The Ultrastructure of Pathogenic Bacteria under Different Ecological Conditions

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By

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This book is dedicated to

George Pavlovich Somov
(1917–2009)
The outstanding Russian epidemiologist and microbiologist, and founder of the Scientific School of Research Institute of Epidemiology and Microbiology in Vladivostok (Russia)

"A life is good only when it is a continuous movement forward from the adolescence to the grave"

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FOREWORD

George Pavlovich Somov was born on October 1, 1917 in Yalta city, Russia (Crimea). His childhood and youth were spent in Leningrad. Georgy Pavlovich can be considered a microbiologist and epidemiologist by vocation, since he chose his path while still a student in the 2nd year of the I. P. Pavlov First Leningrad Medical Institute, when he began to engage in scientific work at the Cathedra of Microbiology. By the time he graduated from the Institute in June 1941, he had gained considerable experience as a bacteriologist. With the excellent reference from his first teacher, professor V. N. Kosmodemiansky, G. P. Somov was called up for military service and appointed head of the sanitary-epidemiological laboratory of the Ladoga Military Flotilla. Here, on the "Road of Life" of besieged Leningrad, from 1941 to 1944, along with practical work ensuring the sanitary well-being of ships and parts of the flotilla, he studied the epidemiological efficacy of new vaccines under the supervision of the Chief epidemiologist of the Baltic Fleet V. I. Ioffe.

Since 1953, George Somov's life and work has been associated with Vladivostok. In his post as the chief epidemiologist of the Pacific Fleet, he worked on special issues of military epidemiology. After retiring with the rank of Colonel (medical services), in 1961, G. P. Somov became the deputy director of the Research Institute of Epidemiology and Microbiology and launched an extensive research project on natural focal infections – an urgent regional problem, which has become the main scientific direction of the Institute.

The purposeful leadership of G. P. Somov in scientific work, and an integration with practical and educational institutions, allowed the Institute of Epidemiology and Microbiology to obtain priority results, including on the etiology and epidemiology of rickettsial infections, and the tsutsugamushi fever, whose natural foci were found in south-western regions of Primorsky Krai, on the Southern Kuril Islands, and the southern Sakhalin. In 1980, the Research Institute of Epidemiology and Microbiology received the status of an academic institution in the system of the USSR Academy of Medical Sciences (and later – the Siberian Branch of the Russian Academy of Medical Sciences). From 1983 to 1988, he worked as a director of the institute, and then as an advisor to the directorate, without leaving the institute until the end of his life.

Having proved himself in his youth as a prominent scientist, G. P. Somov followed the best traditions of the national sciences all his life. A man of high culture and encyclopedic knowledge, he invariably attracted the attention of others. Being constantly in a state of curiosity, George Pavlovich charged his pupils and colleagues with a boiling energy and purposefulness. For them, he was not only a scientist, but also a friend, and a living example of an intellectual. No wonder his life credo was the statement of Napoleon Bonaparte: "A life is good only when it is a continuous movement forward from the adolescence to the grave".

G. P. Somov became one of discoverers of Far Eastern scarlet-like fever (FESLF) – a new clinical-epidemic manifestation of pseudotuberculosis, first recorded on the territory of Russia in the late 1950s. A close study of this previously unknown disease contributed to the development of progressive biomedical ideas about the "World of Microbes". Based on these ideas, G. P. Somov created an original concept regarding the psychrophilicity and adaptation mechanisms of pathogenic bacteria which enable them to live both in humans and warm-blooded animals, and in the environment. Specialists of the G. P. Somov Scientific School managed to obtain priority scientific data that formed the basis of the Doctrine on Saprozoonoses (sapronoses), developed by G.P. Somov.

The validity of judgments and at the same time the rare courage in making original scientific decisions – this was what essentially characterized George Pavlovich. He was convinced: "Paradoxical things are seldom perceived immediately and unconditionally, and are irritating in their apparent contradiction of generally accepted propositions and well-established theories. However, they are the things that move the science, because ... the science develops not by accumulating finally established truths, but by changing the dominant concepts."

Nowadays, the enormous scientific achievements of G. P. Somov – an academician of the Russian Academy of Medical Sciences, an honored worker in science, and the laureate of the State Prize of the Russian Federation – are generally recognized. He is rightfully ranked among the coryphaeus of the Russian and world epidemiology and microbiology. G. P. Somov was the creator of the scientific school, and in 2012 the Institute of Epidemiology and Microbiology was named after him.

As early as the mid-twentieth century, it was recognized that under the influences of external effects, the hereditary characteristics and properties of microbes, their species qualities and their features may change. Even then, the variability of bacteria began to be seen as an expression of adaptation processes. By the beginning of the 2000s, on the basis of many years of research conducted at the Institute of Epidemiology and

Microbiology of the Siberian Branch of the Russian Academy of Medical Sciences under the direction of the academician G. P. Somov, the genetic-biochemical mechanisms of pathogenic bacteria adaptation to abiotic environmental factors were revealed on models of pseudotuberculosis and listeriosis microbes. These mechanisms determine the dual – saprophytic and parasitic – nature of the causative agents of saprozoonoses: their ability to multiply and persist for a long time in environmental objects. This unique feature of these pathogens is due to a wide metabolic plasticity that ensures their adaptation to different habitat conditions.

In the Russian-language edition of the monograph by L. M. Somova et al., "Ultrastructure of pathogenic bacteria under different ecological conditions" (Vladivostok, 2009), for the first time, the results of microbiological and electron microscopic studies of saprozoonoses' causative agents, carried out under conditions of model microecosystems, were generalized and systematized. Over the past five years, the Russian academy of natural sciences has presented this monograph at International book exhibitions in Germany (Munich), Russia (Moscow), France (Paris), Great Britain (London), Spain (Barcelona), and in "*BOOKEXPO AMERICA 2019*" (New York), "*HONG KONG BOOK FAIR 2019*", (China, Hong Kong, July 17–23, 2019), and *BUCH WIEN 2019* (November 6-10, 2019, Vienna, Austria).

The decision to publish the book in the English-language edition (with changes and additions) was dictated by the desire to make the priority data of Russian scientists on the problem of saprozoonoses a property of world science. The expediency of this decision was connected with the transition of research on medical microbiology and ecology to a qualitatively new stage of study associated with the development of approaches to a broad study of non-cultivated forms of microorganisms directly related to the multiple antibiotic resistance of bacterial strains, and to the development of persistent and chronic infections.

I hope that our developments about the morphological bases of heterogeneity and adaptation of infectious pathogens will be in demand at the present time, when this problem has acquired a global significance in biology and medicine. I send my best regards to the readers of my book.

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PREFACE

To date, the problem of bacteria variability occupies one of the key positions in microbiology. As far back as the 20th century, it was noted (Timakov, 1958) that, in contrast to the previously existing concepts of the constancy of bacterial species, it should be recognized that external influences may change innate characteristics and properties of microbes, and their specific qualities and features. Even then, the variability of bacteria began to be considered mainly as an expression of adaptation processes. Particular attention was paid to the need to expand research in order to determine the variability of bacteria in their natural habitats, especially in various associations.

However, there is still no solid data on the morphological variability of bacteria and its essence. In the system of basic research in the field of biology and medicine, electron microscopy methods do not lose their significance for the identification and taxonomy of human and animal pathogens.

The new knowledge on the biology of pathogenic bacteria accumulated by the end of the 20th century formed the basis of the theory of saprozoonoses (sapronoses), a new group of infectious diseases whose causative agents have two habitats – the human body and warm-blooded animals on the one hand, and the external environment on the other (Somov, 1985; Somov et al., 1991). This unique feature of saprozoonoses' pathogens is caused by a wide metabolic plasticity, which enables their adaptation under different conditions during the transition between the parasitic (in an organism) and the saprophytic (in the external environment) phases of their existence (Tafelshtein et al., 1995; Somov and Litvin, 1988; Buzoleva and Terekhova, 2002; Somov and Buzoleva, 2004).

In this regard, it became necessary to expand the understanding of the submicroscopic organization of this group of pathogenic bacteria in various environmental conditions. In relation to non-organism populations of saprozoonoses' causative agents, electron microscopic studies are of fundamental importance, since they contribute to the disclosure of the morphological basis of their adaptation to changing environmental factors in natural conditions. Until now, the ultrastructural aspects of pathogenic bacterial adaptations in the environment have been studied in fragments (Litvin et al., 1998; Okolelov et al., 1989; Pavlova, 1998). The established

ideas about the submicroscopic organization of microbes are based exclusively on the data obtained when they are cultivated in a thermostatically controlled environment at 37°C (Avakyan et al., 1972), which only reflects the state of the bacterial population under the same conditions as would be found in a warm-blooded organism.

Based on years of research conducted in the Somov Research Institute of Epidemiology and Microbiology, on models of pseudotuberculosis and listeriosis microbes, adaptive genetic and biochemical mechanisms are revealed that determine the dual – saprophytic and parasitic – nature of saprozoonoses' causative agents, and their ability to multiply and persist in environmental objects. The main mechanisms include (Somov and Buzoleva, 2004):

* the ability of bacteria to multiply in a wide range of temperatures (from 0 to 40° C) due to the synthesis of "cold" and "thermal" isoenzymes, and changes in the conformational structure of their active surface, which ensures the maintenance of the necessary level of metabolism during the bacteria's transition from the environment to the warm-blooded organism and back;
* the deterioration of the nutritional conditions of bacteria when they are removed from a warm-blooded organism into the environment causes a change in their metabolism from a heterotrophic to an autotrophic pathway, allowing carbon dioxide and other C_1-compounds to be assimilated, and their carbon can be used to synthesize the necessary organic compounds (DNA, RNA, proteins, carbohydrates, and lipids).

These mechanisms underlie the adaptation of microbes to varying trophic and temperature factors. Naturally, the adaptation process is accompanied by changes in the physiological state of the bacterial populations. As shown in our works on salmonella (Isachkova et al., 1994), pseudotuberculosis and listeriosis pathogens (Isachenko et al., 2000; Isachenko, 2004; Somova et al., 2004; Somova et al., 2004, 2006), this can be judged by the ultrastructure of pathogenic bacteria in appropriate environmental conditions.

This monograph-atlas is the result of the generalization and systematization of the data of long-term microbiological and electron microscopic research projects at the Somov Research Institute of Epidemiology and Microbiology (Vladivostok) – relatively little-studied aspects of the above problem. For the studies, museum strains of *Yersinia*

pseudotuberculosis, Listeria monocytogenes and *Salmonella enteritidis* were taken from the Institute's collection.

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I express my deep gratitude to the doctor of biological sciences L. S. Buzoleva, post mortem (1958–2018), and A. S. Isachenko for carrying out microbiological studies and preparing samples of bacterial suspensions in the laboratory of pathogenic bacteria ecology (the head was academician G. P. Somov). For assistance in the preparation of illustrative materials, thanks to O. G. Polushin, N. G. Plekhova, I. N. Lyapun, and graphic designer A. G. Ilyushina. The Russian-language edition of the monograph (Vladivostok: Medicine DV, 2009) was carried out with the financial support of the Russian Foundation for Basic Research, project no. 09-04-07009.

LIST OF ABBREVIATIONS

Ag	–	electron-dense agglomerate
BDS	–	bubbles in dividing septum of bacteria
Cap	–	capsule
CB	–	coryneform bacteria
ChF	–	chromatin fibrils
Cov	–	cover
CpM	–	cytoplasmic membrane
CW	–	cell wall
CWD	–	cell wall detachment
D	–	electron-dense deposit
DB	–	dividing bacteria
FEdM	–	flocculent electron-dense masses
FLBC	–	focal lysis of bacterial cytoplasm
FsS	–	feather-shaped structures
GCW	–	growth of the cell wall
HEdM	–	high electron-density material
IcC	–	intercellular contact
IcM	–	intercellular matrix
ICW	–	invagination of the cell wall
Inc	–	electron-dense inclusion
LB	–	lysed bacteria
Mc	–	microcapsule
MEdM	–	medium electron-density material
Mes	–	mesosome
MlS	–	mesosome-like structure
Muc	–	mucus
Nuc	–	nucleoid
OG	–	osmiophil granules
Pp	–	protoplast
Pr	–	prostake (outgrowth on the surface of bacteria)
PS	–	periplasmic space
R (Rs)	–	ribosomes
RNZ	–	rarefaction in the nucleoid zone
RS	–	reticular structures

Sp – spheroplast
Vac – vacuole (pseudovacuole)
WD – waist of division

CHAPTER ONE

FUNCTIONAL AND MORPHOLOGICAL FEATURES OF BACTERIA IN DIFFERENT PHASES OF GROWTH OF A PERIODIC CULTURE

Abstract. Information is provided on the morphological changes of pathogenic bacteria in different phases of periodic cultivation, due to the rearrangement of the bacterial cells' metabolism in accordance with the environmental parameters. In the lag phase and exponential phase, these changes are associated with the intensity of DNA and RNA replication and the protein synthesis during the accumulation of bacterial biomass, and are manifested by a pronounced heteromorphism of bacteria. With a maximum increase in the population density, growth in the number of bacteria is inhibited, which coincides with the start of the synthesis of reserve substances (carbohydrate, lipid, polyphosphates). In the stationary phase of periodic cultivation, when the growth rate of microorganisms is equal to the rate of their death, bacterial cells are more uniform in size than in the exponential phase. Many of them have excreted secondary metabolites into the environment. At the end of this phase, a decrease in the intensity of the DNA and RNA synthesis is observed in the cells, and the growth of bacteria stops. The consequence of these processes is the presence of microorganisms of different quality – living but starving, living but inhibited – as well as the die-off of some bacteria due to starvation or poisoning. Cell division is disturbed, with the appearance of filamentous and branching forms, as well as coccoid forms. In the late stationary phase, the culture dies out, with the appearance of involutional forms and a large number of lysed bacteria. The population of starving bacteria is extremely heterogeneous. In the die-off phase, resting bacteria accumulate. The main cellular structures of these remain intact, but the content of protein, lipids and RNA in them is significantly reduced. Therefore, these cells have a reduced size and compacted cytosol. Morphological and functional changes in the bac-

teria in the die-off phase of a periodic culture contribute to the economical utilization of nutrients remaining in the environment.

The periodic culture of bacteria is actually a model that is close to natural conditions, because in any phase it is in a state of restructuring its metabolism in accordance with changing environmental parameters (Vaysman, 1985; Golovlev, 1983). In the process of periodic cultivation, the bacterial culture goes through the following phases of growth and development:

1) the phase of adaptation to the medium (lag phase);
2) the phase of exponential (logarithmic) growth;
3) the stationary phase;
4) the die-off phase.

Depending on the phases of growth and development of the population, the morpho-functional state of microbial cells changes (Kaminsky, 1985).

In the lag phase, which covers the time interval between the inoculation of microorganisms in a nutrient medium and the exponential growth phase, there is gigantism of bacteria, with a significant increase in their biomass due to intensive accumulation of DNA, RNA and protein. In this case, due to the delay of division, the size of microorganisms increases several times.

The formation of polyergic filamentary forms of bacteria is aimed at the preservation and multiplication of the gene pool of the population. The leading element here is the multiple replication of DNA, which is lagged behind by the processes of building up membrane structures and the synthesis of cell wall substances necessary for the formation of transverse septums during division. The so-called “gigantism of young forms” is a sign of high vital potencies of inoculum. In the lag phase, the maximum number of mesosomes is revealed in bacterial cells.

At this time, changes in the composition of the bacterial cell are most strongly reflected in the RNA content, which is greatly enhanced due to the rapid synthesis of proteins and other substances (Kuznetsova et al., 1991). The lag phase duration is determined by the optimal composition of the growth medium – the more balanced the medium, the shorter this phase (Rybalchenko and Tets, 1987). According to I. L. Rabotnova et al. (1981), it is not an obligatory growth phase and its occurrence depends on

non-observance of optimal conditions for the reproduction of the seeding material.

A seeding culture on a fresh medium is accompanied by a period during which not only is cell division not observed, but sometimes even a reduction in the number of cells occurs (Baskanyak et al., 1981; Brunschede al., 1977). Depleted cells of the old seeding material should go from starvation or self-poisoning to the state corresponding to the ability to reproduce, which is determined by the required number of ribosomes, the ability and conditions for DNA replication, cell wall synthesis, etc. (Hmel, 1970). A change in the rate of RNA synthesis and the formation of ribosomes is accompanied and often precedes changes in the growth rate of bacteria (Korotyaev, 1973, 1998; Tets and Kaminsky, 1984). The less stress a culture experiences at the beginning of cultivation, the more resistance it has to subsequent stressful effects (Rybalchenko and Tets, 1987; Cheroutre-Vialette and Lebert, 2000).

The exponential (logarithmic) growth phase is characterized by a constant maximum speed of bacterial cell division, depending on the type of microorganism, on the environment, and on the conditions of cultivation (Kuimova, 1984). This phase most reflects the ability of the culture to reproduce. The transition to the exponential phase of growth occurs abruptly through the fragmentation of the filamentous forms of bacterial cells. The division of a multitude of individuals that appeared during a short period of time by fragmentation leads to a kind of "explosion" of growth, which provides a high population. Bacterial cells, in which structural, plastic and metabolic processes predominate at this stage, produce biologically active substances, probably of an enzymatic type, called "fission protein" and "growth activators" in *Escherichia coli* and *Salmonella typhi* (Vaysman, 1985; Egorov, 1977), "schizokines" in cultures of *Bacillus subtilis* and *B. megaterius* (Liu et al., 2000), enhancing cell division, in particular, in *Clostridium perfringens*. The presence of these substances is necessary, apparently, not only to initiate, but also to maintain the cell division.

During the exponential phase, in the period of phenotypic adaptation, bacterial cells are at their most vulnerable, and in some individuals, pathological changes may develop (Vaysman, 1985; Timakov et al., 1983). For example, in a culture of meningococcal bacteria in this phase, occasionally there are giant, non-dividing cells, the cut-off area of which is 2–2.5 times larger than the cut-off area of normal cells.

This, and a number of other signs (smoothed contours, a nucleoid poor in the fibrillar component), indicate the non-viability of such bacteria (Vysotsky et al., 1991). In this regard, according to I. Sh. Vaysman (1984),

a clear distinction should be made between normal and pathological heteromorphic growth in early stages of the bacterial culture's development.

During the period of exponential growth, there are marked fluctuations in the levels of metabolic processes, their qualitative shifts, and the uneven growth of bacterial biomass. Morphological analysis shows that in most bacterial cells, the growth of the body as a whole is strictly coordinated with the growth of the corresponding structural and functional components, but the rate of these processes is variable. In the course of multiple divisions in this phase, during the formation of transverse septa, the build-up of the cytoplasmic membrane and the enhanced synthesis of the cell wall substance, which is used to form them, clearly outpace the growth of the cell body, which is somewhat delayed (Vaysman, 1985).

The growing and thickening of the bacterial population with exponential growth causes a corresponding cell response. Regarding *Lactobacillus plantarum*, for example, it is first expressed in the hyperplasia of cytoplasmic membrane structures, which is not observed in the early exponential phase (Vaysman, 1984). An increase in the density of the bacterial population causes a cell reaction, which can be judged not only by morphology, but also by the production of biologically-active substances by the microorganisms, which serve as a kind of metabolic signal to inhibit the growth of the population (Liu et al., 2000). The beginning of slowing bacterial growth coincides with the beginning of the synthesis of reserve substances – carbohydrates, lipids, polyphosphates, etc. (Kulaev et al., 1974; Rabotnovaet al., 1981; Ratner et al., 1988).

The beginning of the stationary phase is the period when the number of microbes stops increasing and the maximum accumulation of their biomass is observed. Its amount remains constant for some time: the rate of growth of microorganisms is equal to the rate of their deaths. At the beginning of the stationary phase, bacterial cells are more uniform in size than in the logarithmic growth phase (Vysotsky et al., 1991). However, an increase in the degree of cellular polymorphism is observed in the dynamics of the development of the bacterial population, with a decrease in the concentration of the substrate in a culture medium, and the accumulation of toxic metabolic products (Nikolaev et al., 2000). In cells, a decrease in the intensity of DNA and RNA synthesis is observed (Tets and Kaminsky, 1984).

The period of slowing of the growth of a periodic culture can be varied and complicated. It may be completely absent when bacteria are cultivated on simple synthetic media, when their growth immediately stops due to the absence of any element of nutriment, in particular a carbon source. With an excess of nutrients, the growth of microorganisms slows down due to

the accumulation of metabolic products. When culture growth is limited due to the lack of nutrition, the metabolism of the bacteria changes as a result: instead of synthesizing normal cell components and forming new daughter cells, their enzyme systems switch to the synthesis of spare substances (Rabotnova, 1990; Rabotnova et al., 1981). In particular, it is believed that poly-β-hydroxybutyrate accumulates in cells under conditions of unbalanced growth, when the processes of protein and nucleic acid synthesis are limited, and it is a depot of energy and carbon for cells (Volova et al., 1992, 1996; Preiss, 1989). There is also evidence that polyphosphates, found in bacterial cells in the exponential growth phase, disappear from the cells of aging cultures when their deficiency occurs in the nutrient medium (Nesmeyanova, 2000; Novik et al., 1992; Hmel, 1970).

In the stationary phase, the growth of bacteria stops and many of them show the release of secondary metabolites into the environment, like exocytosis in eukaryotic cells (Vysotsky et al. 1995; Mynbayeva et al., 1987; Reshilov et al., 1983). At the same time, exoproducts are displaced from intracytoplasmic membrane structures through the internal cytoplasmic membrane into the periplasmic space, where they accumulate in the form of an amorphous substance (Bezborodov and Astapovich, 1984; Kuimova, 1984). Their excretion from the cell can also occur through pores of the cell wall or by dissolving it in separate areas (Afanasyev et al., 1976; Oleskin et al., 2000; Pavlova and Ratgauz, 1970).

On complex media containing several carbon sources, their sequential utilization may occur with a gradual slowdown in the bacterial growth. In this case, a simultaneous poisoning and starvation of the cells is possible. Therefore, the most diverse microorganisms can be contained in the stationary phase: living, but starving; living, but inhibited; as well as bacteria which are dying due to starvation or poisoning (Rabotnova and Ivanova, 1971). Specifically, in the stationary phase of the bacterial culture development, many bacteria are found with impaired cell division (Kuimova, 1984). There are thread-like and branching forms of microorganisms (Vysotsky et al., 1984; Nikolaev and Voronina, 1999; Nikolaev et al., 2000; Tets and Kaminsky, 1984). Morphologically, the starvation state can manifest itself in a change of microbial forms: the rod-shaped bacteria are often rounded, although the cell volume does not change (Akayzin, 2000; Pavlova, 1998; Pavlova et al., 1990a, 1990b). The fragmentation of the rod-shaped bacteria into coccoid forms may be observed. In *L. monocytogenes*, the appearance of coccoid forms takes place, which are capable of reverting to rod-shaped bacteria when introduced into a fresh nutrient medium (Pomanskaya, 1961). In coryne-like bacteria during starvation, the following transformation of the cell form is observed: cocci – rods –

branched cells and aggregates (Pavlova et al., 1990). *Bacillis brevis* forms giant multicellular masses capable of fragmentation to bacillary individuals when transplanted into a medium with normal cultivation conditions. The cell wall in many species of bacteria is often thickened. It is believed that the branching forms of microorganisms are modifiers and/or spontaneous mutants that are selectively resistant to nutritional deficiencies, as well as to changes in physico-chemical growth parameters (Nikolaev et al., 2000). V. V. Tets and G. D. Kaminsky (1984) also suggested an increase in the mutability of bacterial cultures during the slowdown of reproduction and at the beginning of the stationary phase.

In the late stationary phase, the death of the bacterial culture begins. There is a significant slowdown in the growth of bacteria; the consumption of the energy substrate increases to maintain their vital activity. The death rate of the cells becomes higher than the speed of their reproduction. When the population reaches a critically high level for these conditions and goes into a state of starvation, biologically-active metabolites that act at the level of cell wall structures are formed. This causes a disturbance of the organization and metabolic functions of bacteria, suppressing autolytic processes at the level of the formation of transverse septums, and blocking division.

In this phase, bacterial cells that are larger than normal, curved and swollen – called involutional forms – are often found, and a large number of lysed microorganisms are also observed (Kuimova, 1984; Feofilov, 1992). However, at the same time, a considerable number of the individuals in the bacterial population are resistant to damage and enter resting (anabiotic) states (Bespalov et al., 2000; Demkina et al., 2000, 2000a; Mukamolov, 1995; Akerman et al., 1993).

In non-spore-forming bacteria, adaptive manifestations under fasting conditions include their division without growth (fragmentation) up to the appearance of ultramicroscopic cells, and the formation of outgrowths (prostakes, hyphae, stems) that increase the surface of cell membranes. (Konstantinova and Rakovskaya, 1980; Coronelli and Nesterova, 1990; Morita, 1988; Moyer and Morita, 1989; Nanninga, 1988; Novitsky and Morita, 1977). However, as a result of crushing, small non-viable cells can also form (Pavlova et al., 1990). Thus, the population of starving bacteria is heterogeneous and may include various groups of microorganisms (Mukamolova et al., 1995).

The resting bacteria retain intactness, basic cellular structures (nucleoid, cytoplasmic membrane, cell wall) and chemical components, although the content of proteins, lipids and RNA in them is significantly reduced. Typically, such microorganisms have a lessened size and coccoid form;

their cytoplasm is more electron-dense than that in the cells of the original culture; electron-dense material is determined in the nucleoid zone; the cytoplasmic membrane is poorly contoured; the cell wall thickens; and its viscosity increases (Ginsburg and Romanova, 1997; Demkina et al., 2000, 2000a; Milko and Egorov, 1991; Mulyukov et al., 1997; Romanova, 1997).

In the stationary phase of the culture growth, bacteria exhibit the ability to utilize the autolyzed products of lysed cells (Buzoleva, 2001). An elimination of bacteria by the lysis of a significant number of individuals (which is where pathological changes in some cells lead) and the transformation of another part of the population to dormant forms both also contribute to the economical utilization of nutrients remaining in a medium (Vaysman, 1985).

Thus, according to I. Sh. Vaysman (1984), the effect of endogenous inhibitory metabolites can be considered, to a certain extent, as a health tool of the bacterial population and the means of selecting the most viable individuals. The autolysis of microbial cultures should be considered as a stage preceding and necessary for the generation of resting forms of microorganisms and the preservation of their species.

CHAPTER TWO

INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON THE SUBMICROSCOPIC ORGANIZATION OF BACTERIA

Abstract. In both natural and laboratory conditions, bacteria are exposed to various abiotic factors (changes in temperature, humidity and the mineral composition of habitats) and biotic factors (influence of other natural biota, the host organism). The temperature of the environment is one of the most decisive factors for the growth and development of bacterial cells. Thermophiles, mesophiles and psychrophiles adapted to their existence under certain temperature conditions. Under natural conditions, very often the temperature values are far from optimal for the vital activity of a particular type of microorganism. Therefore, at elevated (supraoptimal) or lowered (suboptimal) temperatures, a disruption of cell division and an inhibition or enhancement of RNA and DNA synthesis can occur, affecting the growth of the bacterial population and the formation of biomass.

Under stressful conditions, disturbances in biosynthetic processes are accompanied by corresponding ultrastructural changes in bacteria (nucleoid, ribosomes and polysomes), as well as their heteromorphism (changes in the shape and size of bacteria, the appearance of inclusions). Some important structures of the bacterial cell, the formation of which can be influenced by habitat conditions, are the capsule, microcapsule, and mucous membranes. These structures are constructed from carbohydrate-containing biopolymers, which make them intermediaries in intra- and interpopulation interactions. Many pathogenic bacteria, including *Salmonella*, *Listeria* and *Yersinia*, are able to adapt to the long-term effects of biotic and abiotic factors, transforming to a non-cultivated (resting) state, which is considered a mechanism for existence in the environment.

Currently there is no doubt that the viability of bacteria is closely dependent on the environmental conditions, since an exchange of the organic and inorganic substances necessary to maintain the cell metabolism at a certain level continuously takes place between microbial cells and the environment (Rabotnova and Ivanova, 1971). Bacteria in both natural and laboratory conditions are exposed to a variety of factors. Seasonal factors (periodic changes in temperature, moisture, pH, and the mineral composition of habitats) can be attributed to both long-term and short-term factors – e.g. day and night changes (changes in light, temperature, humidity, etc.), changes in the levels of oxygen and other gaseous substances. The influence of other natural biota (bacteria, protozoa, invertebrates, fungi, algae, higher plants – symbionts), as well as the organism of a warm-blooded or other host carrier, is significant (Litvin et al., 1998). Most environmental factors have a decisive influence on the characteristics of the bacterial metabolism, primarily acting on the integrity of the cellular organization.

The temperature of the environment is one of the decisive factors for the growth and development of bacterial cells. Thermophiles, mesophiles and psychrophiles adapted to their existence in the corresponding ecological conditions. In natural conditions, very often the temperature values are far from optimal for the vital activity of a particular type of microorganism. Therefore, an inhibition of the bacterial population growth by an excess of thermal energy (at supraoptimal temperature) or a growth inhibition by lack of heat (at suboptimal temperature) may occur. The temperature range above that in which bacteria grow to their maximum biomass formation is called supraoptimal. At the optimum temperature, cultures grow slowly but have a longer growth period at a high rate than cultures under elevated temperature conditions.

The beginning of the cell cycle is most sensitive to the action of elevated temperatures. If temperatures are elevated at this time, there is a disturbance in the formation of a division septum, up to a complete absence, and this results in the formation of long filamentous bacteria (Lyakh, 1976) or the non-total separation of cells from each other. This is due to a disturbance in the balance of constructive processes, which leads to abnormal growth and the loss of the cell's ability to divide normally (Zhdan-Pushkina et al., 1986; Puchkov et al., 1987). The formation of filamentous cells was observed both in temperature-sensitive mutants of *E. coli* mesophilic bacteria and in obligately psychrophilic Antarctic yeasts. D. Gilichinsky et al. (1993) noted that the process of the filamentous cells' formation at elevated temperature is reversible. When bacteria are incubated under conditions that are optimal for growth, their ability to divide is

restored, but this process may be disturbed: both normal-sized and very short bacterial cells are formed.

Under the action of elevated temperature, the synthesis of RNA and DNA is inhibited, thereby reducing the optical density of the bacteria cytoplasm. At supraoptimal temperatures, a coarsening of chromatin fibrils occurs in the nucleoid zone, with a tendency to cause their parallel folding, and the condensation of the hereditary material in the zone of formation of the partition walls between daughter cells (Peshkov and Mashkovtseva, 1977; Puchkov et al., 1987; Samoylenko et al., 1997).

At low (suboptimal) cultivation temperatures in some soil bacteria, as well as in the pathogenic bacteria *E. coli* and *Y. pseudotuberculosis*, an increase in RNA synthesis was noted (Aseeva and Lysak, 1981). At the same time, the amount of RNA clearly exceeds that in cultures grown at the optimum temperature. However, a lowering of the cultivation temperature of bacteria leads to a decrease in the ribosome productivity. As shown by the authors, in order to maintain the growth rate at a certain level, bacteria compensate for the low efficiency of ribosomes by their additional synthesis, due to which no fundamental changes occur in the most important intracellular process – protein biosynthesis. In mesophiles at low temperatures, the rate of polysome formation first decreases and then stops (Innis and Ingraham, 1978). Differences in the content of DNA in microbial cells cultivated at optimal and low temperatures were not found. Suboptimal temperatures inhibit the speed of reproduction and growth of bacteria, but they can stimulate the formation of reserve high-energy biopolymers. Thus, a decrease in temperature to 5°C (at a growth optimum of 25°C) leads to a pronounced accumulation of polyphosphates in *Acinetobacter spp*. cells, which is apparently one of the methods for the diverting of unused energy from the original substrate (Pozmogova, 1983).

Thermophilic *B. brevis* cells grown at a low temperature (28°C) contain an increased amount of lipid inclusions compared with control cells (Peshkov and Mashkovtseva, 1977). There is evidence that microorganisms containing polyoxybutyrate better tolerated a lower temperature of cultivation than bacteria deprived of its reserve (Volova et al., 1996). It is assumed that this compound is involved in the reparation process of bacterial cells which have undergone a disturbance of their division (Pronin and Zhukov, 1986).

The causative agents of saprozoonoses infections are psychrophiles, which are adapted to the environment both in a warm-blooded organism and in the external environment, with its changing temperature and trophic conditions (Somov and Buzoleva, 2004). There is a division of psychro-

philes between obligate and facultative, depending on the optimum temperature of their growth – below or above 20°C, respectively.

When comparing the equivalent growth phases of cultures of facultative psychrophilic strains of the *Pseudomonas* bacteria at temperatures of 2°C and 30°C, no differences in the sizes of their cells were found, despite the fact that at 2°C the growth rate was 10 times lower. It was suggested that in this case the absence of the thermal-morphological reaction was provided by the functional flexibility inherent in facultative psychrophiles (Lyakh, 1976).

S. P. Watson et al. (1998) noted that at a low (between –1.5 and 0°C) temperature, the generation time and cell sizes of facultative psychrophilic microorganisms depend on the concentration of an organic carbon. At a low concentration, the generation time was two to three times longer than at its high concentration. Thus, in the case of growth in the cold, facultative psychrophilic bacteria have an increased need for nutrients.

Negative temperatures (down to –30°C) are suboptimal for psychrophilic bacteria, but a cell's development can still occur in a nutrient medium with glycerol and high salt concentrations, which lower the freezing point of the aquatic environment. The lower the temperature, the slower the biological processes in the cells, which contributes to the pronounced increase in the length of time that the viability of microorganisms can be maintained (Kochkina and Ivanushkina, 2001). It seems likely that the bacterial stress caused by low temperatures – and recorded as a state of suppressed growth – is fundamentally different from a stressor of another origin (e.g. extreme values of pH, high temperatures, freezing, exposure to toxic substances, radiation and other factors damaging to subcellular structures). However, the reaction of cells in all of the above situations is to reduce the growth rate of the bacterial population. At low temperatures, metabolic processes are slowed down, but cell components are not damaged (Nikitin, 1985).

During freezing and defrosting, part of the microbial population dies, but some bacteria that have been frozen in natural conditions can remain in this state for a long time, retaining their viability (Kochkina and Ivanushkina, 2001). Freezing causes deformation, folding and cell wall damage, reducing the volume and size of cells due to dehydration (Sventitsky et al., 1988; Morita, 1975). During defrosting, the pronounced activation of respiration and reproduction of microorganisms can be observed. This feature plays an important role in the successful survival of bacteria in cold seasonal conditions. It is assumed that during the long-term storage of bacteria at a negative temperature, the selection of those variants which have developed a resistance to stress occurs (Demkina et al, 2000).

In natural habitats, in the daytime most bacterial cells are more exposed to the dry air than to direct sunlight, since microorganisms are shielded by dust particles, soil, leaves, etc. At different times of the day in spring, summer and autumn, the periodic drying and wetting of most bacteria is possible. When dried, bacterial cells become dehydrated; some of them die, while others, when exposed to sufficient moisture, can restore their properties, including the ability to reproduce. Dried cells are very resistant to the effects of various extreme physico-chemical factors, as a result of which the viability of bacterial populations in general remains (Becker, 1979).

Using the example of the yeast *Saccharomyces cerevisiae*, it was shown that during dehydration there is a decrease in the cells' volume and a change in their form (Rabotnova et al., 1981). The bacteria were lengthened when the glucan component of the cell wall changed. Changes in the structure of the cytoplasmic membrane – its protrusion, deepening and rupture; areas of cytoplasm damage, delimited by membranes – are observed. Bacterial cells in the exponential phase (during intensive growth and reproduction) are more sensitive to drying than those in the stationary phase. The period after rehydration is characterized by an extended lag phase (Becker M.E., 1979). During the revival of dried microorganisms, in the first generations, unstable forms of the heteromorphic growth, similar to L-forms, are observed (Dobritsa et al., 1987).

Extremely low or high pH values of a medium lead to the existence of a bacterial population in an inhibited state. A feature of cultures inhibited by extreme (but not lethal) pH values of a cultivation medium is the accumulation of reserve substances (Pozmogova and Medvedeva, 1978). Such cultures are characterized by the extreme heterogeneity of the population – a high content of hypertrophied (swollen, elongated) cells and a relatively small number of cells of normal size, due to which, probably, reproduction occurs, and the bacterial culture continues to exist (Podgorsky et al., 1989). In *B. megaterium*, cultivated in an acidic medium, rod-shaped bacteria turn into balls, forming conglomerates; the division process is disturbed, and in the culture long filamentary forms of bacteria with rare division septums appear. At the same time, cells grown in alkaline media are not significantly different from bacteria that developed under optimal conditions (Rabotnova and Ivanova, 1971). Many types of bacteria alkalize or acidify the nutrient medium in the process of developing a periodic culture, which can cause autolytic changes in the culture (Akayzin, 1998; Reshilov et al., 1983).