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REVIEW PAPERS

Colonial Organization and Intercellular Communication in Microorganisms

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Abstract—This review covers the modern concepts and recent data demonstrating the integrity and coherence of microbial populations (colonies, biofilms, etc.) as peculiar "superorganisms." Special attention is given to such relevant phenomena as apoptosis, bacterial altruism, quorum effects, collective differentiation of microbial cells, and the formation of population-level structures such as an extracellular matrix. Emphasis is placed on the channels and agents of intercellular communication in microbial populations. The involvement of a large number of evolutionarily conserved communicational facilities and patterns of intercellular interactions is underscored. Much attention is also given to the role of colonial organization and intercellular communication in parasite/commensal/symbiont–multicellular host organism systems.

Key words: microorganisms, colonies, apoptosis, quorum, biocommunication, matrix, peptides, homoserine lactones, neuromediators, hormones

This paper deals with the data indicating that bacteria and eukaryotic microorganisms exist as integral structured colonies. There are important reasons for regarding microbial colonies as "superorganisms" like the colonies of multicellular animals (*Coelenterata* and *Bryozoa*) and the communities of social insects and some mammals (molerats). Microbial colonies are characterized by the functional specialization of the cells they contain. These cells enjoy the advantages of a social lifestyle, including enhanced resistance to antimicrobial agents and efficient utilization of nutrient substrates, particularly in spatially limited ecological niches such as a multicellular animal (plant) host organism. Research on microbial colonies as coherent entities came into fashion in the 1990s (see, e.g., [1]). However, one should not disregard the relevant works by the classical scientists of microbiology. The renowned Russian microbiologist N.D. Ierusalimskii actually foreshadowed the current debates on the subject of the organizational patterns of microbial colonies (films, zoogloeas, flocks, etc.). In his dissertation [2], Ierusalimskii criticized primitive organicism based on the straightforward comparison of the microbial colony to the multicellular organism (see comments by E.L. Golovlev [3] on Ierusalimskii's works). Ierusalimskii's ideas were more consistent with the concept of the microbial colony as a *supraorganismic (biosocial) system* [4, 5], which, like ant or even mammal communities, is characterized by (i) the spatial isolation of the microcolonies of each microbial species ("microbial anthills") in natural habitats; (ii) the phenotypic heterogeneity of the culture as the basis for cell differentiation in terms of quasi-social roles; (iii) culture integrity during the developmental process (a culture possesses integral features not exhibited by individual cells); and (iv) the colony's capacity to influence the properties of the environment at a sufficient population density (the relevant notion *quorum* is considered below). In the 1980s, S.G. Smirnov held similar views on the microbial colony, which he described as a "spatial-temporal continuum" consisting of "cell clusters" with different properties. Each of the stages of culture development corresponds to another subcolonial cluster [6].

Microbial colonial organization was previously considered by different authors in a number of papers [3, 4, 7]. However, this review concerns itself with data that are more recent. It also deals with additional areas of research, including such currently popular subjects as bacterial programmed cell death (PCD), altruism, quorum effects, etc.

Recently, a number of extensive reviews on microbial colonies and biocommunication have been published (see, e.g., [1, 8–16]). Nevertheless, the point that has not yet received sufficient attention concerns the role of evolutionarily conserved (i.e., chemically identical or clearly homologous in various forms of life) signal molecules. They operate as factors of intercellular communication and social behavior and perform the functions of histohormones, hormones, and neuromediators in multicellular animals. The evolutionarily

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conserved nature of a large number of signal molecules was earlier emphasized by A.M. Ugolev as evidence for his theory of the evolution of life, "based on combining a limited number of universal functional blocks" [17, p. 143]. Ugolev viewed chemical signals and receptors as impressive examples of functional blocks which are identical or at least very similar in organisms at different levels of biological evolution.

As for the available literature on microbial communication, much attention to evolutionarily conserved signal molecules is given in the works by A.S. Kaprelyants *et al.* (reviewed in [14]). Nevertheless, these works primarily emphasize the role of the protein/peptide signal substances, termed *cytokines* in an analogy with the intraorganismic informational agents of animals. The present review, in contrast, concentrates on nonpeptide factors of communication (although it pays tribute to peptides and proteins). Nonpeptide factors comprise both unique microbial products and evolutionarily conserved agents, including those performing neuromediator functions in multicellular animals (the subject of our own research [18, 19]). This review paper is also concerned with the role of colonial organization and intercellular (especially density-dependent) communication in the interactions between symbiotic/parasitic microbiota and the host macroorganism.

FORM AND STRUCTURE OF MICROBIAL COLONIES

The current gradual change in the paradigm of microbiology—the transition from the concept of microbial unicellularity to the idea that microbial colonies are coherent "superorganisms"—manifests itself in the increasing attention given to the shape, ornament, and micro- and macrostructure of bacterial colonies. "The colonies of virtually all prokaryotic species display capacities for cellular differentiation and multicellular organization. These capacities are, of course, also available to bacteria in natural settings, where biofilms, chains, mats, and microcolonies are the predominant modes of existence" [1, p. 598]. Modern microbiology is, therefore, characterized by a gradual transition to the biosocial ("biopolitical" [3, 4, 19, 20]) approach to microorganisms. This transition is facilitated by detailed research on intercellular (interpopulational) interactions, using genetic engineering, flow cytofluorimetry, scanning electron microscopy, and time-lapse video monitoring.

Numerous studies conducted on the organization of microbial colonies have yielded data on the morphological and physiological heterogeneity of the colony cells. A colony seems to consist of several different "tissues" [1, 21], cell clusters in Smirnov's usage [6]. In *Shigella*, he distinguished such typical clusters as (i) actively dividing cells; (ii) dormant cells; and (iii) cells undergoing spontaneous autolysis [17]. Kaprelyants and his associates obtained similar data. For instance, upon starving for 3–6 months, a *Micrococcus luteus* population consisted of active, dormant, and nonviable cells. This fact was established by sorting cells according to their capacity to bind rhodamine [23] and in a biphasic system containing aqueous solutions of biopolymers [14, 22–24].

A colony is characterized by both vertical stratification and horizontally separated sectors and concentric zones. Vertical stratification is easy to observe in colony sections stained with toluidene blue or methylene blue. *Escherichia coli* [1, 25] and *Shigella flexneri* [21] colonies were shown to consist of three strata: (i) the lower stained stratum (6 μm thick in the tested *E. coli* colony [25]); (ii) the middle stratum, chiefly light in color and apparently composed of nonviable (mostly irregularly shaped [21]) cells (this stratum also contains solitary stained viable cells and is 16 μm thick in *E. coli* [25]); and (iii) the upper stained (40 μm thick) stratum, which, in *E. coli*, consists of two substrata, the lower thin (1 to 3 cell layers) with a sharp boundary and the uppermost thick (40 μm in *E. coli*), intensely stained layer containing solitary unstained cells [25]. If genetically manipulated *E. coli* strains with the *lacZ* gene are stained for β-galactosidase, a similar pattern results. On top of a thin stratum consisting of β-galactosidase-containing cells (adjacent to the substrate), there exists a β-galactosidase-free stratum, followed by a β-galactosidase-containing stratum. The uppermost colony stratum is characterized by a mixed composition [25]. Strata composed of morphologically and biochemically distinct cells were detected in the colony of the cholera pathogen *Vibrio cholerae* as early as in 1920 [26].

A large number of researchers also described systems of air-containing microcavities, often with cell strands running across them. The intricate microcavity system virtually transforms a colony into a group of partially isolated aggregates (microcolonies). Microcolonies embedded in the mucous matrix and separated by open (frequently water-filled) canals are characteristic of the internal structure of biofilms. This is a peculiar analog of the circulatory system, which delivers nutrients and removes metabolic products [27]. The colonies of the bacterium *Alcaligenes* sp. strain d_2 contain pores (gas channels) and specialized extracellular hemoprotein-containing structures ("hemosomes") coated by a special "membrane." Such structures presumably facilitate O_2 transport to cells in colonies (aggregates); i.e., they represent analogs of the respiratory system [28–30].

Apart from vertical stratification, sectors and concentric zones are also characteristic of microbial colonies. Sectors correspond to genetically distinct clones differing in color, structure, shape, growth rate, enzyme activities, etc. This is exemplified by bacterial phase dissociation into R, S, and M forms with different cell wall thicknesses (the cell wall is thicker in the R variant than in the S variant with *Brucella* [31]) and properties of the fibrillar (in the R and S variants) or vesicular and

tubular (in the M variant) extracellular matrix. Another distinctive feature is the microcapsule, which occurs in some of the variants. Phase dissociation is responsible for the differences in the architectonics of colony sectors in this situation. In the S variant of rhodococci, the cells are evenly distributed throughout the colony thickness, and the number of contacting cells is insignificant [31]. As for the R variant, the cells of the lower strata of the corresponding sector are characterized by a perpendicular or slanting position relative to the nutrient medium surface. The cells of the upper strata are arranged radially and are parallel to the agar surface. In the M sector, cells form large groups and do not contact one another [32].

Concentric zones reflect the stages of the "ontogeny" of a bacterial colony. They correspond to different developmental steps. Concentric zones can be visually detected, e.g., in *E. coli* grown on minimal synthetic medium (M9) [33]. On agar-containing medium with tryptone and glucose, concentric circles can be discerned upon adding 2,3,5-triphenyltetrazolium, which is reduced by the cells in some sectors to the red substance formazan. As a result, the colony consists of white and red concentric rings [33]. Shapiro [1, 34] visualized the rings on *E. coli* colonies on a test medium for β-galactosidase activity (vertical strata also differ in this respect, see above).

If a spreading bacterial colony encounters mechanical barriers, e.g., glass fibers or natural obstacles such as folds and crypts in the gut as an ecological niche for microbiota, this only results in a local deformation of the concentric rings, which do not lose their continuity. Once the obstacle is left behind the colony front, the subsequent rings form according to the same geometri cal laws as in the rest of the colony [35]. A nonmechanical disturbance of the colony development results from a mutation in a developmentally important gene. An *E. coli* mutant with an impaired (insertion-containing) DNA polymerase I gene forms anomalous microcolonies with filamentous cells during the first hours of development. However, the colony succeeds in compensating for the deficiency: in two–four days, the mutant colonies and their cells become morphologically indistinguishable from normal colonies composed of wild-type cells [36]. The amelioration of the genetic damage is markedly accelerated by mature (2-day-old) adjacent colonies, which apparently release diffusible chemical factors of communication [36]. The older colonies cause the younger colonies to "adjust" their age to that of the "senior citizens" by, e.g., forming the outer concentric rings without prior formation of the inner rings [1] (for more detail on microbial communication, see the relevant review section).

If concentric rings coexist with sectors, then the concentric rings of the faster-growing sectors shift towards the periphery [34]; i.e., concentric ring formation is subject to temporal (biological clock–dependent) regulation, not spatial coordination (due to interactions between adjacent cells). This is particularly easy to observe in *Escherichia coli, Proteus, Serratia,* and *Salmonella*, which periodically form swarmers (nondividing cells with excessive flagellation) [37]. Swarmers form a colony structure consisting of concentric terraces resulting from the following alternating processes: (i) the growth and division of vegetative cells (the lag phase before swarmer formation); (ii) mass formation of centrifugally migrating swarmers; and (iii) conversion of the swarmers into vegetative cells with the formation of a new terrace (the consolidation stage) [38].

The data obtained on the dependence of the "biological clock" rhythm on the population cell density, e.g., data on the relationship between inoculum density and the lag phase before the formation of the first swarmer wave in *Proteus mirabilis*, point to the operation of a complex system of intracolony communication. In *Serratia liquefaciens*, the signal molecule was identified as an acylated homoserine lactone [39] (a representative of a widespread group of signal molecules of gram-negative bacteria, see below). Colony development is accompanied by the increasing synchronization of the behaviors of individual cells, resulting in a regular circular pattern of the colony as a whole, despite the disturbing factors [40]. This tendency towards synchronous behavior persists upon decreasing the substrate (glucose) concentration and increasing the agar concentration in the medium. An increase in the agar concentration results in a decrease in the swarmer migration rate, since swarmer motility depends on the moisture that the flagella absorb from agar gel due to the capsule polysaccharide [40, 41]. In *Serratia marcescens*, cells form a moistening cyclic lipopeptide [42]. A genetic trigger switches the cells over from synthesizing the proteins of the later cell division stages to producing flagellar protein (flagellin) and, therefore, controls the swarmers–dividing vegetative cell interconversion [43].

Of interest in terms of colony organization is the fact that only a swarmer group as a whole can migrate over the agar surface that has not yet been occupied by the growing colony. Individual cells that cross the colony borders lose their motility until a swarmer group comes into contact with them [1]. These data suggest behavioral coordination at the group level. In addition, swarmer migration is also coordinated at the level of the whole colony. Therefore, the bacterial colony is characterized by at least two integration levels: (i) the level of a group of concertedly migrating swarmers and (ii) the level of the whole colony, which includes a large number of such cell groups. This organizational pattern is analogous to that of a multicellular organism possessing both coordination systems operating on the tissue level (paracrine systems producing local histohormones such as serotonin, histamine, etc.) and generalized systems acting on the whole organism (including the nervous and the endocrine system) [44].

Microbial colonies, like many other biosocial systems, form supraorganismic functional organs which belong to the whole system and are collectively employed by all of its elements (individuals). Of particular interest is the fact that individual cell envelopes, including capsules and extracapsular mucus, merge into a biopolymer *matrix*, which typically consists of glycosylphosphate-containing polymers like teichoic acids, glycoproteins, and polyglutamic acid (in bacilli and some other species) [41]. The microbial matrix, like the intercellular matrix of animal tissues, contains fibrillar elements [45]. In addition, microbial and animal matrix structures share some chemical components, e.g., sialic acids. The matrix of a microbial colony operates as its functional organ. It performs functions related to the supracellular organization level:

(i) *Structuring* role. A matrix-containing colony consists, strictly speaking, of subcolonial associations, not of individual cells. Subcolonial associations occur with both gram-positive and gram-negative bacteria (including pathogenic species of both groups); they are particularly prominent in capsulated species such as *Klebsiella* when observed in the electron microscope [46]. Colony structures also include hollow fibers of extracellular polysaccharides and other biopolymers (e.g., in *Pseudomonas aeruginosa*), which presumably serve as microchannels for transporting substances. In addition, colony cells (typically their petite L-forms) migrate through such hollow fibers [47]. Such "offshoots" are particularly characteristic of bacterial species forming part of human and animal symbiotic microbiota [47].

(ii) *Protective* role. The cell-enveloping matrix is an internal buffering milieu that protects individual cells and the entire colony from deleterious environmental factors (desiccation, heating/cooling, hydrolytic enzymes, etc.). Polysaccharide and peptide matrix components contain a number of cryo-, thermo-, and xeroprotectors [48].

(iii) *Communicative* role. Exometabolites, cell autolysis products, and signal molecules, including those used by microorganisms to estimate the density of their own population, are released into the matrix and spread therein. Only insignificant concentrations of some signal molecules occur in the culture supernatant, because they are trapped in the matrix where they perform their functions. Importantly, a large number of bacterial species retain their supracellular organization patterns and extracellular matrix when cultivated in liquid media.

Prokaryotes form intercellular contacts, like eukaryotic cells in the tissues of a multicellular organism. These contacts probably promote the transfer of signal molecules among cells in a population, especially if these communication agents are not diffusible. Intercellular contacts form on the basis of various surface structures, including microfibrils, cone-shaped protrusions, cell wall evaginations, and glycocalyx elements, which reflect the "genetically determined regularity in the development of microbial populations as self-regulating multicellular systems" [49, p. 222].

The colonial structure of microorganisms, therefore, is a direct manifestation of their multilevel social organization, which enables microorganisms to perform collective behaviors. This implies that "an individual's (cell's) will" submits to "the collective will." In fact, " ... bacteria, although unicellular organisms, are also social creatures that form multicellular associations" [8, p. 184].

MICROBIAL APOPTOSIS AND ALTRUISM

Apoptosis, the programmed death of individual cells in the interests of a whole population, represents an impressive example of socially controlled processes (on the population level). Apoptosis was earlier researched in animals and, to a lesser extent, in plants. In these cases, apoptosis may be regarded as a normal stage of the individual development of an organism. For instance, apoptosis is involved in tail elimination associated with the tadpole–frog conversion. Brain development involves the programmed death of some neurons, and the mutations preventing the apoptosis of embryonic brain cells may be lethal. The apoptosis of plant cells invaded by a pathogenic agent prevents the dissemination of the infection. Much attention has been recently given to the genetic and biochemical apoptosis mechanisms involving the activation of a cascade of caspases (evolutionarily conserved cysteine proteases), ultimately responsible for the activation of nucleases and other enzymes degrading cellular structures [50, 51]. Interestingly, apoptosis of animal cells can involve mitochondria, whose ancestors were symbiotic bacteria. Stress-induced damage of mitochondrial membranes poses a threat not only to the cell containing the mitochondria but also to its neighbors, due to the accumulation of radical oxygen species. The cell senses mitochondrial damage because cytochrome *c* extrudes from the mitochondria. Cytochrome *c* binds to the cytoplasmic protein Apaf1, which binds to procaspase-9, converting it to the active caspase-9. This initiates the caspase cascade and apoptosis [50, 51].

As for apoptosis-like programmed cell death (PCD) in microorganisms, this phenomenon has not yet been well understood. Extensive research was carried out with an eukaryotic system, the myxomycete *Dictyostelium discoideum*. The transition from single-celled ameboid individuals to a multicellular motile pseudoplasmodium and, subsequently, to a fruiting body with spores represents a collective response to the starvation of the cell population (this system was considered by us earlier [4, 5]). Once the multicellular pseudoplasmodium begins to form a fruiting body, the cells in its front part undergo the PCD process. The resulting dead cells form the stem of the fruiting body [52, 53]. The process is subject to regulation by a number of signal molecules. On the whole, cyclic adenosine monophosphate

is the most important communication agent; however, DIF (1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-L-hexanone) is of the foremost importance in terms of stem cell differentiation (involving PCD) [52, 53]. Myxobacteria, prokaryotic analogs of myxomycetes in terms of the life cycle, also display programmed death of many cells during cell aggregation, followed by the formation of fruiting bodies. Some cell groups inside the ripening fruiting body are also doomed to die [54].

The death of part of an *E. coli* cell population under stasis (bacterial growth arrest resulting, e.g., from nutrient depletion) can be considered another prokaryotic analog of apoptosis. Phenomenologically, this process was described relatively long ago [55] (see also review [4]). A starving *E. coli* population gradually subdivides into two subpopulations. One subpopulation perishes and is autolysed, whereas the other subpopulation utilizes the autolysis products and continues growing and giving rise to new colony-forming units [55]. Recently, the genetic mechanism of PCD in this system was revealed [56, 57]. The *E. coli* genome contains an operon with the *mazE* and *mazF* genes. *mazF* codes for a stable cytotoxic protein, and *mazE*, for an unstable antidote to MazF. The antidote is rapidly degraded by the protease clpPA. The depletion of the amino acid pool available for a cell results in the activation of the *rel* operon, whose protein product RelA is responsible for guanosine tetraphosphate synthesis by ribosomes. Guanosine tetraphosphate blocks the *maz* operon, and the synthesis of the antidote is suppressed. Under these conditions, protein MazF causes the death and autolysis of a part of the cell population, thereby replenishing the amino acid pool and reactivating the synthesis of the MazE antidote in the surviving cells [56, 57]. Therefore, the system functions as a chromosome-based analog of bacterial plasmids (addiction modules) encoding a stable cytotoxic agent in combination with an unstable antidote.[2]

The above example of PCD in *E. coli* can also be regarded as an example of bacterial altruism, since a part of the starving cells dies, promoting the survival of the rest of the cell population [56, 58]. The authors of this review paper do not ignore the involvement of other, possibly more potent, mechanisms sustaining the life of a starving microbial population. These mechanisms include the economization of the energy-supplying metabolic processes, the subject of N.S. Panikov's works [59–61].

[2]For instance, one of the *E. coli* plasmids contains the genes encoding a stable restriction endonuclease and an unstable DNA methylase which protects the DNA from the restriction enzyme (methylated DNA is not recognized by this enzyme) [54]. Obviously, the elimination of this plasmid prevents methylase synthesis and results in DNA fragmentation by the stable restriction endonuclease, i.e., death of the plasmid-free cells. Such addiction modules, therefore, are viewed as "selfish DNA molecules" (the term coined by the sociobiologist R. Dawkins) which kill all the cells trying to get rid of it, so that the only survivors are the cells containing these DNA molecules.

Modern *sociobiology*, a modified version of Darwin's theory of evolution emphasizing social interactions in the populations of various forms of life, supports the concept of *kin altruism* [62]. This concept deals with self-sacrificial behavior in the interests of direct kins, which have many genes in common with the altruistic individual. Strictly speaking, this behavior is not purely altruistic, because it is aimed at cloning the individual's own genes, which are transmitted to the next generation via an alternative carrier (the kin, not the individual per se). The term kin altruism has been adopted by sociobiologists; it does not imply conscious self-sacrifice and signifies that the genes programming "death for the sake of kins" spread as a result of natural selection [62].

Colonies of prokaryotes and many single-celled eukaryotes with a predominantly asexual mode of reproduction represent almost ideal clones. The sociobiolological kin selection concept, therefore, predicts that altruistic behaviors such as programmed death (provided that it contributes to the survival of the population) should be widespread among microorganisms [9, 58].

Of interest in this context is the fact that the above PCD processes are not the only microbial altruistic behaviors. Like infected eukaryotic cells, which die to prevent the dissemination of a pathogen in a multicellular organism, some *E. coli* strains contain genes which trigger cell death in response to the intrusion of phage T4 [63]. The *lit* gene blocks the synthesis of all cell proteins upon the onset of the expression of the late genes of phage T4, because it codes for a protease degrading the elongation factor EF-Tu involved in protein synthesis [64]. The *prrC* gene codes for a nuclease that cleaves lysine tRNA. The nuclease is activated by the product of *stp*, a gene of phage T4 [63]. The *rex* genes induce the formation of ion channels in T4-infected cells, resulting in a loss of vital ions by the cells and in their altruistic death, unless the phage blocks the channels by its own proteins encoded by the *rII* genes [65].

Curiously enough, the genes involved in PCD in response to phage infection are unlikely to become permanently integrated into the chromosome. The *rex* genes belong to the phage genome, and they are expressed in lysogenic cells only [63]. Presumably, such altruistic genes located in mobile genetic elements only function in a part of a bacterial population. If this suggestion is valid, then a bacterial population is a mixture of "altruistic" and "selfish" cells. This mixed composition is also characteristic of populations of higher animals (e.g., rats) and people [66].

QUORUM SENSING AND INTERCELLULAR COMMUNICATION IN MICROORGANISMS

Over the last decade, researchers have been making important additions to the list of microbial processes

proceeding at sufficiently high population densities only (*quorum*-dependent processes). Actually, this field was already under study about a century ago. It was at this early stage of microbiological research that, e.g., the question arose as to why bacteria fail to grow if the inoculum density is too low. In 1988, J. Shapiro [34] also pointed out that myxobacterial spores germinate only at sufficient spore concentrations in the medium. In the early 1980s, density-dependent activities of microbial populations were intensely investigated by V.I. Duda, G.I. El'-Registan, and coworkers [67, 68]. A number of chemical factors (autoregulators) accumulating in a microbial culture and producing biological effects, e.g., causing cell autolysis (the fatty-acid d_2 factor [68]), were identified. The relevant studies conducted in Russia and abroad in the 1980s were generalized in A.S. Khokhlov's monograph *Low-Molecular-Weight Microbial Autoregulators* [69].

The research done in this field in the 1990s stirred up interest in the quorum effects in microbial populations. Following are important examples of the relevant processes described until now, which require a sufficiently high population density [1, 8–15, 70]: (i) bioluminescence in the marine bacteria *Vibrio* (=*Photobacterium*) *fischeri* and *V. harveyi*; (ii) myxobacterial cell aggregation with subsequent formation of fruiting bodies with myxospores; (iii) conjugation with plasmid transfer in the nodule bacteria of the genus *Agrobacterium* and in *Enterococcus faecalis* and related species; (iv) formation of swarmer cells in the *Proteus* and *Serratia* species; (v) synthesis of exoenzymes and other virulence factors in plant (*Erwinia carotovora, E. hyacinthii*, etc.) and animal (*Pseudomonas aeruginosa*) pathogens; (vi) antibiotic formation in *Streptomyces* and *E. carotovora*; (vii) sporulation in bacilli and actinomycetes; and (viii) growth stimulation in streptococci and a variety of other microorganisms.

The mechanisms of a significant part of the above processes have been revealed and the respective intercellular communication factors involved in density-dependent processes have been identified. A few remarks on biocommunication in general, the subject of the biological science referred to as *biosemiotics*, should be made at this point. Among the interorganismic communication channels investigated in terms of biosemiotics, three channels are so highly conserved that they are fully functional even in single-celled forms of life [4, 5]: (i) direct physical contact between organisms; (ii) production of chemical agents diffusing in the medium; and (iii) generation of physical fields. All of the above communication channels seem to be involved in quorum effects.

Physical contact between organisms. Some density-dependent processes include stages controlled by nondiffusible chemical factors. These factors are attached to the cell generating them. Their interaction with the receptors of another cell requires establishing direct intercellular contacts. A starving *Myxococcus xanthus* population initiates cell aggregation with the subsequent formation of fruiting bodies with myxospores (the *D. discoideum* pseudoplasmodium briefly discussed above represents a eukaryotic analog of the myxobacterial system). This process is under the control of both diffusible and nondiffusible chemical factors. The late stages starting 6 h after cell aggregation and providing for a compact cell arrangement (mandatory for myxospore formation) are regulated by the nondiffusible, cell surface-bound proteinaceous C factor, the product of the *csgA* gene [10, 71, 72]. A cell with a mutant *csgA* gene is incapable of coordinate cell movements, a prerequisite for a compact arrangement of rod-shaped *M. xanthus* cells; such mutants do not form fruiting bodies. The expression of at least 16 genes is factor C-dependent [72].

Physical contacts between cells are also required for communication via surface organelles, such as pili and components of the exopolymer matrix coating individual cells, their groups, or a whole colony. The aggregation and sporulation process in *M. xanthus* depends on (i) the type IV pili (whose homologs are also involved in socially coordinated cell movements in the pathogenic bacteria *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*) [73], (ii) polysaccharide–protein fibrils, and (iii) the lipopolysaccharide O antigen of the external layer of the outer membrane [1, 73, 74]. The synthesis of these cell surface structures depends on the *S* (social) genes, which are responsible for concerted collective cell movements and the formation of supercellular structures. In contrast, the myxobacterial *A* (adventurous) genes enable individual cells to move across the border of a growing colony.

An intermediate role between nondiffusible and diffusible communication factors is characteristic of biopolymer trails separating from the cell synthesizing them and thereby paving the way to other cells forming daughter colonies [75]. An analogous role in an ant community is performed by toribons, which mark the trails of forager ants [76]. Contact- (and trail-) dependent communication is not confined to myxobacterial systems. Pili are involved in cell aggregation in *Neisseria gonorrhoeae* colonies [1]. Apart from diffusible chemical agents, morphogenesis in eukaryotic microorganisms, e.g., *D. discoideum*, also depends on cell contacts. Of particular importance for these interactions are glycoproteins determining the fate of cell subpopulations. The cells programmed to form spores contain glycoprotein PsA, while the cells of the prospective stem (whose formation involves PCD) contain the MUD9 antigen [77].

Distant chemical communication and its involvement in quorum effects. Chemical communication in microorganisms is such a broad area of research (partly considered in our earlier works [4, 5, 78]) that this section will deal only with the diffuse chemical agents whose involvement in quorum effects has been firmly established. These agents include: (i) acylated

homoserine lactones regulating a wide range of density-dependent collective activities of gram-negative bacteria; (ii) peptide and protein factors regulating the conjugative plasmid transfer in *Enterococcus*, the development of aerial mycelium in *Streptomyces*, sporulation in bacilli, and a variety of other processes; and (iii) amino acids and related amines, which are involved in bacterial cell aggregation (*E. coli, Salmonella typhimurium*, and *Myxococcus xanthus*) and swarmer formation in *Proteus mirabilis*.

1. Quorum-dependent systems using homoserine lactones as intercellular communication agents ("luxI–luxR" type systems). Initially, we shall focus on the bacterial systems employing acylated homoserine lactone, which are relatively well understood. The marine luminescent bacterium *Vibrio* (=*Photobacterium*) *fischeri* is a classic model system [8, 12, 70]. Luminescence is a density-dependent process. It does not occur in dilute cell suspensions, e.g., in seawater, where the cell density is less than 10^2 cells per ml [70]. *V. fischeri* luminescence requires a concentrated cell suspension, which is typical of the natural habitat of this bacterium—the light organ of the squid *Euprymna scolopes*, containing 10^{10}–10^{11} cells per ml [70]. This system is apparently characterized by mutually beneficial interspecies interactions. "The squid, a nocturnal forager, benefits because the luminescing bacteria camouflage it from predators below; the glow, resembling moonlight, erases the shadow that would normally be cast when the moon's rays struck the squid from above. And the bacterium benefits because the squid provides a nourishing, sheltering haven" [10, p. 69]. The research on the biochemistry and genetics of *V. fischeri* luminescence included several consecutive stages. Initially, the luminescence of *V. fischeri* cultures at the early exponential developmental stage was induced with culture fluid separated from a stationary-stage *V. fischeri* culture. Subsequently, the "*luxI–luxR*" genetic system was characterized in detail [12, 69, 70]. This system proved typical of the majority of the density-dependent systems of gram-negative bacteria investigated up to now.

The system includes two main gene clusters. One gene cluster, the *luxICDABEG* operon, contains genes with the following functions:

(i) The *luxI* gene codes for a protein (LuxI, 193 amino acids), apparently operating as the synthase of the intercellular communication agent whose accumulation in the medium signals that the threshold (quorum) cell concentration required for bioluminescence has been attained. The communication agent, *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL), is synthesized from *S*-adenosylmethionine and 3-oxohexanoyl-coenzyme A.

(ii) The *luxA* and *luxB* genes code for α and β subunits of luciferase (the enzyme complex responsible for bioluminescence), respectively.

(iii) The *lux C, D, E* genes encode fatty-acid reductase (dealing with one of the oxidizable substrates used in the luciferase reaction resulting in light quantum emission).

(iv) The *luxG* gene codes for flavin mononucleotide reductase (flavin mononucleotide is the other substrate oxidized in the luciferase reaction).

The other gene cluster includes the *luxR* gene, whose protein product LuxR (250 amino acids) binds 3-OHHL. The LuxR–3-OHHL complex attaches to the promoter site of the *luxICDABEG* operon and activates its transcription. In the absence of 3-OHHL, the *luxICDABEG* operon is expressed at a low baseline level. Protein LuxR operates as a gene repressor without 3-OHHL. It inhibits the expression of its own gene *luxR*. With an increase in the concentration of *V. fischeri* cells in the medium, the 3-OHHL accumulating in the medium starts functioning as an autoinducer. Along with the transcription of the structural genes, the LuxR–3-OHHL complex activates *luxI* transcription, i.e., the synthesis of 3-OHHL per se [10, 12, 70], which, upon complexation with LuxR, enhances *lux* operon transcription in an increasing number of *V. fischeri* cells. This results in the avalanche-like synthesis of all components of the luciferase system and intense bacterial luminescence.

The quorum-sensing regulatory systems in a large number of other gram-negative bacteria are based on the same principle as the *luxI–luxR* system (table). Acylated homoserine lactones also operate as diffuse chemical communication factors. One bacterial species may use several density-dependent systems. It has been recently shown that *V. fischeri* actually possesses a second density-dependent system involved in bioluminescence, *ainI–ainR*, with its own transcription activator (AinR) binding the diffuse factor *N*-octanoyl-L-homoserine lactone [8].

Two quorum-sensing systems with *N*-(3-hydroxybutanoyl)-L-homoserine lactone and AI-2, an unidentified compound, as intercellular communication agents regulate the luminescence of the marine bacterium *Vibrio harveyi*. However, apart from the transcription activator (LuxR), *V. harveyi* also possesses a repressor (LuxO). Its inactivation results from the combined effects of the diffuse products of both systems. *N*-(3-hydroxybutanoyl)-L-homoserine lactone binds to protein LuxN, and AI-2 binds to proteins LuxP and LuxQ with histidine kinase activities. They initiate the operation of a kinase cascade, resulting in the modification (phosphorylation) of the LuxO repressor and, therefore, activation of the bioluminescence system [79].

Bacteria of the genus *Erwinia* (*E. carotovora, E. chrysanthemii*, etc.) are soft-rot pathogens in potatoes, chrysanthemums, and other plants. They degrade plant cell walls with pectinases and cellulases. These enzymes are important virulence factors, and their formation is a density-dependent process [12, 70, 80]. At a sufficient bacterial population density, the enzymes are synthesized so intensely that plant cells are destroyed before the immune system responds to the

pathogen. The *expI–expR* gene system, an analog of the *V. fischeri luxI-luxR* system, operates in *Erwinia*. Protein ExpI, a partial homolog of protein LuxI, is required for the synthesis of the diffuse communication factor 3-OHHL (identical to the *V. fischeri* factor). Since the communication factors in *Erwinia* and *V. fischeri* coincide, luminescence in *E. carotovora* can be induced by a plasmid with all *V. fischeri lux* genes except *luxI* [80].

Apart from *expI–expR*, *E. carotovora* also contains another analogous gene system, *carI–carR*. The *carI–carR* system is responsible for the population density-dependent synthesis of the antibiotic carbapenem. Activation of antibiotic synthesis at high population densities is presumably involved in the elimination of microbial competitors that attempt to utilize the products of degradation of plant cell components by the quorum-dependent exoenzymes of *E. carotovora* [12, 70].

In addition to 3-OHHL, *E. carotovora* also uses other pheromones[3] (see the table). Based on the studies with this bacterium, density-sensing gene systems are also under the control of other regulatory systems, some of which depend on cAMP and the cAMP-binding protein CRP [80]; similar data were obtained with *V. fischeri*. Quorum-sensing systems, therefore, measure not only the population density, but also other environmental parameters via respective gene regulatory systems.

The animal/human pathogen *Pseudomonas aeruginosa*, like *E. carotovora*, synthesizes its virulence factors, including toxin A, elastases LasA and LasB, alkaline protease, hemolysins, and the surfactant rhamnolipid, in a quorum-dependent fashion [70, 82], using two gene systems, *lasI–lasR* and *vsmI–vsmR*.

The *V. fischeri*, *E. carotovora*, and *P. aeruginosa* systems demonstrate that microbial cells interact with a macroorganism (a plant or an animal) if the pheromone concentration signals that the microbial population is sufficiently dense. The microorganisms may be parasites or symbionts of the host macroorganism. Following are additional relevant examples:

(i) Nodule bacteria of the genus *Rhizobium*. For instance, the strains of *R. leguminosarum* bv. *viciae* cause the formation of nitrogen-fixing nodules on the roots of legumes. The quorum-sensing *rhiI–rhiR* system is responsible for intense expression of the *rhiABC* genes at high population densities. The protein products of these genes are involved in the bacterial symbionts–rhizosphere cells interactions, although their functions have not yet been well understood. Interestingly, the related species *R. etli* possesses an additional gene system, *raiI–raiR*, involved in limiting the nodule number on the roots of the host plant (bacteria with mutant *raiI–raiR* systems form two times more nodules on kidney bean roots than the wild-type bacteria) [83].

[3]The term *pheromone* is used here to mean "a chemical agent of intercellular communication" [81].

(ii) The bacterium *Agrobacterium tumefaciens*, which forms crown galls in a large number of plant species. The galls represent a plant analog of malignant tumors. Their formation is due to the transfer of oncogene-containing DNA fragments from the bacterium to plant cell nuclei via Ti plasmids. Some Ti plasmid genes cause the synthesis of opines, nutrient substrates for *A. tumefaciens*. The *traI–traR* gene system, an analog of the *luxI–luxR* system, facilitates the spread of Ti plasmids in a bacterial population. Since the *traI–traR* system per se is located on a plasmid, its behavior, like that of addiction modules, is consistent with the "selfish DNA" theory advanced by the sociobiologist R. Dawkins. Plasmid DNA attempts to spread in the bacterial population, and, therefore, it causes plasmid-carrying cells to conjugate with other cells once the quorum density has been attained [13]. Nevertheless, the conjugal transfer of Ti plasmids depends on opines, and, for this reason, it depends on successful interactions between microbiota and the macroorganism (the plant with the opine-producing tumor). For example, *traR* transcription is stimulated by the OccR factor, which is activated by octopine, one of the opines [70].

Swarmer formation, which promotes the spread of a bacterial population over a solid medium and the colonization of various ecological niches (including a macroorganism), is regulated by *luxI–luxR* type systems in some bacterial species. The *swr* gene system stimulates swarmer migration on a solid medium in *Serratia liquefaciens*. Presumably, the density-dependent expression of the *swr* genes yields an extracellular surfactant (an analog of *P. aeruginosa* rhamnolipid), which facilitates swarmer migration [84].

The data on the density-dependent systems of the *luxI–luxR* type and their pheromones are summed up in the table. A large number of such systems are essential for regulating the behaviors of symbiotic (parasitic) microflora, aiming to optimize its interactions with the host macroorganism. Moreover, acylated homoserine lactones are also involved in interspecies communication. For instance, the pheromone *N*-(3-oxo)-dodecanoyl homoserine lactone of *P. aeruginosa* interacts with human epithelial cells, causing them to synthesize interleucine-8, a factor of the human immune system [8].

Some homoserine lactone-based quorum-sensing systems are involved in the elimination of competing microflora by synthesizing antibiotics and bacteriocins. The *phzI–phzR* gene system regulates the synthesis of antifungal antibiotics in *Pseudomonas aerofaciens* [12]. Actinomycetes of the genus *Streptomyces* possess population density-dependent systems which regulate antibiotic synthesis, the development of aerial mycelium, and spore formation. γ-Butyrolactones (the *A* factor of *S. griseus*) bind to a repressor (resulting in its inactivation) [70] and not to a transcription promoter. One of the *Rhizobium* homoserine lactones, the *N*-3R-

hydroxy-7-*cis*-tetradecanoyl)-L-homoserine lactone, represents a bacteriocin (a bacterial growth inhibitor) [83].

Eukaryotic cells as competitors/antagonists of prokaryotes can produce compounds resembling the pheromones of prokaryotic density-dependent systems. Being "aware" of the informational function of such chemical agents in prokaryotes, eukaryotes probably cause an interference in the prokaryotic communication channel, thereby "bluffing" bacterial cells. This is a possible reason why halogenated furanons, structurally similar to acylated homoserine lactones, are formed by *Delysea* (red algae) as efficient antimicrobial agents [85].

Interestingly, microbial pheromones, including acylated homoserine lactones, can be used in interspecies interactions as signal molecules and not only as antibiotics/bacteriocins. This is possible because different species of microorganisms in many cases have identical or very similar pheromones [8]. For example, the extracellular substances produced by *P. aeruginosa* enhance the virulence of the facultative pathogen *Burkholderia cepacium* [8].

2. *Quorum-sensing systems with peptide/protein pheromones.* The system responsible for conjugal plasmid transfer in *Enterococcus faecalis* and related bacterial species can be regarded as a classic quorum-sensing system with a peptide pheromone [70, 86]. Like the above *luxI–luxR*-type systems, this system promotes the spread in the population of properties which are relevant to the microbiota–host interactions and enable the microbes to eliminate their microbial competitors. For example, *E. faecalis* plasmids pAD1, pCD1, and pCF10, whose transfer depends on peptide quorum-sensing systems, are responsible for hemolysin synthesis, bacteriocin formation, and tetracycline resistance, respectively [86].

Each pheromone (a hexa- or octopeptide) induces clumping of bacterial cells and their conjugation with the transfer of a specific plasmid from the donor to the recipient. For instance, the octapeptide cPD1 stimulates the conjugal transfer of plasmid pPD1. This plasmid codes for the pheromone receptor located on the repressor protein of the respective operon. This function is performed by the *traA* gene of plasmid pPD1 [86]. The pheromone interacts with the receptor, inactivating it and enhancing the synthesis of the gene product. Plasmid pPD1 also includes gene *traC*, whose product is a pheromone-binding protein promoting the transfer of the peptide pheromone across the cell wall (the effect of the pheromone on spheroplasts does not depend on the expression of the *traC* gene [82]). Pheromones are intensely synthesized only by cells that do not contain the corresponding plasmid. Pheromone synthesis is suppressed in plasmid-carrying donor cells; moreover, the plasmid encodes an inhibitory protein. For example, plasmid pPD1 codes for peptide iPD1, which inactivates pheromone cPD1 [69, 86].

Sporulation in *Bacillus subtilis* actively proceeds at a high density of the cell population or upon the addition of the culture fluid from another, sufficiently dense, population. This process is subject to regulation by a density-dependent system involving an oligopeptide pheromone. The *pfrA* gene codes for an inactive peptide precursor (41 amino acids) of the pheromone. The *N*-terminal sequence is detached upon excretion of the peptide from the cell. An extracellular peptidase attacks the remaining chain of 19 amino acids, resulting in the formation of an active communication molecule, pentapeptide PEP_5 [87].

The mechanism of PEP_5-dependent activation of sporulation in *B subtilis* has been elucidated. *B subtilis* cells take up PEP_5 with the help of an oligopeptide permease. Intracellular accumulation of PEP_5 results in the complexation with and inhibition of phosphatase RapA. With the phosphatase inhibited, Spo0A and Spo0F, the key factors of sporulation, remain in the active (phosphorylated) state. Interestingly, *rapA*, the phosphatase-encoding gene, is cotranscribed with the *pfrA* gene. They belong to the same operon. At low cell densities, the intracellular concentration of the peptide PEP_5 is well below the threshold value, and Spo0A and Spo0F are dephosphorylated by RapA. Sporulation, therefore, does not occur. The formation of the PfrA–PEP_5 complex and, therefore, the initiation of the sporulation program are quorum-dependent events [72, 86].

The idea that PEP_5 serves as a pheromone in a density-dependent system raised serious doubts [87], because the culture fluid contained very insignificant amounts of this peptide. Is PEP_5 trapped by the cell wall and do the processes of precursor excretion, processing, and PEP_5 uptake operate as a kind of timer setting the tempo of sporulation [87]? We suggest that the low PEP_5 concentration in the supernatant points to its preferential location in the extracellular matrix. Diffusion of a substance in the matrix seems quite compatible with its pheromone function in the matrix-coated bacterial population.

Genetic transformation competence in *B. subtilis* and *Streptococcus pneumoniae* and virulence in *Staphylococcus aureus* are subject to control by density-dependent systems involving peptide pheromones [72, 88]. The *S. pneumoniae* quorum-sensing system also decides whether antibiotic resistance genes from other oral pathogens of the genus *Streptococcus* will actively transform *S. pneumoniae* cells [72, 88]. Like systems of the *luxI–luxP* type, peptide density-dependent systems function in a large number of symbiotic/parasitic microorganisms.

The host macroorganism itself produces intraorganismic peptide regulators. In response to the intrusion of *Rhizobium* bacteria, host plants (pea, soybean, etc.) form a peptide (10 amino acids) that modifies the effect of the hormone auxin on plant cells, changing the concentration dependence of the auxin-induced stimulation of cell division. Without this peptide, the maxi-

Some pheromones involved in the density-dependent communication systems of microorganisms [1, 8–10, 12–16, 69, 70, 80–83, 86–88, 110–113]

Microorganism	Function	Pheromone
1. Systems of the *luxI–luxR* type and other lactone derivative-based systems		
Vibrio fischeri	Bioluminescence	*N*-(3-oxohexanoyl)-L-homoserine lactone
	Bioluminescence	*N*-octanoyl-L-homoserine lactone
V. harveyi	Bioluminescence	*N*-(3-oxobutanoyl)-L-homoserine lactone
	Bioluminescence	AI-2, an unidentified compound
Erwinia carotovora	Synthesis of extracellular hydrolytic enzymes (pectinases, cellulases, etc.)	*N*-(3-oxohexanoyl)-L-homoserine lactone
	Synthesis of the antibiotic carbapenem	*N*-(3-oxohexanoyl)-L-homoserine lactone
Pseudomonas aeruginosa	Synthesis of virulence factors (the *lasI–lasR* system)	*N*-(3-oxododecanoyl)-L-homoserine lactone
	Synthesis of virulence factors (the *vsmI–vsmR* or the *rhlI–rhlR* system)	*N*-butanoyl-L-homoserine lacton
Agrobacterium tumefaciens	Conjugal transfer of Ti plasmids	*N*-(3-oxo-octanoyl)-L-homoserine lactone
Serratia liquefaciens	Stimulation of swarmer movement over agar	*N*-butanoyl-L-homoserine lactone
Yersinia enterocolitica	Infection involving Yop proteins	*N*-(3-oxohexanoyl)-L-homoserine lactone and *N*-hexanoyl-L-homoserine lactone
Streptomyces griseus	Streptomycin synthesis, the development of aerial mycelium and sporulation. Note. Streptomycete systems differ from typical *luxI–luxR* systems (see text)	2-isocaproyl-3-hydroxymethyl-γ-butyrohomoserine lactone
S. virginiae	Virginiamicin synthesis	Various butyrolactones and butanolides
2. Systems with peptide/protein pheromones		
Enterococcus faecalis	Conjugal plasmid transfer	Hexa- or octopeptides, e.g., cPD1 (H-Phe-Leu-Val-Met-Phe-Leu-Ser-Gly-OH)
Bacillus subtilis	Sporulation. Transformation competence	The pentapeptide H-Ala-Arg-Asn-Glu-Thr-OH and related peptides
Streptococcus pneumoniae	Transformation competence	The heptadecapeptide H-Glu-Met-Arg-Leu-Ser-Lys-Phe-Phe-Arg-Asp-Phe-Ile-Leu-Gln-Arg-Lys-Lys-OH
Xanthomonas maltophila	Growth stimulation	Homolog of chorionic gonadotropin
Micrococcus luteus	Growth stimulation after a dormancy period	Protein (19 kDa)
Volvox carteri (a green alga)	Sexuality	Glycoprotein
Paramecium tetraurelia (an infusorian)	Growth stimulation	Protein (17 kDa)
3. Systems with amine/amino acid pheromones		
Myxococcus xanthus	Aggregation and formation of fruiting bodies (early stages)	Factor A, a mixture of amino acids (predominantly, tyrosine, proline, phenylalanine, leucine, and isoleucine) with short peptides as an admixture
Proteus mirabilis	Swarmer formation	Glutamine
E. coli	Colony macro- and microstructure	Aspartic acid

mum stimulation occurs with approximately 5 μM auxin, and this effect is attenuated by a further increase in the auxin concentration. However, in the presence of the peptide regulator, the concentration dependence is characterized by a plateau persisting up to an auxin concentration of about 20 μM [89]. The peptide pheromone operating in the density-dependent system of *Volvox carteri*, a colonial eukaryotic microalga, stimulates its growth at a concentration as low as 10^{-16} M [14].

Growth autoinduction, which provides for the viability of microorganisms after a period of dormancy, appears to be a widespread microbial phenomenon [9, 14, 16]. After starving for 3–6 months with subsequent inoculation into a nutrient-rich medium, a *Micrococcus luteus* culture accomplishes only a few cell divisions followed by growth arrest. The addition of 20–30% supernatant of another culture which has reached the early stationary stage in a rich medium prevents growth arrest and provides for the normal growth of a starved *M. luteus* population [14, 22].

3. Quorum-sensing systems with amine/amino acid pheromones. Apart from the nondiffusible C factor (see above), the myxobacterium *Myxococcus xanthus* produces a diffusible factor, A. It is responsible for the quorum-dependent initiation of cell aggregation with the subsequent formation of fruiting bodies [90]. Cell aggregation does not occur at cell densities below 3×10^8 cells/ml. Factor A is an amino acid mixture [72, 90] resulting from the action of extracellular proteases on the cell surface proteins [90]. Factor A accumulation in combination with nutrient depletion activates the two-component *sasS–sasR* gene system, which initiates cell aggregation and fruiting body formation [72]. Factor A includes ketogenic amino acids, which are subsequently utilized by the cells via the glyoxylate shunt [72].

The density-dependent systems of the *luxI–luxR* type can also be regarded as systems based on amino acid (homoserine) derivatives. Homoserine is not contained in proteins, but it occurs in various organisms as an intermediate in the biosynthetic pathways of some amino acids. The only reason why we consider quorum-sensing systems involving acylated homoserine lactones in a separate section of this paper is that these communication systems are regarded as classic.

The macro- and microstructure of *E. coli* colonies form under the influence of the spatial gradients of an attractant, aspartic acid, synthesized by *E. coli* cells [91]. Intricate patterns (concentric rings, hexagonal lattices) result from the superposition of two aspartic acid gradients, which are generated by the cells (i) in the colony center and (ii) on the colony perimeter. Aspartic acid represents an evolutionarily conserved signal molecule. In animals, it serves as a neurotransmitter, a substance transferring excitation waves between neurons.

Importantly, other neurotransmitter amines also represent evolutionarily conserved signal molecules contained in microorganisms. Their addition to microbial cultures produces developmental and structural effects on microbial colonies [18, 19, 92–95]. Serotonin (5-hydroxytryptamine), a neurotransmitter and histohormone in higher organisms, probably functions as a microbial communication agent. This suggestion is based on the data on the stimulation of cell aggregation in *E. coli, Rhodospirillum rubrum*, and *Polyspondilum* sp. (a myxobacterium) by exogenous serotonin [18]. Similar serotonin concentrations (10^{-7}–10^{-5} M) accelerate the growth of microorganisms [18, 95].

Norepinephrine (noradrenaline), which also represents an animal neurotransmitter and hormone, stimulates the growth of pathogenic enterobacteria and the synthesis of adhesin K99 and Shiga-like toxins I and II by their cells [92]. However, norepinephrine fails to stimulate the growth of nonpathogenic *E. coli* strains (our unpublished data). This substantiates Lyte's hypothesis [92] that the norepinephrine-dependent stimulation of growth represents an adaptive phenomenon in terms of evolution. Pathogenic enterobacteria use the host's response (stress-induced intense norepinephrine synthesis associated with an infectious disease) for their own benefit. Microorganisms contain a variety of other neurotransmitters and hormones (histohormones) of higher animals, such as γ-aminobutyric acid, β-alanine, and insulin[4] [92, 93], which are probably involved in (i) microbial intercellular communication processes and (ii) symbiotic/parasitic microbiota-host interactions (see review [19] for more detail).

The authors of this paper are currently engaged in research on the role of evolutionarily conserved amines and amino acids in processes (i) and (ii). Using high-performance liquid chromatography with electrodetection, we have recently detected serotonin in *Bacillus cereus* and *Staphylococcus aureus* [96] (it had previously been found in *Enterococcus faecalis* by Strakhovskaya *et al.* [95]), norepinephrine in bacilli, *Proteus vulgaris, Serratia marcescens, Saccharomyces cerevisiae*, the fungus *Penicillium chrysogenum*, and dopamine in a wide range of tested prokaryotic species [96].

Also of considerable interest are the data that microorganisms contain homologs of neurotransmitter receptors. The purple phototrophic bacterium *Rhodobacter sphaeroides* contains a homolog of the benzadipine receptor (one type of the receptors binding the inhibitory neurotransmitter γ-aminobutyric acid) [97]. The mitochondria of eukaryotic cells are descendants of free-living prokaryotes, and the bacterial subgroup including *R. sphaeroides* is closely related to the probable ancestor of mitochondria. Therefore, research on bacterial neurotransmitter-binding receptors and on the effects of evolutionarily conserved neurotransmitters on microbial systems is of paramount importance for brain neurochemistry, since there are data on the involvement of the mitochondria of brain neurons in

[4]Exogenous insulin stimulates the growth of a number of microorganisms [76].

neurotransmitter binding. Mitochondria of neurons contain glutamate receptors (of the NMDA subtype) [98]. When glutamate is present at a high concentration, its binding to these mitochondrial receptors results in a substantial Ca^{2+} influx into mitochondria, dissipation of the membrane potential, a decrease in the intracellular ATP concentration, and, ultimately, in cell apoptosis. Apoptosis of brain neurons caused by excessive concentrations of glutamate and other neurotransmitters probably occurs during neurodegenerative diseases, such as ischemic apoplectic strokes and Parkinson's, Alzheimer's, and Huntington's diseases [98].

Of relevance in this context are also oligosaccharines, microbial signal molecules and evolutionarily conserved agents. These are short chains of monosaccharide residues. Lipid fragments are attached to some of them. For example, the Nod factors produced by the nodule bacteria *Rhizobium* (whose density-dependent *luxI–luxR* type system was considered above) are involved in the signal exchange between the bacteria and the cells of the host plant (a legume). Plant-synthesized flavonoids activate the transcription of bacterial *nod* genes. The *nodD* gene is the direct flavonoid target, and its products activate other *nod* genes. The products of these genes (e.g., NodC) are responsible for the synthesis of Nod factors, acylated short chitin fragments consisting of 2–5 chitin monomers. They produce multiple effects on the root cells, resulting in their differentiation, active division, and nodule formation. The nodules contain bacterial cells, which convert to nitrogen-fixing bacteroids under the influence of plant-generated signals [10, 99, 100].

Based on the recent data, oligosaccharines and related compounds are also produced by higher plants and animals. Protein DG42, a NodC homolog, occurs in the embryos of the frog *Xenopus* during the period from the middle blastula stage to the neurula stage. DG42 is also capable of catalyzing the synthesis of chitin oligosaccharides [101].

E. coli, Bacillus subtilis, and the yeast *Candida utilis* release a number of chemically similar compounds that promote the adaptation of these microorganisms to various kinds of stress, including changes in growth conditions, supraoptimal temperatures, and the presence of antibiotics or *N*-ethylmaleimide [102–104]. These compounds include: (i) factor X_{II}, which decreases the bacterial growth rate and, therefore, helps the bacteria overcome stress according to the principle of "improving the stability of a car's movement by shifting gears;" (ii) factor X_I, which accelerates cell adaptation to *N*-ethylmaleimide (not detected in *C. utilis*); and (iii) the factor accelerating adaptation to new media. Like homoserine lactones, these signal compounds are also active at the interspecies level. For instance, *E. coli* pheromones produce specific effects in *B. subtilis* and *C. utilis*. *E. coli* factor X_{II} inhibits the growth of *B. subtilis* cells [104].

We have considered a number of important chemical factors of communication among microbial cells, but their list necessarily remains incomplete. Moreover, additions to this list have been frequently made recently, largely due to the investigation of evolutionarily conserved factors of intercellular/interorganismic communication. Apart from the biogenic amines considered above, these include reactive oxygen species (ROS) such as O_2^-, H_2O_2, OH^*, and their derivatives. ROS probably function as pacemakers in oscillatory processes regulating the activities of diverse biosystems. Their effects can be mediated by resonance excitation waves spreading in the matrix. The matrix is also capable of generating its own ROS, albeit with a low efficiency (V.L. Voeikov, unpublished). Nitric oxide, a neurotransmitter and evolutionarily conserved regulator of various processes in pro- and eukaryotic systems, can also be regarded as a ROS derivative.

Physical factors of intercellular communication in microorganisms. Data on interactions between microbial colonies across barriers cutting off chemical communication channels have been recently accumulating in the literature. A *Vibrio costicola* culture dying under the influence of chloramphenicol emits a signal that stimulates the growth of another culture separated from it by a glass layer [105]. Presumably, several communication channels (chemical signals and physical fields) produce synergistic effects in such systems. This suggestion is based on the studies on the influence of one bacterial colony on the adhesive properties of another colony [114, 115]. The antibiotic resistance of *Bacillus carbonifillus* cells is increased, and their growth is stimulated by signals from other microbial cultures of the same or different species. The donor and the recipient of the signals were cultivated on two glass partition-separated parts of one Petri dish [106, 107]. The following physical factors have been suggested to be involved: (i) electromagnetic waves [105], on the analogy of eukaryotic cells, which are known to respond to UV light (the Gurwitch effect promoting cell division) and (ii) ultrasonic waves [106, 107].

The current stage of the research on physical factors of distant communication among microbial cells and on their role in density-dependent processes can be compared to the stage of "primary accumulation of capital" in the development of a market economy. Further studies in this field can yield results going beyond the scope of purely microbiological research, since analogous data are already available on cultivated cells from multicellular organisms (including the human organism) [108, 109]. The data on the physical factors of intercellular or interorganismic interactions (particularly electromagnetic waves) can promote the adoption of a coherent, integral vision of living things, emphasizing the relevant resonance phenomena, the role of physical fields, and the continuity of living matter within organisms and their associations. According to this concept, uni- or multicellular organisms are viewed as focal

points of physical fields (and gradients of chemical communication factors) which merge into one general field structure without any sharp internal borderlines. The intercellular matrix represents a material simulacrum of the organism-enveloping field(s).

There is yet another item to be underlined, also already dealt with in this paper. The data obtained over recent decades point out that the colony organization and intercellular communication of microorganisms cannot be adequately understood unless we take into account the whole gamut of both intra- and interspecies ecological relationships. Biosocial microbial systems are embedded in intricate ecological networks, which in many cases include micro- and macroorganisms. Therefore, agents of microbial communication in cell density-dependent systems are predominantly involved in regulating activities that are essential for establishing acceptable relationships between microflora and the host macroorganisms.

If the host organism is a human being, then the symbiotic microbiota behaves like a tuning fork, highly sensitive to the somatic state, the stress level, and even the mood of the human host. Because the state of a human individual is influenced by his relationships with other people in society, microbial symbionts are expected to indirectly respond to the socio-psychological atmosphere. Therefore, research on human symbiotic microbiota is of value in biosociological and biopolitical terms.

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