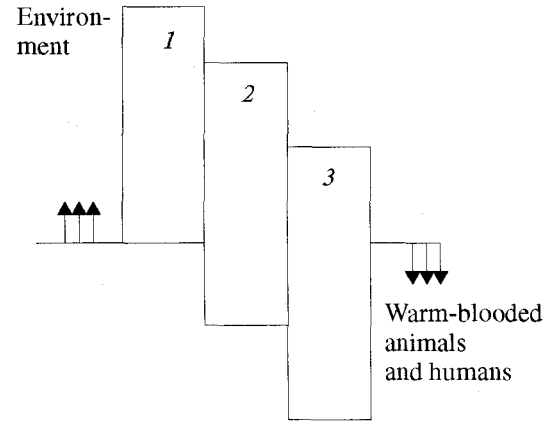

Fig. 1. Evolution-ecological relationships between pathogenic and potential pathogenic bacteria with environment and the warm-blooded organism. *1*) pathogenic saprophytes, sapronosis pathogens; *2*) facultative parasites, saprozoonose pathogens; *3*) obligate parasites, anthroponose and zoonose pathogens.

with that at 37°C indicates that pyridine-dependent dehydrogenases play a more important role in cell respiration at low than at high temperatures. Low temperature activates hydrogen transport in the respiratory chain and ATP synthesis.

Our findings agree with previous reports [11], which describes intensification of the pentose cycle in PTM cells at low temperature due to 1.5-2-fold activation of NADP-dependent glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These similar data attest to additional mechanism of adaptation to low environmental temperature in pathogenic bacteria.

Gas chromatography demonstrated that PTM and LM cultures grown on a synthetic medium without carbogen compounds absorb carbogen dioxide from the gas-air mixture [7].

Participation of absorbed CO_2 in constructive cell metabolism was proved by radioisotope assay with labeled carbogen (^{14}C). Experiments showed that bacteria during the logarithmic phase utilize 31.24±4.6% ^{14}C at 5-7°C and only 20.1±2.3% ^{14}C at 37°C.

The soil-adapted PTM stain absorbs 72.2±2.2% ^{14}C at 5-7°C and only 45.7±2.2% at 37°C. ^{14}C-labeled carbogen dioxide is incorporated into all basic cell biopolymers (Table 1). At a low temperature, ^{14}C is primarily incorporated into RNA, which indirectly attests to activation of protein synthesis and correlates with the above data on accumulation of isozymes under these conditions [8].

The peculiarities of utilization of gaseous substrates by pathogenic bacteria at a low temperature reflect their adaptation to relatively low content of nutrients in soil and water. As most saprophytic bacteria, they are myxotrophic and can switch from chemo-organoheterotrophic nutrition within a warm-blooded

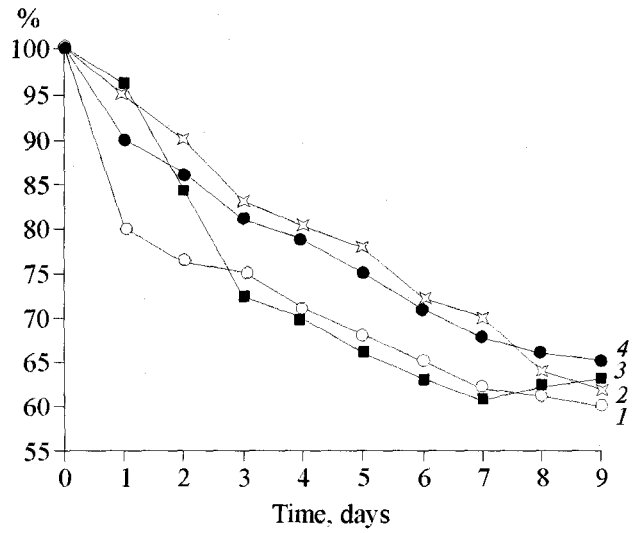

Fig. 2. Assimilation of hydrogen (*1, 2*) and oxygen (*3, 4*) by growing culture of pseudotuberculosis microbes at low (4-6°C, *1, 3*) and high (37°C, *2, 4*) temperatures.

organism to chemolithoautotrophic nutrition in the environment [7].

We also demonstrated propagation of PTM and LM on synthetic mineral media in the absence of nitrogen compounds. It substantiates the hypothesis that the examined bacterial species absorb nitrogen released during autolysis of the dead cells or molecular nitrogen from air (nitrogen fixation is characteristic of many species of saprophytic bacteria).

Using conventional acetylene method, we examined PTM and LM for possible nitrogen fixation from air. The nitrogen-fixing bacteria express a nitrogenase enzyme complex, which performs fixation of nitrogen by breaking the triple covalent bond in molecular nitrogen. The same nitrogenase complex can reduce acetylene and convert it into ethylene. This reaction is used as the test for nitrogen fixation in various biological objects. Chromatography and acetylene method showed bacteria grown on nitrogen-free synthetic medium can reduce minor amount (0.01%) of acetylene to ethylene. Ethylene production was proved by introducing of pure ethylene into the system, which increased the amplitude of initial chromatographic peak proportionally to amount of added ethylene. Therefore,

TABLE 1. Effect of Environmental Temperature on Incorporation of ^{14}C (%) from Carbon Dioxide into Cell Biopolymers (*M±m*)

Biopolymers	5-7°C	37°
CRNA	8.2±0.42	4.4±0.21
DNA	2.4±0.12	2.1±0.12
Lipids	29.3±1.1	9.4±0.39
Proteins and carbohydrates	45.8±2.3	53.8±2.9

the examined bacteria possess nitrogenase activity and probably fix minor amount of nitrogen from the air. Further studies showed that insignificant nitrogen fixation proceeds only at 7°C, but not at 37°C. High content of molecular oxygen in the system inhibits nitrogen fixation, while addition of CO_2 (to 3% v/v) 2-fold increases ethylene production only in PTM. The most favorable conditions for nitrogen fixation were observed at 3% CO_2 and 10% H_2 [7].

It should be noted that some test bacterial stains possessed no nitrogenase activity. This activity was observed only in 5 of 10 PTM strains and only in 3 of 10 LM stains. No nitrogenase activity was detected in the control strains of *Shigella flexneri* and *Salmonella enteritidis*. When bacteria were cultured in a medium containing 0.01% ammonia (primary product in the process of nitrogen assimilation), bacteria exhibited no nitrogenase activity. High energy expenditure for fixation of molecular nitrogen in the presence of ammonia are not justified, because it covers all nitrogen demands of the cell.

Our data suggest that evolution of saprozoonotic pathogens originated from and repeatedly returned to the environment from warm-blooded organisms is associated with the development of adaptive genetical and biochemical mechanisms ensuring their survival in varying environmental conditions. Inhibition of bacterial metabolism is caused by drop of environmental temperature, intensified absorption of hydrogen by some saprozoonotic pathogens, which serves as a fuel for the cell respiratory chain. This process is associated with activation of NAD- and NADP-dependent dehydrogenases involved in electron transfer from substrate hydrogen to flavoenzymes and other components of the respiratory chain coupled with ATP synthesis. In this connection it is interesting to study the function of other elements of the respiratory chain (flavoenzymes, ubiquinone, and cytochromes) and the intensity of ATP synthesis at low temperature.

Nutritional depletion associated with migration of bacterial population from warm-blooded host to the environment induces metabolic rearrangement from heterotrophic to autotrophic pathways characterized by assimilation of CO_2 or sodium bicarbonate dissolved in soil water, as well as fixation of molecular nitrogen from the air.

The inhibition of all enzymatic processes at low environmental temperatures induces synthesis of new isozymes that are more adapted to low temperatures.

We considered some adaptation mechanisms of pathogenic bacteria, belonging to saprozoonotic pathogens, to unfavorable environmental factors responsible for the formation of a reservoir of these pathogens in the environment. The revealed mechanisms are only a small part of the integral adaptation system in ecto-

thermic organisms involved in their complex interrelations with varying environment.

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