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REVIEW

Allolysis in Bacteria

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Abstract—The review deals with the phenomenon of allolysis, i.e., lysis of a part of a bacterial population induced by a group of epigenetically differentiated cells of the same species or phylotype. Allolysis is best studied in two species of gram-positive bacteria, *Streptococcus pneumoniae* and *Bacillus subtilis*. In *S. pneumoniae*, allolysis is associated with the onset of the competence stage, while in *B. subtilis* it is associated with transition to the stage of spore formation. The mechanisms of allolysis are considered, as well as its possible role in the populational and symbiotic relationships of bacterial cells. The relation between allolysis ant the programmed death of a part of the cells within a bacterial population (apoptosis) is discussed.

Keywords: allolysis, pneumococci, competence, cannibalism, bacilli, spore formation, apoptosis.

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In the course of bacterial growth and differentiation, death of some cells occurs accompanied by lysis, i.e., their degradation beginning with decomposition of their cell envelope. This usually involves various kinds of autolysis (self-destruction of bacteria). This phenomenon is known for practically all microbial groups. Apart from its basic importance, investigation of the mechanisms of lysis is of practical value, especially for biotechnology. Allolysis is an instance of bacterial lysis, which is similar but not identical to autolysis. Allolysis is cell lysis induced by other cells of the same species [1, p. 456] or of closely related species of the same phylotype. This phenomenon is somewhat similar to autolysis, since the same autolytic enzymes of the murein hydrolase group are involved, which decompose bacterial cell walls. However, during autolysis these enzymes decompose (completely or partially) the surface layers of the producer's cell wall, not attacking the neighboring cells. Allolysis is similar to heterolysis or, broadly speaking, to predation, when the cells of another genus or species act as prey. The term "cannibalism" is practically completely identical to the term "allolysis." However, since the term "allolysis" was used to designate this phenomenon in most publications dealing with the genus *Streptococcus*, it was used in the title of our review. Allolysis is probably associated with one of the types of differentiation of the cells within a bacterial population into subpopulations.

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1. ALLOLYSIS IN *STREPTOCOCCUS PNEUMONIAE*: HISTORICAL PREREQUISITES FOR DISCOVERY OF THE PHENOMENON AND ITS MECHANISMS

Bacteria of the genus *Streptococcus* are attracting ever-increasing attention in the microbial ecology of humans. In infants, they colonize the nasopharynx and the upper air passages, forming complex biofilms and coexisting with the host as symbionts [2, 3]. This equilibrium, however, is often disturbed, and *S. pneumoniae* may cause pneumonia, meningitis, or such less dangerous diseases as otites and sinusites. Every year, pneumococci-caused diseases are considered responsible for about 1.6 million deaths worldwide, especially among young children in developing countries [4, 5]. The mechanisms of virulence of *S. pneumoniae* are presently subject to intense investigation [4].

Many *S. pneumoniae* strains, especially the clinical isolates, have a thick polysaccharide capsule, which is the main virulence factor. Over 90 serotypes of capsule antigens are known [6]. Apart from the capsule, however, other virulence factors exist in pneumococci, including the autolytic enzymes responsible for release of the Ply toxic protein (see below) and also for the phenomenon of allolysis. These enzymes are briefly characterized below.

The LytA protein (36 kDa), the main pneumococcal autolysin, is N-acetylmuramoyl-L-alanine amidase, the product of the *lytA* gene. This protein consists of two domains. It decomposes teichoic and teichuronic acids of the cell wall, targeting choline residues. While the products of cell wall decomposi-

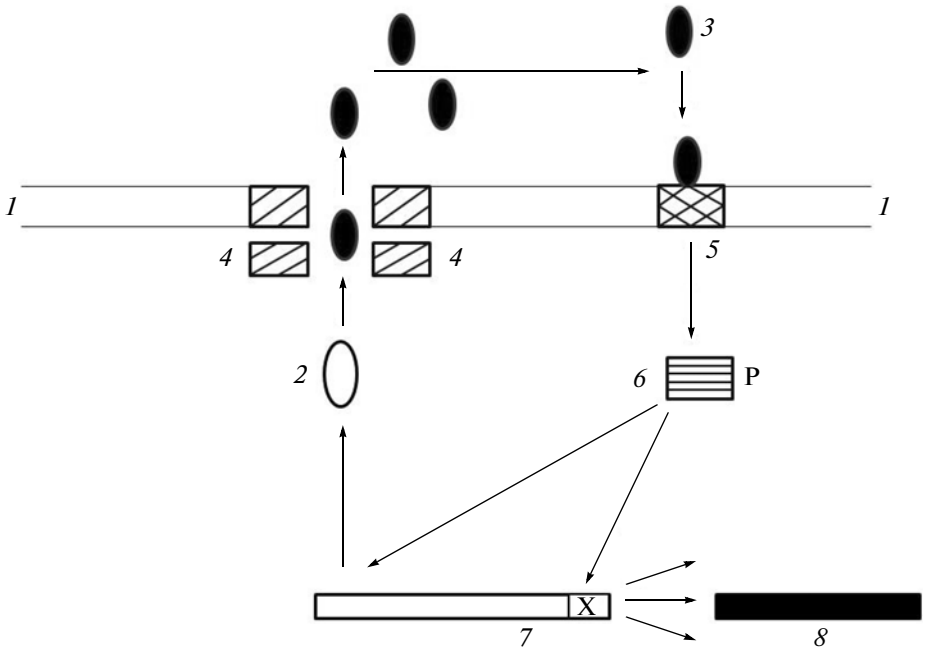

Fig. 1. Synthesis of the competence pheromone in *S. pneumoniae* and control of the competence genes (from [13], modified). Surface cell layers (*1*); precursor of the competence pheromone (*2*); mature competence pheromone (*3*); products of the *comAB* genes (*4*); histidine kinase, product of *comD* (*5*); phosphorylated product of *comE* (*6*); block of the early competence genes (including *comX*) (*7*); and block of the late competence genes (*8*)

tion possess toxic qualities, release of pneumolysin (Ply protein), which has a cytotoxic effect and causes β hemolysis of erythrocytes, is responsible for most of the toxic effect. The strains with impaired LytA activity have lower virulence. LytA is among the best-studied autolytic enzymes not only in pneumococci, but among bacteria in general [4, 7].

The LytC autolysin (55 kDa), consisting of two domains, is a muramidase (lysozyme) [8].

CbpD (*choline-binding protein*, about 50 kDa) is a product of the *cbpD* gene. It consists of three domains. Together with LytA and LytC, this protein is involved in release of pneumolysin from the pneumococci cells [9]. In the cells of pneumococci, autolysins perform the same functions as in other bacterial cells. They are responsible for turnover and maturation of peptidoglycan and participate in cell division and the separation of divided cells. Moreover, they are involved in allolysis, associated with the series of events related to the onset of the competence state and genetic transformation. The sequence of the stages resulting in the competence state in pneumococci and to absorption of transforming DNA will be therefore briefly described below.

1.1. Genetic Control of Competence in S. pneumoniae

In the case of genetic transformation, competence is usually understood as the capacity of cell for sorption of transforming bacterial DNA and for its incorporation into the chromosome via recombination.

The competence state in pneumococci is determined by 22 genes, with their products being absolutely required for transformation [1]. They are usually subdivided into the earlier and later ones. The earlier ones comprise eight genes attuning the cell to the acquisition of competence. For example, the *comM* gene is an early one. Its product, the ComM protein located on the cell surface, prevents the competent cells from being destroyed by the LytA, LytC, and ClpD autolysins (see below). The remaining 14 genes belong to the later ones. They are responsible for the mechanisms of DNA binding and absorption and DNA processing in the course of absorption, as well as for recombination of DNA with the chromosome. The sequence of events during development of competence in the culture of pneumococci may be described as follows (Fig. 1).

At the onset of culture growth, the product of the early competence gene *comC* appears in the cytoplasm, a peptide of 45 amino acid residues, the precursor of the competence pheromone. It interacts with the system of peptide export formed by the products of the *comAB* genes. The pheromone precursor is cleaved at the leader sequence site, and the "mature" pheromone is released from the cell. Synthetic preparations of the competence pheromones have been obtained for a number of streptococci. In order to initiate competence in the experiments of transformation, addition of a synthetic pheromone to the culture of "immature" cells results in improved synchronization of the subsequent stages of competence.

Then, the two-component transmission system begins to act: the pheromone contacts with its receptor on the cell surface, the transmembrane part of histidine kinase, the product of the *comD* gene (the sensor protein). Activated histidine kinase phosphorylates the product of the *comE* gene (the response regulator protein), and the phosphorylated protein is accumulated in the cell. The concentration of the pheromone in the medium increases in the course of culture growth, and the intracellular level of phosphorylated ComE protein increases correspondingly. Starting from a certain concentration, it binds with the promoter of the *comCDE* operon and stimulate its activity via positive regulation. Self-induction thus occurs, and the cycle closes: the more pheromone is released into the medium, the more phosphorylated ComE protein is produced, which stimulates additional synthesis of the pheromone [10–13].

The phosphorylated ComE protein has also other functions: above a certain concentration, apart from the stimulation of its promoter, it activates also the synthesis of the *comX* gene product, sigma factor 70. This sigma factor, in turn, by reaction with the promoters of numerous (about 120) genes, initiates the synthesis of their products. Some of these genes (the later competence genes) are required for manifestation and completion of the transformation, while most of them are responsible for other processes, so that their inactivation has little or no effect on the transformant yield [14]. Due to such polyfunctionality of the *comX* gene, substitution of the broader term "state X" instead of "competence state" was suggested in one publication [1].

The pheromones of *Streptococcus* may differ from each other both in size and in amino acid composition. In 42 clinical isolates of *S. pneumoniae*, two types of pheromones (pherotypes) were found [15], while, among 11 isolates of the *mitis* group streptococci, 9 had different pherotypes [16]. The pheromones are able to efficiently induce the competence state only within their pherotype [17].

1.2. Phenomena of Fratricide and Sobrinicide

In addition to the ability of some cells to absorb experimentally introduced DNA, competent cultures are characterized by emergence of proprietary DNA in the culture liquid. It was evidently released from the lysed cells of the pneumococci. This phenomenon, described for pneumococci about 50 years ago [18], has been known even earlier for other bacteria and is termed spontaneous or natural transformation. A causal relationship between the onset of competence and lysis of some of the cells has been suggested by a number of authors (see [19] for details). To prove these suggestions, Hövarstein et al. (Norway) used a strain of pneumococci with an inactivated *comA* early competence gene [20]. This gene is responsible for release of the competence pheromone from the cell. Although the strain did not form competent cells, the competence was restored on addition of a synthetic pheromone to the growing culture. The *E. coli* β-galactosidase gene under a constitutive promoter was inserted into the chromosome of this strain. Since β-galactosidase could not penetrate through the cell envelope, it was synthesized in the cell and was not released into the culture liquid. It could be released only after cell lysis. The strain was resistant to novobiocin.

During cultivation of this strain, the medium did not contain free β-galactosidase and extracellular DNA. The latter was confirmed by the absence of transformational activity with the novobiocin resistance marker. Immediately after addition of the synthetic pheromone, both the extracellular enzyme and extracellular transformational DNA were found. This finding could be explained only by the pheromone-induced lysis of some of the cells. The β-galactosidase content indicated the lysis of about 20% of the cells in the population. Since both competent and incompetent cells were present in the culture, it was necessary to determine whether the lysed cells belonged to any of these fractions or were uniformly distributed among them.

The next work of the same laboratory provided the answer [21]. Two strains of pneumococci were used in the experiments. One of them, similar to the previous work, carried a mutation in *comA*, while the other carried both *comA* and *comE* mutations. The product of *comE* is a response regulator protein (see above). Unlike *comA*, the absence of this protein was not compensated by addition of the competence pheromone. Moreover, the β-galactosidase gene and the novobiocin resistance mutation were inserted into the chromosome of this strain. The strains were initially cultivated independently; then the cultures were mixed and supplemented with the synthetic competence pheromone. It was able to induce competence only in the strain with *comA* mutation. Soon after addition of the pheromone, both extracellular DNA with the novobiocin resistance marker and free β-galactosidase emerged in mixed cultures. The higher the number of potentially competent cells was, the greater was the concentration of the enzyme. The only explanation for this result was that the cells unable to develop the competent state were lysed, their lysis being directly related to induction of competence in the partner strain. The degree of lysis decreased significantly when the cells of the potentially competent strain were mutants incapable of synthesis of autolysin LytA, the main autolysis enzyme in pneumococci [7]. Importantly, the wild strain did not release LytA into the culture liquid; the enzyme was, at least during the first stages of the process, fixed at the cell surface. Contact of the cells of two partner strains resulting in agglutination was required for lysis. At the initial stage of this research, it was not clear why the cells of the competent strain, unlike the partner cells, were protected from lysis. This was explained later (see below). The

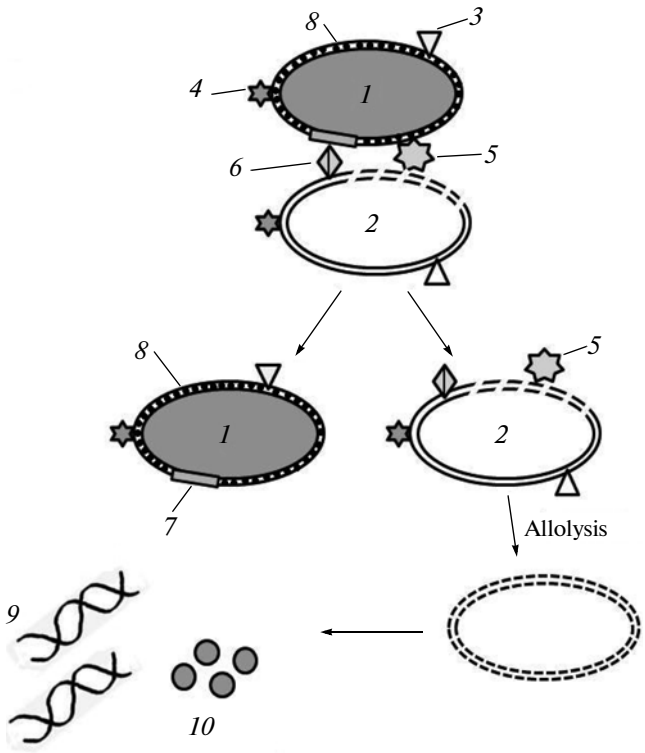

Fig. 2. Allolysis in *S. pneumoniae* (from [25], modified). The competent (*1*) and incompetent (*2*) cells are in contact with each other. During the contact, various lytic agents destroy the surface layers of the incompetent cell, and its content, including DNA and pneumolysin, is released into the environment. The competent cell is protected by the cell immunity factors. Lytic agents: LytA (*3*), LytC (*4*), CbpD (*5*), CibAB (*6*). Cell immunity factors: CibC (*7*), ComM (*8*). DNA released from the lysed cell (*9*), Ply protein (pneumolysin) (*10*).

works carried out by Claverys and coworkers at about the same time yielded similar results [22]. Related works followed, carried out mainly in the same laboratories [9, 23, 24 and reviews 1, 25, 26]. Their results may be generalized as follows (Fig. 2).

Apart from LytA, other lytic enzymes, LytC and CbpD, are involved in the lysis of incompetent cells [8, 9]. A bacteriocin-like peptide CibAB (competent-induced bacteriocin) joins the lytic activity of these enzymes. Production of this peptide is controlled by the gene *comX*. The competent cells producing the relevant proteins and peptides localized at the cell surface are resistant to their action due to the ComM and CibC proteins of their cell wall. Incompetent cells probably also produce LytA and LytC, although not the resistance factors ComM and CibC. Incompetent cells are, therefore, defenseless against the whole complex of lytic factors. Lysis of incompetent, nonimmune cells occurs at direct contact with the competent killer cells. The lysed cells release DNA, which is immediately absorbed by the competent cells, as well as various proteins, including pneumolysin (Ply protein) [4]. In [23], an elegant experiment is described. The suspension of cells which did not reach the competence state was applied to a plate of blood agar, and then a drop of the solution of the competence pheromone was placed in the center of the dish. The zone of β-hemolysis proportional in size to the pheromone concentration appeared around the drop. This zone resulted from the hemolytic action of pneumolysin released from the lysed cells which had not attained competence. Their destruction was in turn induced by the part of the population that acquired competence due to the action of the pheromone. Thus, lysis of incompetent cells could be observed, apart from emergence of DNA or β-galactosidase, also as release of pneumolysin from the cells.

Lysis of incompetent pneumococci cells induced by the competent cells within the population fits completely to the term "allolysis." This is neither autolysis in the narrow sense, since cell death was induced *in trans,* by their neighbors, nor heterolysis, since a part of the same cell population dies. In [24], this kind of allolysis in pneumococci was termed fratricide. This term indicates that the victims belong to the same species as their killers.

Allolysis (fratricide) in *S. pneumoniae* is probably a part of the chain of processes terminated by exchange of genetic material (DNA). The competent cells obtain this DNA from their neighbors by inducing their death. Release of virulence factors, primarily pneumolysin, from the lysed cells is another probable reason for allolysis [7, 27]. Thus, induction of allolysis in pneumococci, as well as induction of competence in general, favors horizontal gene transfer [28, 29] and plays a certain part in pathogenesis of the diseases caused by these bacteria [30].

Together with fratricide, the phenomenon of sobrinicide (killing of relatives, rather than brothers; from the Latin *sobrinus*, "cousin") belongs to manifestations of allolysis in streptococci. In the case of sobrinicide, lysis of one species of streptococci is induced by the competent cells of another, closely related species [1, 26]. Sobrinicide is closer to heterolysis (and predation) than fratricide and, strictly speaking, is somewhat different from allolysis.

Sobrinicide involves the same [28] or somewhat different [31] mechanisms as fratricide. Sobrinicide facilitates gene transfer from the lysed incompetent cells of *S. mitis* and *S. oralis* to the competent cells of *S. pneumoniae* [28], of from *S. gordonii* to *S. mutans* [31].

Both fratricide and sobrinicide create conditions for intense gene exchange between the *Streptococcus* cells in complex biofilms of human oral cavity and nasopharynx. Since these biofilms are cemented by bacterial polysaccharides, nucleic acids, and proteins, the competence of a streptococcal biocenosis in a biofilm may be higher than in the case of free-living, planktonic cells [32].

2. ALLOLYSIS-LIKE PHENOMENA IN *BACILLUS SUBTILIS*

Sporulation in bacteria, which has been most studied for *B. subtilis*, is a complex process comparable to some stages of eukaryotic ontogenesis; it has been discussed in hundreds of experimental articles and tens of reviews and monographs. Eight stages of sporulation are usually singled out, including the zero, preparatory stage. The zero stage is sometimes termed presporulation; during this period, the cell remains morphologically unchanged, while some of the previously inactive genes are translated [32, 33]. Exhaustion of the nutrient medium is the main stimulus for sporulation. The *spoOA* gene, which belongs to response regulator genes, is considered the gene initiating the process of sporulation. Its product is a DNA-binding protein exercising negative or positive control over transcription of 121 gene [34]. Although many of the products of the genes of this regulon are directly involved in sporulation, some of them probably perform other functions [35, 36]. The SpoOA protein becomes functional after phosphorylation, which involves a complex of three different histidine kinases and two other proteins, SpoOF and SpoOB. Some of the genes of the *spoOA* regulon react to low intracellular concentrations of phosphorylated SpoOA, while others react only to high concentrations of it [37]. This "concentration play" is important for determination of the sequence of activation of the genes within this regulon. The cells in the population do not sporulate simultaneously. In [38], which will be analyzed below, the number of sporulating cells was as low as 10%.

Among the operons of the *spoOA* regulon are two operons, *skf* (sporulating killing factor) containing eight genes and *sdp* (sporulating delay protein) containing three genes. Investigation of the cells with deletions of the genes of these operons resulted finally in discovery of cannibalism in bacilli.

The number of viable cells in a *B. subtilis* culture decreases significantly (approximately by 70%) during the first stages of sporulation. This well-known fact has been interpreted as a passive process of dying off under starvation. However, [38] demonstrated that dying off did not occur and sporulation was considerably postponed in the culture of the strains carrying deletions of some genes of the *skf* and *sdp* operons. This finding suggested that the decrease in cell number observed for the wild type during this growth phase was an active, rather than a passive, process associated with the functioning of the genes of these operons; in the case of the relevant mutants, their function was impaired. When the *skfA* gene was controlled by the lactose promoter, its induction by IPTG stimulated cell death. Experiments with mixed populations of the cells with the original genotype and the mutants incapable of sporulation demonstrated predominant death of the vegetative, rather than sporulating cells. The cells involved in sporulation are, therefore, somehow able to cause the death of the individuals not involved in this process.

The product of the gene *skfA* is probably a peptide with bacteriocin properties. The mechanism of its action on the vegetative cells is unclear. The lytic enzymes (of which *B. subtilis* has about 15 [39]) act at a later stage, lysing the cells already killed by the SkfA peptide. The lysis of these cells was confirmed by release of β-galactosidase when the relevant gene was introduced into the genome [38]. According to the hypothesis suggested in [38], the products of the *skfE* and *skfF* genes act as a pump, releasing the SkfA bacteriocin from the cell into the medium and thus preventing the suicide of the presporulating cell. The product of the gene *sdpC* is a toxic protein (63 amino acid residues). It targets the cell membrane. The self-regulated system consisting of two other genes of the *sdp* operon prevents *SdpC* suicide [40].

Thus, presporulating cells are protected from their own toxic products; these compounds kill the vegetative cells, in which the SpoOA protein is still not functioning and the products o the *skf* and *sdp* operons are therefore missing. For some time, the culture therefore consists of two subpopulations, presporulating killers and vegetative victims. This phenomenon was termed cannibalism [38]. Initially, low concentrations of phosphorylated SpoA switch on the "early" operons of the regulon, including *skf* and *sdp*, and the medium becomes temporarily enriched with substrates due to the lysis of dead vegetative cells. Then, after the secondary depletion of the medium, the concentration of phosphorylated SpoOA gradually increases, resulting in the activation of the genes involved in the visually discernable stages of spore formation [41]. Some of the "early" operons participate also in formation of the intercellular polysaccharide–protein matrix in *B. subtilis* colonies grown on solid media [42, 43].

Such subdivision of the cell population into subpopulations at the onset of sporulation resembles the subdivision of a pneumococcal culture into subpopulations of competent and incompetent cells with antagonistic relations between these groups. What is the biological meaning of cannibalism during sporulation? Article [38] cited above, as well as subsequent works [44, 45], initiated an animated discussion in which authors participated who worked with pneumococci and with the toxin–antitoxin (TA) system. The latter is probably involved in the processes of apoptosis in bacteria [25, 46, 47]. For example, the "last supper" hypothesis was proposed: the cells at the onset of sporulation require an additional source of energy, which is obtained by releasing the content of the killed bacterial cells into the medium [47]. Cannibalism in bacteria is likened to apoptosis in eukaryotes, when genetically programmed destruction of certain cells and their replacement with other ones occurs. In this case, the term "self-digestion" is proposed instead of "cannibalism" [39, 46]. Finally, cannibalism is con-

sidered among the mechanisms that help to temporarily overcome nutrient limitation and to postpone the onset of sporulation, together with such responses to medium exhaustion as mobilization of the chemotaxis apparatus for the search of new nutrient sources, utilization of the by-products of bacterial growth, etc. [38, 48].

To end this chapter, we would like to cite Stragier: "When hungry, eat your sister... but beware that she does not eat you first! This is the motto of the soil bacterium *Bacillus subtilis*..." [47, p. 461]. The order in which the required level of SpoOA phosphorylation is achieved determines which cell will be a killer and which a victim. The poorly known patterns of epigenesis in bacterial populations prevent its simultaneous activation in all the cells. The term "bistability" is used to characterize such cell differentiation within the culture grown in the same aerated flask and containing individuals of the same genotype (see reviews [49–52].

3. ALLOLYSIS AND APOPSTOSIS SYSTEMS IN BACTERIA

Programmed cell death (apoptosis) is well-studied in eukaryotic organisms. Recently, increasing attention has been paid to apoptosis in bacteria.

As was mentioned above, some authors investigating toxin–antitoxin systems in bacteria consider them as an apoptosis instrument and are inclined to treat the allolysis systems in bacilli and streptococci as a form of apoptosis [46]. This point of view deserves attention, although certain differences exist between allolysis and the mechanisms responsible for the functioning of TA systems. The action of TA systems of many bacteria, which has been studied in a number of works (see reviews [53–55]), is based on antagonism between the products of the chromosomal modules consisting of two neighboring genes. One of them, a long-lived toxin decomposing mRNA, is constantly neutralized by the product of another gene, a short-lived antitoxin, by formation of a complex of these two proteins. Under stress conditions, however (especially at nutrient limitation), the balance is impaired, since only the short-lived, mRNA-cleaving toxin remains in the cell and can no more be neutralized. The processes of translation are disrupted, and the cells stop growing and finally die. Their disintegration probably occurs due to the action of autolytic enzymes, and the products of their lysis are used by the remaining living cells as a nutrient source, thus enabling them to resume growth. Unlike allolysis, however, the death of the majority of the population is not induced by the subpopulation of specialized cells. Thus, in the case of TA systems (at least in *E. coli*), no differentiation of the population into the killer and victim cells has been found. Moreover, the mechanisms employed in the allolysis system to provoke the death of some cells differ from the toxins in TA systems; although pneumococci possess TA genes of the *relBE* system [56], these systems are not active during allolysis of incompetent cells within the population.

For allolysis, formation of specialized cells with metabolic products lethal to other cells is required. This phenomenon is therefore more closely related to the processes occurring during the differentiation of eukaryotic tissues and in populations of bacteria with pronounced social behavior, especially myxobacteria (see reviews [57–59]). These microorganisms have a complex life cycle, with a stage in which the population exists as autonomous vegetative cells followed by a stage in which fruiting bodies, relatively large (up to 200 μm) multicellular structures of various shape on a stalk above the surface of the medium, are formed. The fruiting bodies consist of numerous myxobacteria. Prior to formation of the fruiting bodies, the vegetative cells aggregate, forming mounds. About 80% of the cells in fruiting bodies are lysed, releasing peptides and amino acids into the environment [60], and the remaining living cells develop to form myxospores. The death and disintegration of most of the vegetative cells is required for myxospore formation. Death of the vegetative cells is known to be caused by the MazFmx protein, a homologue of the *E. coli* MazF toxin (24% identity and 58% similarity). Similarly to MazF, MazFmx cleaves mRNA, although at other sequences: between UU in GUUGC, while the MazF in *E. coli* acts at ACA sequences. The *mazFmx* gene in *Myxococcus xanthus* has no neighbor (a closely located gene of the antitoxin *maze*), and the Mrp protein, a transcription regulator, acts as an antitoxin. The relevant gene is located in another site of the myxobacterial chromosome. Similarly to the *MazEF* system in *E. coli*, both proteins are able to form a complex, in which the toxin protein is inactivated. In *M. xanthus* mutants with deletions of the *mazFmx* gene formation of myxospores decreased by more than an order of magnitude [61].

Actinobacteria also have a complex life cycle including death and lysis of a considerable part of the population. In these microorganisms, step-by-step formation of different types of mycelium is known (see reviews [62, 63]). For example, in the case of *Streptomyces antibioticus*, feltlike substrate mycelium is formed 5 h after plating of the spores; it does not rise above the surface of the medium. After 8–10 h, death and lysis of most of the hyphal segments occurs, while the remaining few segments form aerial mycelium. After 25 h of growth, a second tour of hyphal death occurs, resulting in a thinner aerial mycelium on which sporulation begins after 48–96 h. The death of the hypha of the surface and aerial mycelium probably additionally enriches the nutrient medium [64, 65], similarly to the death of some bacilli in sporulating cultures. The enzyme systems involved in the changes of mycelial type are probably related to those responsible for cell differentiation in eukaryotes, including calcium-dependent serine–threonine protein kinases ([66–68] and review [69]). Thus, apoptosis and differ-

entiation of bacterial cells may be carried out by different mechanisms.

Investigation of associated processes of competence development and allolysis in streptococci and possibly in other bacteria may have applied aspects, since disruption of these processes may affect the virulence of pathogenic microorganisms. For example, inactivation of the structural gene of *S. pneumoniae* serine–threonine protein kinase was shown to result in complete absence of competence in the relevant mutants, accompanied by a drastic decrease in virulence [70]. In mice infected intranasally with these mutants, the number of viable pneumococcal cells in lung tissues and blood was four to six orders of magnitude lower than in the case of animals infected with the wild type strain. Searching for the preparations targeting the proteins involved in various forms of apoptosis is therefore an intriguing task for pharmacology.

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REFERENCES

1. Claverys, J.P., Prudhomme, M., and Martin, B., Induction of Competence Regulons as a General Response ao Stress in Gram-Positive Bacteria, *Annu. Rev. Microbiol.*, 2006, vol. 60, pp. 451–475.
2. Petersen, F.C., Tao, L., and Scheie, A.A., DNA Binding-Uptake System: a Link Between Cell-to-Cell Communication and Biofilm Formation, *J. Bacteriol.*, 2005, vol. 187, no. 13, pp. 4392–4000.
3. Moscoso, M., Garca, E., and López, R., Biofilm Formation by *Streptococcus pneumoniae*: Role of Choline, Extracellular DNA, and Capsular Polysaccharide in Microbial Accretion, *J. Bacteriol.*, 2006, vol. 188, no. 22, pp. 7785–7795.
4. Jedrzejas, M.J., Pneumococcal Virulence Factors: Structure and Function, *Microbiol. Mol. Biol. Rev.*, 2001, vol. 65, no. 2, pp. 187–207.
5. Bogaert, D., De Groot, R., and Hermans, P.W., *Streptococcus pneumoniae* Colonisation: the Key to Pneumococcal Disease, *Lancet Infect. Dis.*, 2004, vol. 4, no. 3, pp. 144–154.
6. Dawid, S., Roche, A.M., and Weiser, J.N., The blp Bacteriocins of *Streptococcus pneumoniae* Mediate Intraspecies Competition Both in Vitro and in Vivo, *Infect. Immun.*, 2007, vol. 75, no. 1, pp. 443–451.
7. Romero, P., López, R., and GarcHa, E., Key Role of Amino Acid Residues in the Dimerization and Catalytic Activation of the Autolysin LytA, an Important Virulence Factor in *Streptococcus pneumoniae*, *J. Biol. Chem.*, 2007, vol. 282, no. 24, pp. 17729–17737.
8. García, P., Paz, González, M., García, E., García, J.L., and López, R., The Molecular Characterization of the First Autolytic Lysozyme of *Streptococcus pneumoniae* Reveals Evolutionary Mobile Domains, *Mol. Microbiol.*, 1999, vol. 33, no. 1, pp. 128–138.
9. Kausmally, L., Johnsborg, O., Lunde, M., Knutsen, E., and Hövarstein, L.S., Choline-Binding Protein D (CbpD) in *Streptococcus pneumoniae* Is Essential for Competence-Induced Cell Lysis, *J. Bacteriol.*, 2005, vol. 187, no. 13, pp. 4338–4345.
10. Pestova, E.V., Hövarstein, L.S., and Morrison, D.A., Regulation of Competence for Genetic Transformation in *Streptococcus pneumoniae* by an Auto-Induced Peptide Pheromone and a Two-Component Regulatory System, *Mol. Microbiol.*, 1996, vol. 21, no. 4, pp. 853–862.
11. Campbell, E.A., Choi, S.Y., and Masure, H.R., A Competence Regulon in *Streptococcus pneumoniae* Revealed by Genomic Analysis, *Mol. Microbiol.*, 1998, vol. 27, no. 5, pp. 929–939.
12. Alloing, G., Martin, B., Granadel, C., and Claverys, J.P., Development of Competence in *Streptococcus pneumonaie*: Pheromone Autoinduction and Control of Quorum Sensing by the Oligopeptide Permease, *Mol. Microbiol.*, 1998, vol. 29, no. 1, pp. 75–83.
13. Prozorov, A.A., Competence Pheromones in Bacteria, *Mikrobiologiya*, 2001, vol. 70, no. 1, pp. 5–14 [*Microbiology* (Engl. Transl.), vol. 70, no. 1, pp. 1–9].
14. Peterson, S.N., Sung, C.K., Cline, R., Desai, B.V., Snesrud, E.C., Luo, P., Walling, J., Li, H., Mintz, M., Tsegaye, G., Burr, P.C., Do, Y., Ahn, S., Gilbert, J., Fleischmann, R.D., and Morrison, D.A., Identification of Competence Pheromone Responsive Genes in *Streptococcus pneumoniae* by Use of DNA Microarrays, *Mol. Microbiol.*, 2004, vol. 51, no. 4, pp. 1051–1070.
15. Pozzi, G., Masala, L., Iannelli, F., Manganelli, R., Havarstein, L.S., Piccoli, L., Simon, D., and Morrison, D.A., Competence for Genetic Transformation in Encapsulated Strains of *Streptococcus pneumoniae*: Two Allelic Variants of the Peptide Pheromone, *J. Bacteriol.*, 1996, vol. 178, no. 20, pp. 6087–6090.
16. Håvarstein, L.S., Hakenbeck, R., and Gaustad, P., Natural Competence in the Genus *Streptococcus*: Evidence That Streptococci Can Change Pherotype by Interspecies Recombinational Exchanges, *J. Bacteriol.*, 1997, vol. 179, no. 21, pp. 6589–6594.
17. Håvarstein, L.S., Gaustad, P., Nes, I.F., and Morrison, D.A., Identification of the Streptococcal Competence-Pheromone Receptor, *Mol. Microbiol.*, 1996, vol. 21, no. 4, pp. 863–869.
18. Ottolenghi, E. and Hotchkiss, R.D., Appearance of Genetic Transforming Activity in Pneumococcal Cultures, *Science*, 1960, vol. 132, pp. 1257–1258.
19. Prozorov, A.A., *Transformatsiya u bakterii* (Transformation in Bacteria), Moscow: Nauka, 1988.
20. Steinmoen, H., Knutsen, E., and Håvarstein, L.S., Induction of Natural Competence in *Streptococcus pneumoniae* Triggers Lysis and DNA Release from a Subfraction of the Cell Population, *Proc. Natl. Acad. Sci. USA*, 2002, vol. 99, no. 11, pp. 7681–7686.

21. Steinmoen, H., Teigen, A., and Håvarstein, L.S., Competence-Induced Cells of *Streptococcus pneumoniae* Lyse Competence-Deficient Cells of the Same Strain During Cocultivation, *J. Bacteriol.*, 2003, vol. 185, no. 24, pp. 7176–7183.

22. Moscoso, M. and Claverys, J.P., Release of DNA into the Medium by Competent *Streptococcus pneumoniae*: Kinetics, Mechanism and Stability of the Liberated DNA, *Mol. Microbiol.*, 2004, vol. 54, no. 3, pp. 783–794.

23. Guiral, S., Mitchell, T.J., Martin, B., and Claverys, J.P., Competence-Programmed Predation of Noncompetent Cells in the Human Pathogen *Streptococcus pneumoniae*: Genetic Requirements, *Proc. Natl. Acad. Sci. USA*, 2005, vol. 102, no. 24, pp. 8710–8715.

24. Håvarstein, L.S., Martin, B., Johnsborg, O., Granadel, C., and Claverys, J.P., New Insights Into the Pneumococcal Fratricide: Relationship to Clumping and Identification of a Novel Immunity Factor, *Mol. Microbiol.*, 2006, vol. 59, no. 4, pp. 1297–1307.

25. Claverys, J.P. and Håvarstein, L.S., Cannibalism and Fratricide: Mechanisms and Raisons d'être, *Nat. Rev. Microbiol.*, 2007, vol. 5, no. 3, pp. 219–229.

26. Claverys, J.P., Martin, B., and Håvarstein, L.S., Competence-Induced Fratricide in Streptococci, *Mol. Microbiol.*, 2007, vol. 64, no. 6, pp. 1423–1433.

27. Martner, A., Dahlgren, C., Paton, J.C., and Wold, A.E., Pneumolysin Released During *Streptococcus pneumoniae* Autolysis Is a Potent Activator of Intracellular Oxygen Radical Production in Neutrophils, *Infect. Immun.*, 2008, vol. 76, no. 9, pp. 4079–4087.

28. Johnsborg, O., Eldholm, V., Bjørnstad, M.L., and Håvarstein, L.S., A Predatory Mechanism Dramatically Increases the Efficiency of Lateral Gene Transfer in *Streptococcus pneumoniae* and Related Commensal Species, *Mol. Microbiol.*, 2008, vol. 69, no. 1 P, pp. 245–253.

29. Henriques-Normark, B., Blomberg, C., Dagerhamn, J., Bättig, P., and Normark, S., The Rise and Fall of Bacterial Clones: *Streptococcus pneumoniae, Nat. Rev. Microbiol.*, 2008, vol. 6, no. 11, pp. 827–837.

30. Gilmore, M.S. and Haas, W., The Selective Advantage of Microbial Fratricide, *Proc. Natl. Acad. Sci. USA,* 2005, vol. 102, no. 24, pp. 8401–8402.

31. Kreth, J., Merritt, J., Shi, W., and Qi, F., Co-Ordinated Bacteriocin Production and Competence Evelopment: a Possible Mechanism for Taking up DNA from Neighbouring Species, *Mol. Microbiol.*, 2005, vol. 57, no. 2, pp. 392–404.

32. Hilbert, D.W. and Piggot, P.J., Compartmentalization of Gene Expression During *Bacillus subtilis* Spore Formation, *Microbiol. Mol. Biol. Rev.*, 2004, vol. 68, no. 2, pp. 234–262.

33. Dworkin, J. and Losick, R., Developmental Commitment in a Bacterium, *Cell*, 2005, vol. 121, no. 3, pp. 401–409.

34. Molle, V., Fujita, M., Jensen, S.T., Eichenberger, P., González-Pastor, J.E., Liu, J.S., and Losick, R., The Spo0A Regulon of *Bacillus subtilis, Mol. Microbiol.*, 2003, vol. 50, no. 5, pp. 1683–1701.

35. Fawcett, P., Eichenberger, P., Losick, R., and Youngman, P., The Transcriptional Profile of Early to Middle Sporulation in *Bacillus subtilis, Proc. Natl. Acad. Sci. USA,* 2000, vol. 97, no. 14, pp. 8063–8068.

36. Bischofs, I.B., Hug, J.A., Liu, A.W., Wolf, D.M., and Arkin, A.P., Complexity in Bacterial Cell-Cell Communication: Quorum Signal Integration and Subpopulation Signaling in the *Bacillus subtilis Phosphorelay, Proc. Natl. Acad. Sci. USA*, 2009, vol. 106, no. 16, pp. 6459–6464.

37. Fujita, M., González-Pastor, J.E., and Losick, R., High- and Low-Threshold Genes in the *Spo0A* Regulon of *Bacillus subtilis, J. Bacteriol.*, 2005, vol. 187, no. 4, pp. 1357–1368.

38. González-Pastor, J.E., Hobbs, E.C., and Losick, R., Cannibalism by Sporulating Bacteria, *Science*, 2003, vol. 301, no. 5632, pp. 510–513.

39. Smith, T.J., Blackman, S.A., and Foster, S.J., Autolysins of *Bacillus subtilis*: Multiple Enzymes with Multiple Functions, *Microbiology (UK)*, 2000, vol. 146.

40. Ellermeier, C.D., Hobbs, E.C., Gonzalez-Pastor, J.E., and Losick, R., A Three-Protein Signaling Pathway Governing Immunity to a Bacterial Cannibalism Toxin, *Cell*, 2006, vol. 124, no. 3, pp. 549–559.

41. López, D., Vlamakis, H., Losick, R., and Kolter, R., Cannibalism Enhances Biofilm Development in *Bacillus subtilis, Mol. Microbiol.*, 2009, vol. 74, no. 3, pp. 609–618.

42. Branda, S.S., Chu, F., Kearns, D.B., Losick, R., and Kolter, R., A Major Protein Component of the *Bacillus subtilis* Biofilm Matrix, *Mol. Microbiol.*, 2006, vol. 59, no. 4, pp. 1229–1238.

43. Vlamakis, H., Aguilar, C., Losick, R., and Kolter, R., Control of Cell Fate by the Formation of an Architecturally Complex Bacterial Community, *Genes Dev.*, 2008, vol. 22, no. 7, pp. 945–953.

44. Allenby, N.E., Watts, C.A., Homuth, G., Prágai, Z., Wipat, A., Ward, A.C., and Harwood, C.R., Phosphate Starvation Induces the Sporulation Killing Factor of *Bacillus subtilis, J. Bacteriol.*, 2006, vol. 188, no. 14, pp. 5299–5303.

45. Nandy, S.K., Bapat, P.M., and Venkatesh, K.V., Sporulating Bacteria Prefers Predation to Cannibalism in Mixed Cultures, *FEBS Lett.*, 2007, vol. 581, no. 1, pp. 51–56.

46. Engelberg-Kulka, H. and Hazan, R., Microbiology. Cannibals Defy Starvation and Avoid Sporulation, *Science*, 2003, vol. 301, no. 5632, pp. 467–468.

47. Stragier, P., To Kill but Not Be Killed: a Delicate Balance, *Cell*, 2006, vol. 124, no. 3, pp. 461-463.

48. Rice, K.C. and Bayles, K.W., Molecular Control of Bacterial Death and Lysis, *Microbiol. Mol. Biol. Rev.*, 2008, vol. 72, no. 1, pp. 85–109.

49. Dubnau, D. and Losick, R., Bistability in Bacteria, *Mol. Microbiol.*, 2006, vol. 61, no. 3, pp. 564–572.

50. Veening, J.W., Smits, W.K., and Kuipers, O.P., Bistability, Epigenetics, and Bet-Hedging in Bacteria, *Annu. Rev. Microbiol.*, 2008, vol. 62, pp. 193–210.

51. Davidson, C.J. and Surette, M.G., Individuality in Bacteria, *Ann. Rev. Genet.,* 2008, vol. 42, pp. 253–268.

52. Gardner, A. and Kümmerli, R., Social Evolution: This Microbe Will Self-Destruct, *Curr. Biol.*, 2008, vol. 18, no. 21, pp. R1021–R1023.

53. Pandey, D.P. and Gerdes, K., Toxin-Antitoxin Loci Are Highly Abundant in Free-Living but Lost from Host-Associated Prokaryotes, *Nucleic Acids Res.*, 2005, vol. 33, no. 3, pp. 966–976.

54. Prozorov, A.A. and Danilenko, V.N., Toxin-Antitoxin Systems in Bacteria: Apoptotic Tools or Metabolic Regulators?, *Mikrobiologiya*, 2010, vol. 79, no. 2, pp. 238-250 [*Microbiology* (Engl. Transl.), vol. 79, no. 2, pp. 129–140].

55. Van Melderen, L. and Saavedra, De Bast, M., Bacterial Toxin-Antitoxin Systems: More Than Selfish Entities?, *PLoS Genet.*, 2009, vol. 5, no. 3, pp. 1–6.

56. Nieto, C., Pellicer, T., Balsa, D., Christensen, S.K., Gerdes, K., and Espinosa, M., The Chromosomal relBE2 Toxin-Antitoxin Locus of *Streptococcus pneumoniae*: Characterization and Use of a Bioluminescence Resonance Energy Transfer Assay to Detect Toxin-Antitoxin Interaction, *Mol. Microbiol.*, 2006, vol. 59, no. 4, pp. 1280–1296.

57. Wireman, J.W. and Dworkin, M., Morphogenesis and Developmental Interactions in Myxobacteria, *Science*, 1975, vol. 189, no. 4202, pp. 516–523.

58. Kaiser, D., Signaling in Myxobacteria, *Annu. Rev. Microbiol.*, 2004, vol. 58, pp. 75–98.

59. Berleman, J.E. and Kirby, J.R., Deciphering the Hunting Strategy of a Bacterial Wolfpack, *FEMS Microbiol. Rev.*, 2009, vol. 33, no. 5, pp. 942–957.

60. Zhang, H., Dong, H., Zhao, J., Hu, W., and Li, Y.Z., Characterization of Developmental Autolysis in Myxobacterial Fruiting Body Morphogenesis with Profiling of Amino Acids Using Capillary Electrophoresis Method, *Amino Acids*, 2005, vol. 28, no. 3, pp. 319–325.

61. Nariya, H. and Inouye, M., MazF, an MRNA Interferase, Mediates Programmed Cell Death During Multicellular *Myxococcus* Development, *Cell*, 2008, vol. 132, no. 1, pp. 55–66.

62. Kalakoutskii, L.V. and Agre, N.S., Comparative Aspects of Development and Differentiation in Actinomycetes, *Bacteriol. Rev.*, 1976, vol. 40, no. 2, pp. 469–524.

63. Hodson, D., Differentiation in Actinomycetes, in *Procaryotic Structure and Function: a New Perspective*, Mohan, S. et al., Eds., Cambrige Univ. Press, 1992, pp. 407–440.

64. Manteca, A., Fernández, M., and Sánchez, J., A Death Round Affecting a Young Compartmentalized Mycelium Precedes Aerial Mycelium Dismantling in Confluent Surface Cultures of *Streptomyces antibioticus, Microbiology (UK)*, 2005, vol. 151, no. 11, pp. 3689–3697.

65. Manteca, A., Fernandez, M., and Sánchez, J., Cytological and Biochemical Evidence for an Early Cell Dismantling Event in Surface Cultures of *Streptomyces antibioticus, Res. Microbiol.*, 2006, vol. 157, no. 2, pp. 143–152.

66. Elizarov, S.M. and Danilenko, V.N., Multiple Phosphorylation of Membrane-Associated Calcium-Dependent Protein Serine/Threonine Kinase in *Streptomyces fradiae, FEMS Microbiol. Lett.*, 2001, vol. 202, no. 1, pp. 135–138.

67. Petrícková, K. and Petrícek, M., Eukaryotic-Type Protein Kinases in *Streptomyces coelicolor*: Variations on a Common Theme, *Microbiology (UK)*, 2003, vol. 149, no. 7, pp. 1609–1621.

68. Danilenko, V.N., Mironov, V.A., and Elizarov, S.M., Calcium as a Regulator of Intracellular Processes in Actinomycetes: A Review, *Prikl. Biokhim. Mikrobiol.*, 2005, vol. 41, no. 2, pp. 319–329 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 41, no. 4, pp. 319–329].

69. Kennelly, P.J., Protein Kinases and Protein Phosphatases in Prokaryotes: a Genomic Perspective, *FEMS Microbiol. Lett.*, 2002, vol. 206, no. 1, pp. 1–8.

70. Echenique, J., Kadioglu, A., Romao, S., Andrew, P.W., and Trombe, M.C., Protein Serine/Threonine Kinase StkP Positively Controls Virulence and Competence in *Streptococcus pneumoniae, Infect. Immun.*, 2004, vol. 72, no. 4, pp. 2434–2437.