UDC 579.262:577.12:577.151.05

EXTRACELLULAR DNA IN BACTERIAL BIOFILMS. PART I: ORIGIN

Krestetska S.L.

Mechnikov Institute of microbiology and immunology

Introduction

Bacterial biofilms are spatially and functionally structured, usually surface-adhered multicellular communities encased in a self-produced extracellular polymeric matrix (EPM). The last one provides mechanical stability, mediates adhesion to surfaces, and forms cohesive three-dimensional polymer network that transiently retains bacterial cells and theirs exoproducts (e.g. exoenzymes, metabolites, etc.). EPM usually consists of polysaccharides, proteins, glycolipids and nucleic acids, and each constituent has its own specific functions [1]. Composition and structure of EPM vary depending on the surface and interface properties, nutrient availability, hydrodynamic and other environmental, as well as species- and strain-specific parameters. Moreover, significant changes occur during biofilm growth and maturation.

For a long time, the presence of eDNA in EPM had been considered to be an occasional phenomenon with no structural and functional significance; however, numerous recent evidences suggest it is not exactly so.

The importance of eDNA in biofilm establishing and stabilizing was first demonstrated by Whitchurch et al. (2002) [2]. During the studies of alginate biosynthesis in *P. aeruginosa* PAO1 strain, they found the majority of the extracellular material reacted in the carbazole colorimetric assay was not exopolysaccharide but DNA. As it was demonstrated, strong inhibition of biofilm formation (but not bacterial growth per se) occurred in presence of DNase in culture medium; furthermore, the ability to disperse completely 2-day-old biofilms upon DNase treatment was also shown. 84 hours biofilms were affected by such treatment to a minor degree, therefore, it was supposed that other substances may strengthen the matrix in mature *P. aeruginosa* biofilms, or that mature biofilms may contain proteolytic exoenzymes in quantities sufficient to inactivate locally the DNase activity.

Subsequent evaluation of matrix sensitivity to DNase confirms functional importance of eDNA in EPM structure development and stability in a wide variety of bacterial and fungal species [3-6]. It was demonstrated that eDNA in biofilms tends to form macromolecular 3D structures (usually described in terms “lattice-like network”, “grid-like patterns” or even “skeleton”) and interact with polysaccharides and proteins [4, 5]. It was also shown eDNA modulates its interaction with other matrix constituents to control its contribution to viscoelastic relaxation under mechanical stress [6].

Definitely, there are significant species- and even strain-specific differences. For example, some strains of *N. meningitidis* form biofilms without eDNA (meningococcal clonal complexes (cc) ST-11 and ST-8) [7], suggesting eDNA is not essential for *N. meningitidis* biofilm establishment per se. However epidemiological observations show that strains unable to use eDNA for biofilm build up have poor colonization properties compensated by high transmission rates, while meningococcal cc that form eDNA-containing biofilms (e.g. ST-41/44 cc and ST-32 cc) display a settler phenotype, which results in a stable interaction with the host [7].

Furthermore, quantity and distribution of eDNA vary significantly during biofilm development. As it was shown for different bacterial species, the control of eDNA incorporation has been carried out by secreted or membrane-bound nucleases (bacterial DNases) [8-11]. Enzymatic degradation of eDNA allows to modify biofilm structure, to disperse or to prevent de novo formation of biofilms by competitors [12, 13]. Besides that DNase production interfere with such host innate immune defense mechanism as neutrophil extracellular traps (NETs) produced by activated polymorphonuclear leukocytes and composed of chromatin DNA, histones, and antimicrobial proteins (AMP) [14, 15].

It is noticeable that the NETs formation is a result of leukocytes (preferentially neutrophils) programmed cell death (PCD) and that eDNA in NETs form lattice structure clearly resembling those observed in bacterial biofilms [15]. In addition to obvious structural importance eDNA in NETs was shown exhibit independent of AMP antibacterial action due to its ability to sequester surface bound cations disrupting membrane integrity [16]. The effect require direct contact and depend on cation chelating properties of phosphodiester backbone as it can be fully abrogated by treatment with the excess of cations or phosphatase enzyme [ibid]. Unfortunately, many successful pathogens evolve different strategies allowing them to survive in NETs despite AMP and eDNA antibacterial potential [17]. Hence, this innate defense mechanism seems predominantly aimed at limiting of bacterial cell dissemination from the initial site of infection, which clearly resembles similar function of biofilm matrix – to retain bacterial cells encased in. In both cases the strategy is not very successful when bacterial DNase production is upregulated.

Quantitative PCR of mRNA demonstrate significantly higher expression of the nucleases in planktonic organisms compared to those in biofilm phase of growth [13]. Predictably, nuclease mutants tend to form a thicker biofilms with increased adherence and impaired ability to dispersal [13, 18, 19].

So, distribution and quantity of eDNA in matrix have temporal and spatial variations within the same biofilm, are dependent on the balance between oppositely directed processes, and have obvious functional consequences for biofilm structure maintenance and colonization fitness.

As it was shown in numerous observations, eDNA can appear in matrix through different ways, but given that its composition is usually similar to that of genomic DNA of biofilm forming bacterial population [3] the main source is widely considered to be cell death and lysis.

Programmed cell death

Recent studies of induced cell death in prokaryotes have revealed that these processes are genetically regulated and self-destructive behavior in bacterial populations is not as counterintuitive as it might seem considering individual cell benefit. Prevailing paradigm in this field supposes that, analogous to PCD in eukaryotes, controlled cell death and lysis in bacteria play an important role in the development of complex multicellular communities [20, 21]. Studies that have been carried out last decade gave enough evidences to support this assumption [20-23] and move our perception towards analogy with tissues of multicellular organisms which routinely use PCD to remove damaged or excessive cells.

In addition to obvious functional analogy to eukaryotic PCD systems there are some similarities at the molecular level, as bacterial proteins presumably relative to the apoptosis regulator Bax/Bcl-2 family [24], as well as caspase-like enzymes [25] produced by some bacterial species was identified.

One of the best-characterized examples of regulated bacterial death originates from studies of the lytic cycle control during a bacteriophage infection. In infection cycle of double-stranded DNA phages of Gram-negative bacteria the timing of cell lysis is dictated by the holin (transmembrane protein, essential for the inner membrane permeabilisation), which allows bacteriophage encoded murein hydrolase (endolysin) to escape from the cytoplasm and to attack the cell wall peptidoglycan. Historically, the fatal membrane lesions formed by holin triggering have been called “holes”, as opposed to channels or pores forming by transporters or porins. The activity of holin is usually regulated by holin-specific inhibitor (antiholin) [26].

The dynamics of these holin/antiholin systems are not the key to bacteriophage induced lysis timing: the current model supposes the holins exhibit critical concentration behavior for membrane lesion forming and precisely-timed triggering still occurs in mutants with altered antiholin production [27]. Nevertheless sufficient protraction of infection cycle after secondary T4 phage infection is certainly mediated by antiholin, as it was shown in Lysis Inhibition of T4-infected cells (LIN) - one of the oldest experimental systems in molecular genetics. For a long time LIN considered to be the only case where it is known that environmental information is used for real-time antiholin-mediated control of holin triggering [27-29].

There are two main points making abovementioned observations relevant to current topic.

The first one is that prophages are ubiquitous elements within bacterial chromosomes and phage-induced lysis is successfully adapted to controlled eDNA release and was shown require for normal biofilm formation in different bacterial species [30-32].

The second point is consideration that phage “holin/antiholin” system has some obvious similarities with molecular mechanisms providing auto- and hetero-destructive behavior in bacterial populations – toxin-antitoxin (TA) systems and other conditionally inducible regulators, especially those using bacterial murein hydrolases (autolysins) as effectors for controlled cell lysis execution.

Toxin-antitoxin systems

TA stems belong to the best-characterized molecular tools considered being involved in bacterial PCD auto-induction [33]. The effector part of such system is represented by the pair of stable toxic protein and its inhibitor, more labile antitoxin (protein or noncoding RNA) that antagonizes the toxin activity or prevents its synthesis. Conditions that provoke antitoxin loss could trigger self-destructive processes, as toxin usually interferes with vital cellular function (e.g. membrane integrity, cell wall synthesis, replication, ribosome assembly) with RNA cleavage as the most prevalent mode of action [34].

Genetic modules coding such pairs originally discovered on low copy number plasmids [35] and prophages [36]. They were coined “addiction” modules because of participation in plasmid maintenance by a mechanism called “addiction” or “post-segregational killing” (selective killing of plasmid-free cells to increase plasmid prevalence in population). The progeny of plasmid bearing cells are “addicted” to the short-lived product, because its de novo synthesis becomes essential for survival. Cells that lost or do not inherit this module are killed by unleashed toxin protein. This provides some evolutional pressure, which compels bacterial population to reproduce and propagate additional genetic information in the absence of external selective pressure [33].

Later sequencing of bacterial genomes led to the discovery of chromosomally encoded TA modules and revealed their abundance, high diversity, and poorly explainable distribution. Some obligate intracellular organisms like Mycoplasma or Chlamydia have no or very few ones, while 88 putative TA systems predicted in *M. tuberculosis* [37].

To date, six classes of such systems have been identified based on the nature and mode of antitoxin action [38, 39]. Attempts to classify the most abundant in bacterial genomes type II TA systems (in which the both toxin and antitoxin are proteins) using structural features of these proteins, allow identifying at least 18 antitoxin and 23 toxin families [40]. Besides that, variability of combinations between the proteins of different families makes the number of possible type II TA variants poorly predictable [40].

While the destination of TA systems encoded by extrachromosomal genetic elements is quite clear, functionality of theirs numerous chromosomally encoded counterparts remains controversial.

Stabilization of associated genetic information (preferentially those belonging to flexible genome represented by integrated conjugative elements conferring pathogenicity traits or antibiotic resistance) was shown [41] indicating that at least some TA systems might have retained their “addictive” properties.

Some of TA modules act as a part of phage resistance mechanism (abortive infection systems (Abi)) that limits viral replication due to induction of premature bacterial cell suicide upon phage infection [42].

PezAT, the member of the only known family of three-component TA systems omega-epsilon-zeta (ω-ε-ζ), as was shown, certainly focused on PCD via cell lysis induction [43]. Pneumococcal zeta toxin PezT phosphorylate the ubiquitous peptidoglycan precursor uridine diphosphate-N-acetyl glucosamine, inhibiting activity of the MurA, enzyme catalyzing the initial step in bacterial peptidoglycan biosynthesis. Impaired cell wall integrity and breakdown of the osmotic barrier provokes an autolytic phenotype. Fast growing part of population is obviously much more sensitive to such influence that predisposes to selection of slow growing forms [43].

However, numerous observations suggest the majority of TA systems evolve toward new functions rather promoting cell survival under specific conditions then facilitating individual cell death. Having direct relation to the transcriptional and post-transcriptional regulation of important physiological processes, they supplement conventional mechanisms of stress response by altering gene expression patterns (for excellent review see Bertram R. and Schuster C.F. (2014) [38]).

Toxin induced changes usually have the character of physiological downshifting and while precise conditions determining whether these changes would be reversible or lead to cell death is not clear, an essential role of TA systems in persister (or dormant) phenotype formation have been well established [44-48]. These isogenic subpopulations of slow growing or nonculturable cells with markedly increased resistance to antibiotics (as well as nutrient limitations and other stressors) preexists in any bacterial population with very low frequency (10^{-4} to 10^{-6}) reaching its maximum in stationary phase or during biofilm mode of growth [46]. The phenomenon now considered to be directly responsible for recalcitrance and relapsing nature of biofilm-associated bacterial infections [46]. Several reports suggest overproduction of some type II toxins could substantially increase persister frequency. Gain-of-function mutants in the E.coli *hipA* (toxin gene of hipBA TA system) lead to augmentation of ampicillin- and fluoroquinolone-tolerant persister amount in a growing population from 1 in 10,000 cells or less (wild-type levels) to 1 in 100 cells [47]. Successive deletion of the 10 mRNase-encoding TA loci of *E.coli* progressively reduce the frequency of persisters, suggesting their cumulative contribution to the effect and reflecting possible degree of regulatory network complexity [48].

An additional dimension for this complexity adds the fact that identical or homologous TA systems in different species are integrated in genome by different ways and diversely interacts with conserved stress response regulators [49, 50]. Besides that, chromosomally encoded TA systems being a part of flexible genome apparently spread via horizontal gene transfer; consequently, different isolates of the same species could have different sets of functional TA systems. This obviously predisposes to different phenotype selection during adaptation to specific growth conditions influencing niche-specific colonization.

Good example of such influence is provided by extraintestinal pathogenic *E. coli* (ExPEC). Only three of at least 36 known for *E.coli* TA systems are strongly associated with its ability to cause urinary tract infections [51]. Precisely YefM-YoeB and YbaJ-Hha independently promote colonization of the bladder by the reference uropathogenic ExPEC isolate CFT073, while PasTI is critical to ExPEC survival within the kidneys [51].

Mature biofilms are highly compartmentalized environment with gradients of nutrients, pH, oxygen and toxic metabolites, so, biofilm mode of growth definitely supposes adaptation to quit variable niches, and phenotypic heterogeneity (or cellular differentiation) in such populations is a commonly accepted concept [52]. Thus, involvement of TA systems in regulation of stress response and cell viability during biofilm development looks quite predictable and experimental evidences of this fact is widely reported [53, 54].

However, information regarding direct participation of TA systems in controlled eDNA release during biofilm formation is limited. Actually, the only reference was found in NCBI database, reporting the deletion of the HipBA in *E. coli* BW25113 significantly reduce the biofilm biomass, eDNA content and sensitivity of biofilm to DNAse treatment [55]. However, any other published information concerning influence of TA systems (even those with confirmed ability to provoke an autolytic phenotype, unlike the *hipAB* TA system, which is commonly considered as being related to persistence only [44, 47, 48]) on controlled eDNA release in biofilm growing populations was not found.

This confirms the assumption that transcriptional diversity, generated by the presence of some sets of functional TA systems represents stress-inducible mechanism promoting rather cell survival, then death. Thus, if drawing analogy with the processes occurring in multicellular organisms is pertinent at all, there are much more similarities with stress-inducible irreversible growth arrest (designated as cellular senescence [56]), than with PCD per se. This compels to suppose that cell death and lysis induction in bacterial populations, as a form of collective behavior focused on eDNA supply, seems must be governed by another, probably more conserved and less dependent on horizontal gene transfer mechanisms.

Transcriptional control of murein hydrolase functions

A bulk of auto- and hetero-destructive molecular mechanisms is based on controlling of murein hydrolase activity. Bacterial murein hydrolases (autolysins) is a family of enzymes that specifically cleave structural components of peptidoglycan and participate in a cell growth and division processes, including daughter cell separation and peptidoglycan recycling and turnover [57]. Similar to bacteriophage encoded murein hydrolase (endolysin) the activity of these enzymes can become detrimental to the cell in certain conditions. For example, as it was shown [58] penicillin interfere with regulatory mechanism(s) that normally keeps murein hydrolase activity in check, and precisely this effect is responsible for the cellular autolysis that often occurs following penicillin exposure.

Different molecular mechanisms that interfere with murein hydrolases activity providing partial lysis of bacterial population have established impact on biofilm formation [59-63]. Process is strongly influenced by

multiple environmental factors and this influence is mediated by transcriptional regulators, – usually alternative sigma factors, which control global switches of the genetic expression program in bacteria, and signal-transducing two-component systems (TCS), which is considered to be more specialized tools of functional adaptation to environmental changes [64-65]. Canonical TCS is composed of transmembrane sensor histidine kinase and a cognate transcriptional response regulator (for comprehensive review of precise structure, functioning and the multiple possibilities for regulation see [65]).

In *Staphylococcus aureus*, two homologous operons cidABC and lrgAB have opposing effects on murein hydrolase activity and provide regulation of "suicidal" behavior observed during biofilm formation [66-73]. Although the exact mode of action has not been demonstrated, a model based on the similarities of the cidA and lrgA gene products to the bacteriophage holin and antiholin was proposed, and several common structural features was reported [69]. A lrgAB mutant exhibits increased matrix-associated eDNA and biofilm adherence consistent with presumptive role of lrgAB as an inhibitor of cidA-mediated lysis [70]. Cid/Lrg system was shown is under control of at least two regulatory networks: LytSR signal-transducing TCS which responds to changes in membrane potential [71], and the LysR-type transcriptional regulator known as CidR that respond to carbohydrate metabolism-mediated acetic acid accumulation in a culture medium [72]. It was also reported the expression of some protein products of cid/lrg murein hydrolase regulators depend on the activation of the alternative sigma factor σ^B [73].

An additional layer of autolysin-induced cell death regulation in *Staphylococcus aureus* is provided by secreted protease SspA that can prevent lysis by specific cleavage of murein hydrolases [74]. It was also shown the ability to interfere with staphylococcal autolysin activity and biofilm formation by other related species producing functional analogs of SspA. Particularly Esp secreted protease of *Staphylococcus epidermidis* demonstrate the ability to cleavage surface associated autolysins of *Staphylococcus aureus*, diminishing eDNA release and biofilm formation [74].

An example of hetero-destructive (or "fratricidal") mechanisms based on autolysin activity regulation comes from observations of biofilm growing enterococci [75]. The effect is QS-dependent and is triggered by an 11-amino acid cyclized peptide lactone termed gelatinase biosynthesis-activating pheromone (GBAP) – the product of the quorum-sensing TCS encoded by the *fsr* locus. Being homologous to staphylococcal Agr system it participates in control of biofilm formation via positive regulation of two co-transcribed secreted extracellular proteases – the zinc metalloprotease GelE (gelatinase) and the serine protease SprE. GBAP first appears by about 24 h of biofilm growth dividing bacterial population into GBAP-responders (which produce GelE and SprE and could be designated as GelE+SprE+ phenotype) and GBAP-nonresponder (GelE- SprE- cells). Last one subpopulation undergoes lysis as a result of autolysin AtlA activation by GelE. SprE interferes with this process, preventing lysis [75].

So, phenomenon is directly attributed to phenotypic heterogeneity and maintained by adaptation to local environmental signals produced within a spatially structured bacterial community.

Very similar mechanism of fratricidal behavior is observed during competence development in streptococci [76-78], majority of which are competent for natural transformation promoting genetic variability of population in regard to antibiotic sensitivity, pathogenicity and capsular antigens switching [78].

It is widely accepted that the primary control point for development of the competence state in many *Firmicutes* is the alternative sigma factor ComX, which activates transcription of late competence genes encoding constituents for DNA uptake and recombination [79]. In streptococci different murein hydrolases are expressed as a part of the ComX regulon allowing synchronization of homologous eDNA supply with activation of DNA capture and processing machinery. *S.pneumonia* and many other species possess competence-induced murein hydrolase CbpD. ComX of nearly all species lacking a CbpD or CbpD-like proteins contain an alternative murein hydrolase LytF, which is unrelated to CbpD, but apparently serves the same function [80, 81].

Competence development is under control of complex regulatory system induced by a peptide pheromone CSP (competence-stimulating peptide), which is ComC gen product [82, 83]. TCS ComDE in response to a critical external concentration of CSP activates expression of ComX and other early competence genes, including the comCDE operon (generating a positive feedback loop, amplifying the signal, and coordinating competence throughout the population [83]) and ComM (the cognate immunity protein that prevents CbpD induced lysis). ComX then initiates transcription of competence associated murein hydrolase (CbpD or LytF) as well as about 80 known 'late' competence genes, 16 of which are essential for genetic transformation encoding proteins involved in DNA uptake, processing, and recombination [84]. Competent ($CbpD^+ComM^+$) cells execute lysis of noncompetent ($CbpD^-ComM^-$) pneumococci and several other species enabling to capture DNA from sibling or closely related streptococci sharing the same habitat.

Streptococcal CSP pheromone was originally thought to be the QS-like signal passively accumulated with cell density rising (similar to enterococcal GBAP) [85], however later investigations has shown that induction of competence regulons can also occurs as a part of general stress response [86, 87] supporting the concept of stress-induced genetic plasticity [88].

Direct involvement of competence-specific bacteriolytic mechanisms in biofilm development was shown. In particular deletion of CbpD strongly affects eDNA release and biofilm formation in *S.pneumonia* [89].

Some streptococci develop additional, independent of CbpD or LytF competence-associated bacteriolytic mechanisms. In *S. mutans* ComDE controls the expression of many bacteriocins allowing coordinated regulation of competence development with lysis of neighboring relative and non-relative species [90, 91]. Moreover, unprocessed form of mutacin V (CipB) was identified as a major factor in CSP-induced autolysis [92],

considering ~2-log-fold reduction in transformation efficiency of a Δ*cipB* mutant strain compared to a wild-type strain under CSP-induced conditions. Precise mechanisms of CipB mediated autolysis have not been elucidated but it was shown autocidal activity of intracellular CipB can be prevented by cognate immunity protein CipI [92].

Synchronized regulation of stress response, bacteriocins production, competence and biofilm development in *S. mutans* [93, 94] is supposed to be an evolutionary adaptation to the multispecies oral biofilm environment. Such overlapping regulation allows tight control of biofilm matrix enrichment with eDNA through competence-specific stress-inducible bacteriolytic mechanisms [95].

Lysis independent eDNA release

Lysis-independent eDNA release was also described indicating the significance of this polymer for different stages of biofilm formation including those considered to be QS-independent.

In *Enterococcus faecalis*, the ratio of eDNA per cell rises more than 1,000-fold compared to planktonic controls during the first 4 h of biofilm development [96]. Precise mechanisms of this phenomenon is not identified, however absence of cellular debris, the character of eDNA distribution (which is different from those making by cell lysis), and early timing of appearance suggest its lysis-independent and probably QS-independent nature.

Some definite mechanisms of DNA secretion during biofilm formation in different species was determined: *Neisseria gonorrhoeae* secrets single-stranded DNA in the initial phase of biofilm formation throughout its type IV secretion system [97]; *Streptococcus mutans* produces eDNA by multiple ways (as was discussed above [90-94]), including lysis-independent active release via membrane vesicles (MVs) [98].

Actually an ability to produce DNA containing MVs was shown for many Gram-negative bacteria and usually is referred to natural competence development. In this regard the involvement of specific mechanisms that serve to horizontal gene transfer (e.g. MVs and type IV secretion system) in lysis-independent eDNA release during biofilm formation seems very probable and direct link between the activation of these mechanisms and transcriptional switch to biofilm lifestyle is probable as well [99, 100]. A model of the *V.cholerae* competence regulatory network that posits three controlling environmental determinants of this phenotype have been proposed [101] and among positive regulators there are increasing cell density, nutrient limitation, growth deceleration, and stress response, all of which are inherent to biofilm mode of growth.

Moreover the ability of EPM to incorporate DNA from various exogenous sources (including lysed cells of other bacterial species, mammalian cell debris, NETs and DNA from salmon sperm) has been also demonstrated [4,102, 103].

Partial cell lysis as a result of antibiotic treatment also can promote eDNA-dependent biofilm formation. Low levels of β-lactams were shown induce significant auto-aggregation and biofilm formation by *Staphylococcus aureus* and both processes were dependent on cell lysis and release of DNA, as was proved by their DNase sensitivity [104]. Process may be of a great clinical relevance because exposure to sub-minimal inhibitory concentrations during the normal course of antibiotic therapy often occurs [105].

Conclusion remarks

The above list of possible eDNA sources is definitely incomplete, - some species develop rather peculiar ways of cell lysis induction. For example there are two known independent mechanisms of cell death induction in *P. aeruginosa*, - pyocyanin dependent H_2O_2 mediated cell lysis [107, 108] and two-component transcriptional regulator BfmR controlled bacteriophage-mediated lysis [30]. Both pathways are QS-dependent suggesting possible existence of other sources enabling early eDNA appearance observed at initial stages of biofilm formation. Coexistence of several different mechanisms is common, which makes strategies based on interference with one of such molecular mechanisms not quite efficient.

Nonetheless available information clearly indicates eDNA release (whether as a result of induced cell death or active secretion) is definitely intentional and regulated process clearly associated with a switch to biofilm lifestyle. Fractional sacrificing in biofilm growing community may have direct relation to the selection of certain phenotypes (as phenotypic heterogeneity is intrinsic quality of such populations), or to be a part of the mechanisms involved in horizontal gene transfer (as stress induced genetic plasticity concept suppose). At the same time high independent importance of eDNA for biofilm structure and properties (that will be discussed in part II) indicates these mechanisms could be designated as serving dual purpose and having undoubted association with sessile community lifestyle of bacterial population.

References

1. Flemming, H.C. Relevance of microbial extracellular polymeric substances (EPSs)--Part I: Structural and ecological aspects. /H.C. Flemming, J. Wingender // Water Sci Technol. – 2001 . – V. 43 . – N6 . – P. 1-8.
2. Whitchurch, C.B. Extracellular DNA required for bacterial biofilm formation. / C.B. Whitchurch, T. Tolker-Nielsen, P.C. Ragas, J.S. Mattick // Science. – 2002 . – V. 295 . - N 5559 . - P. 1487 . – doi: 10.1126/science.295.5559.1487.
3. Das, T. The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. / T. Das, S. Sehar, M. Manefield //Environ MicrobiolRep .– 2013 . – V. 5 . – N 6 . – P. 778-786 . – doi: 10.1111/1758-2229.12085
4. Wang, S. The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm skeleton in Pseudomonas aeruginosa./ S.Wang, X.Liu, H.Liu, L.Zhang, Y.Guo, S.Yu, Wozniak D.J., Ma L.Z. //Environ MicrobiolRep .– 2015 . – V. 7 . – N 2 . – P.330-340 . – doi: 10.1111/1758-2229.12252.

5. Brockson, M.E. Evaluation of the kinetics and mechanism of action of anti-integration host factor-mediated disruption of bacterial biofilms./ M.EBrockson, L.A. Novotny, E.M.Mokrzan, S.Malhotra, J.A.Jurcisek, R. Akbar, A.Devaraj, S.D. Goodman, L.O.Bakaletz. //MolMicrobiol. – 2014 . – V.93. – N6. – P. 1246-1258 . – doi: 10.1111/mmi.12735.
6. Peterson, B.W. A Distinguishable Role of eDNA in the Viscoelastic Relaxation of Biofilms. / Brandon W. Peterson, Henny C. van der Mei, JelmerSjollema, Henk J. Busscher, Prashant K. Sharma //mBio.. – 2013 . – V. 4. – N 5. – P. e00497-e00513. . –. doi: 10.1128/mBio.00497-13
7. Lappann, M. A dual role of extracellular DNA during biofilm formation of Neisseria meningitidis. / M. Lappann, H. Claus, T. van Alen, M. Harmsen, J. Elias, S .Molin, U.Vogel // MolMicrobiol .– 2010 . –V. 75 . – N 6 . – P.1355-1371 .– doi: 10.1111/j.1365-2958.2010.07054.x.
8. Kiedrowski, M.R. Nuclease modulates biofilm formation in community-associated methicillin-resistant Staphylococcus aureus. / M.R. Kiedrowski, J.S. Kavanaugh, C.L. Malone, J.M. Mootz, J.M. Voyich, M.S. Smeltzer, K.W. Bayles, A.R. Horswill //PLoS One . – 2011 . – V. 6 . – N 11 . – P. e26714 . – doi: 10.1371/journal.pone.0026714.
9. Steichen, C.T. The Neisseria gonorrhoeae biofilm matrix contains DNA, and an endogenous nuclease controls its incorporation./ C.T. Steichen, C. Cho, J.Q. Shao, M.A. Apicella // Infect Immun . – 2011 . – V. 79 . – N 4 . – P. 1504-1511 . – doi: 10.1128/IAI.01162-10.
10. Beenken, K.E. Impact of extracellular nuclease production on the biofilm phenotype of Staphylococcus aureus under in vitro and in vivo conditions. / K.E. Beenken, H. Spencer, L.M. Griffin, M.S. Smeltzer // Infect Immun. . – 2012 . – V. 80 . – N5 . – P.1634-1638. - doi: 10.1128/IAI.06134-11.
11. deBuhr, N. Streptococcus suis DNase SsnA contributes to degradation of neutrophil extracellular traps (NETs) and evasion of NET-mediated antimicrobial activity. / N. de Buhr, A. Neumann, N. Jerjomiceva, M. von Köckritz-Blickwede, C.G. Baums //Microbiology . – 2014 . – V. 160 .– N 2 . – P. 385-395 . – doi: 10.1099/mic.0.072199-0
12. Nijland, R. Dispersal of biofilms by secreted, matrix degrading, bacterial DNase. / R. Nijland, M.J. Hall, J.G.Burgess // PLoS One. – 2010 . – V.5. – N12. – P.e15668. – doi: 10.1371/journal.pone.0015668.
13. Cho, C. Role of the nuclease of NontypeableHaemophilusinfluenzae in dispersal of organisms from biofilms. / C. Cho, A. Chande, L. Gakhar, L.O. Bakaletz, J.A. Jurcisek, M. Ketterer, J. Shao, K.Gotoh, E. Foster, J. Hunt, E. O'Brien, M.A. Apicella // Infect Immun . – 2015 . – V. 83 .– N3 . – P. 950-957 .– doi: 10.1128/IAI.02601-14..
14. deBuhr, N. Streptococcus suis DNase SsnA contributes to degradation of neutrophil extracellular traps (NETs) and evasion of NET-mediated antimicrobial activity. / N. de Buhr, A. Neumann, N. Jerjomiceva, M. von Köckritz-Blickwede, C.G. Baums //Microbiology . – 2014 . – V. 160 .– N 2 . – P. 385-395 .– doi: 10.1099/mic.0.072199-0..
15. Brinkmann, V. Beneficial suicide: why neutrophils die to make NETs. / V. Brinkmann, A. Zychlinsky // Nat. Rev. Microbiol .– 2007 . – V. 5 .– P. 577-582.
16. Halverson, T.W. DNA is an antimicrobial component of neutrophil extracellular traps. / T.W. Halverson, M. Wilton, K.K. Poon, B. Petri, S. Lewenza //PLoSPathog .– 2015 . – V.11 . - N1 . – e1004593 .– doi: 10.1371/journal.ppat.1004593.
17. Menegazzi, R. Killing by neutrophil extracellular traps: fact or folklore? / R. Menegazzi, E. Decleva, P. Dri //Blood .– 2012 . – V. 119 . – N 5 . – P. 1214-1216 .– doi: 10.1182/blood-2011-07-364604.
18. Mann, E.E. Modulation of eDNA release and degradation affects Staphylococcus aureus biofilm maturation. / E.E. Mann, K.C. Rice, B.R. Boles, J.L. Endres, D. Ranjit, L. Chandramohan, L.H.Tsang, M.S. Smeltzer, A.R. Horswill, K.W. Bayles // PLoSOne .– 2009 . – V. 4 .– N6 . – P. e5822 . – doi: 10.1371/journal.pone.0005822
19. Seper, A. Extracellular nucleases and extracellular DNA play important roles in Vibrio cholerae biofilm formation. / A.Seper, V.H. Fengler, S. Roier, H. Wolinski, S.D. Kohlwein, A.L. Bishop, A. Camilli, J. Reidl, S. Schild //MolMicrobiol . – 2011 . – V. 82 . – N4 . – P.1015-1037. - doi: 10.1111/j.1365-2958.2011.07867.x..
20. Rice, K.C. Death's toolbox: examining the molecular components of bacterial programmed cell death. / K.C. Rice, K.W. Bayles //Mol. Microbiol.– 2003. – V. 50. – P. 729–738. – doi: 10.1046/j.1365-2958.2003.t01-1-03720.x
21. Rice, K.C. Molecular Control of Bacterial Death and Lysis. / Kelly C. Rice, Kenneth W. Bayles// MicrobiolMolBiolRev.– 2008. – V. 72. – N 1.– P. 85–109. – doi: 10.1128/MMBR.00030-07
22. Bayles, K.W. Bacterial programmed cell death: making sense of a paradox. / K.W.Bayles // Nat Rev Microbiol. –2014 . – V.12 . –N1 . – P. 63-69 .–doi: 10.1038/nrmicro3136.
23. Bayles, K.W. The biological role of death and lysis in biofilm development. //Nat Rev Microbiol.– 2007. –V.5 . –N9 .– P. 721–726.
24. Pang, X. Active Bax and Bak are functional holins. / X. Pang, S.H. Moussa, N.M. Targy, J.L. Bose, et al. //Genes Dev. – 2011. – V. 25. – P. 2278–2290.
25. Gautam, S. Involvement of caspase-3-like protein in rapid cell death of Xanthomonas. / S.Gautam, A. Sharma // Mol. Microbiol. – 2002. – V.44 . – P.393–401.
26. Saier, M.H. Holins in bacteria, eukaryotes, and archaea: multifunctional xenologues with potential biotechnological and biomedical applications. / M.H. Jr. Saier, B.L. Reddy // J Bacteriol. –2015 . – V.197. –N1. – P. 7-17. – doi: 10.1128/JB.02046-14
27. Young, R. Phage lysis: do we have the hole story yet? / R.Young //CurrOpinMicrobiol. –2013 . – V. 16 . – N 6 . – P. 790-797 .–doi: 10.1016/j.mib.2013.08.008.
28. Moussa, S.H. Genetic dissection of T4 lysis. / S.H. Moussa, J.L. Lawler , R. Young . //J Bacteriol. – 2014 . – V. 196. –N 12. –P. 2201-9.–doi: 10.1128/JB.01548-14.
29. Tran, T.A. Periplasmic domains define holin-antiholin interactions in T4 lysis inhibition. / T.A.T. Tran, D.K. Struck, R. Young //J. Bacteriol. –2005. –V. 187. – P. 6631–6640.

30. Petrova, O.E. The novel Pseudomonas aeruginosa two-component regulator BfmR controls bacteriophage mediated lysis and DNA release during biofilm development through PhdA. / Olga E. Petrova, Jill R. Schurr,Michael J. Schurr, Karin Sauer //MolMicrobiol. – 2011 . –V.81 . – N3 . – P. 767–783 .–doi: 10.1111/j.1365-2958.2011.07733.x.
31. Carrolo, M. Prophage spontaneous activation promotes DNA release enhancing biofilm formation in Streptococcus pneumoniae. / M.Carrolo, M.J. Frias, F.R. Pinto, J. Melo-Cristino, M. Ramirez //PLoSOne.– 2010. – V. 5. – N 12. – P. e15678.–doi: 10.1371/journal.pone.0015678. PMID: 21187931
32. Binnenkade, L. Iron triggers λSoprophage induction and release of extracellular DNA in Shewanellaoneidensis MR-1 biofilms. / L. Binnenkade, L. Teichmann, K.M.Thormann // Appl Environ Microbiol.– 2014. – V. 80. – N 17. – P. 5304-5316. – doi: 10.1128/AEM.01480-14.
33. Aizenman, E. An Escherichia coli chromosomal "addiction module" regulated by guanosine 3',5'-bispyrophosphate: a model for programmed bacterial cell death / Aizenman E., H. Engelberg-Kulka, and G. Glaser. //Proc. Natl. Acad. Sci. USA . – 1996. – V.93. – P.6059–6063.
34. Schuster, C.F. Toxin-antitoxin systems are ubiquitous and versatile modulators of prokaryotic cell fate / C.F. Schuster, R. Bertram //FEMS MicrobiolLett. – 2013 .– V.340 . – N2 . – P. 73-85 .– doi: 10.1111/1574-6968.12074.
35. Ogura, T. Mini-F plasmid genes that couple hosts cell division to plasmid proliferation / T. Ogura, S. Hiraga // Proc. Natl. Acad. Sci. USA.– 1983. –V. 80. – P. 4784–4788. – doi: 10.1073/pnas.80.15.4784.
36. Lehnherr, H. Plasmid addiction genes of bacteriophage P1: doc, which causes cell death on curing of prophage, and phd, which prevents host death when prophage is retained / H. Lehnherr, E. Maguin, S. Jafri, and M. B. Yarmolinsky..// J. Mol. Biol.– 1993. – V. 233. – P.414–428.
37. Ramage, H.R. Comprehensive Functional Analysis of Mycobacterium tuberculosis Toxin-Antitoxin Systems: Implications for Pathogenesis, Stress Responses, and Evolution /Ramage H.R., Connolly L.E., Cox J.S., Rosenberg S.M., ed. //PLoS Genetics .– 2009 .– V.5. – N12. – e1000767 . – doi:10.1371/journal.pgen.1000767.
38. Bertram, R. Post-transcriptional regulation of gene expression in bacterial pathogens by toxin-antitoxin systems / Bertram R, Schuster CF.// Frontiers in Cellular and Infection Microbiology.– 2014.– V.4 . – P. 6-7 . - doi:10.3389/fcimb.2014.00006.
39. Markovski, M. Preventing bacterial suicide: a novel toxin-antitoxin strategy /Markovski M., Wickner S. //Mol. Cell.– 2013 . – V. 52. – P. 611–612 . – 10.1016/j.molcel.2013.11.018
40. Leplae, R. Diversity of bacterial type II toxin–antitoxin systems: a comprehensive search and functional analysis of novel families / Leplae R, Geeraerts D, Hallez R, Guglielmini J, Drèze P, Van Melderen L. //Nucleic Acids Research. – 2011. – V.39. – N13. – P. 5513-5525. – doi:10.1093/nar/gkr131.
41. Bjørkeng, E.K. ICESluvan, a 94-Kilobase Mosaic Integrative Conjugative Element Conferring Interspecies Transfer of VanB-Type Glycopeptide Resistance, a Novel Bacitracin Resistance Locus, and a Toxin-Antitoxin Stabilization System / Bjørkeng EK, Hjerde E, Pedersen T, Sundsfjord A, Hegstad K. // Journal of Bacteriology. – 2013. – V.195. – N 23. – P. 5381-5390. – doi:10.1128/JB.02165-12.
42. Dy, R.L. A widespread bacteriophage abortive infection system functions through a Type IV toxin-antitoxin mechanism /Dy R.L., Przybilski R., Semeijn K., Salmond G.P., Fineran P.C.// Nucleic Acids Res. – 2014 . – V.42. – N7. – P. 4590-605. – doi: 10.1093/nar/gkt1419
43. Mutschler, H. A Novel Mechanism of Programmed Cell Death in Bacteria by Toxin–Antitoxin Systems Corrupts Peptidoglycan Synthesis / Mutschler H., Gebhardt M., Shoeman R.L., Meinhart A.. Waldor M., ed. // PLoS Biology. – 2011. – . V.9. – N 3:e1001033 . – doi:10.1371/journal.pbio.1001033.
44. Germain, E. Stochastic induction of persister cells by HipA through (p)ppGpp-mediated activation of mRNA endonucleases /Elsa Germain, Mohammad Roghanian, Kenn Gerdes, Etienne Maisonneuve //Proc Natl Acad Sci U S A . – 2015 . – V.112 . – N 16 . – P. 5171–5176 . – doi: 10.1073/pnas.1423536112
45. Maisonneuve, E. Molecular Mechanisms Underlying Bacterial Persisters. / E.Maisonneuve, K. Gerdes . //Cell . – 2014 . – V.157 . - N 3 . – P. 539–548 . - doi:10.1016/j.cell.2014.02.050
46. Lewis, K. Multidrug tolerance of biofilms and persister cells/ K. Lewis //Curr Top Microbiol Immunol . – 2008 . – V.322 . – P.107-131.
47. Korch, S.B. Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis/ Korch SB, Henderson TA, Hill TM.// Mol Microbiol . – 2003 . – V.50 . – N 4 . – P. 1199-213.
48. Maisonneuve, E. Bacterial persistence by RNA endonucleases / E. Maisonneuve, L.J. Shakespeare, M.G. Jørgensen, K. Gerdes //Proc. Natl. Acad. Sci. USA . – 2011 . – V.108 . – P. 13206–13211
49. Amitai, S. MazF-mediated cell death in Escherichia coli: a point of no return. / S. Amitai, Y. Yassin, H.Engelberg-Kulka // J Bacteriol. – 2004 . – V.186 . – P. 8295–8300.
50. Donegan, N.P. Regulation of the mazEF toxin-antitoxin module in Staphylococcus aureus and its impact on sigB expression / Donegan NP, Cheung AL. // J Bacteriol . – 2009 . – V.191 . – P. 2795–2805. doi: 10.1128/JB.01713-08
51. Norton, J.P. Toxin-antitoxin systems are important for niche-specific colonization and stress resistance of uropathogenic Escherichia coli / Norton J.P., Mulvey M.A. //PLoSPathog.– 2012. – V.8 . – N 10 . – P. e1002954 .– doi: 10.1371/journal.ppat.1002954.
52. Serra, D.O. Stress responses go three dimensional – the spatial order of physiological differentiation in bacterial macrocolony biofilms / Serra DO, Hengge R. // Environmental Microbiology . – 2014 . – V.16 . – N 6 . – P.1455-1471 . – doi:10.1111/1462-2920.12483.
53. Kolodkin-Gal, I. Differential Effect of E. coli Toxin-Antitoxin Systems on Cell Death in Liquid Media and

Biofilm Formation / Kolodkin-Gal I, Verdiger R, Shlosberg-Fedida A, Engelberg-Kulka H. A. Bereswill S, ed. // PLoS ONE . – 2009 . – V.4 . – N 8 . – :e6785. – doi:10.1371/journal.pone.0006785.
54. Wang, X. Toxin-Antitoxin Systems Influence Biofilm and Persister Cell Formation and the General Stress Response / Wang X, Wood TK. // Applied and Environmental Microbiology . – 2011 . – V.77 . – N16 . – P. 5577-5583 . – doi:10.1128/AEM.05068-11.
55. Zhao, J. Escherichia coli toxin gene hipA affects biofilm formation and DNA release / Zhao J, Wang Q, Li M, Heijstra BD, Wang S, Liang Q, Qi Q. // Microbiology . – 2013 . – V.159 . – Pt 3 . – P. 633-40 . – doi: 10.1099/mic.0.063784-0.
56. Baker, D.J. Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. / D.J. Baker, B.G. Childs, M. Durik, M.E. Wijers, C.J. Sieben, J. Zhong, A. R. Saltness, K.B. Jeganathan, G.C. Verzosa, A. Pezeshki, K. Khazaie, Miller JD, van Deursen JM.// Nature. – 2016 . – Feb 3. - [Epub ahead of print] . – doi: 10.1038/nature16932
57. Perkins, H. R. The bacterial autolysins, p. 437–456. – [In H. J. Rogers Microbial cell walls and membranes: by H.J. Rogers, H.R. Perkins and J B Ward] . - Chapman & Hall, London. - 1980 . - p 564.. – ISBN 0-412-12030-5
58. Heidrich, C. Involvement of N-acetylmuramyl-l-alanine amidases in cell separation and antibiotic-induced autolysis of Escherichia coli. / C.Heidrich, M. F. Templin, A. Ursinus, M. Merdanovic, J. Berger, H. Schwarz, M. A. de Pedro, J.-V. Holtje // Mol. Microbiol . – 2001. – V.41. – P. 167-178.
59. Groicher, K. H. The *Staphylococcus aureus lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. / K.H. Groicher, B. A. Firek, D. F. Fujimoto, K.W. Bayles, // JBacteriol. – 2000 . –V.182. –P. 1794–1801.
60. Ju, C.X. Characterization and Functional Analysis of atl, a Novel Gene Encoding Autolysin inStreptococcus suis. / Cun-Xiang Ju, Hong-Wei Gu, Cheng-Ping Lu // J Bacteriol . – 2012. – V 194. – N 6. – P. 1464–1473. – doi: 10.1128/JB.06231-11
61. Allignet, J. Staphylococcus caprae strains carry determinants known to be involved in pathogenicity: a Gene Encoding an Autolysin-Binding Fibronectin and the ica Operon Involved in Biofilm Formation. / Jeanine Allignet, Sylvie Aubert, Keith G. H. Dyke, Nevine El Solh //Infect Immun.– 2001. –V.69. – N 2. – P. 712–718. – doi: 10.1128/IAI.69.2.712-718.2001
62. Hussain, M. The adhesive properties of the Staphylococcus lugdunensis multifunctional autolysin AtlL and its role in biofilm formation and internalization. / M. Hussain,T. Steinbacher, G. Peters , C. Heilmann, K. Becker // Int J Med Microbiol. – 2015. – V.305. – N 1. – P. 129-139 . – doi: 10.1016/j.ijmm.2014.11.010.
63. Paganelli, F.L. Enterococcus faecium Biofilm Formation: Identification of Major Autolysin AtlAEfm, Associated Acm Surface Localization, and AtlAEfm-Independent Extracellular DNA Release. / Fernanda L. Paganelli, Rob J. L. Willems, Pamela Jansen, AntoniHendrickx, Xinglin Zhang, Marc J. M. Bonten, Helen L. Leavis // mBio. – 2013. – V.4. – N 2 . – P. e00154-13. – doi: 10.1128/mBio.00154-13
64. Galperin, M.Y. Bacterial signal transduction network in a genomic perspective. // Environ Microbiol.– 2004. – V 6. –N 6. –P. 552-567.
65. Schaller , G.E. Two-Component Systems and Their Co-Option for Eukaryotic Signal Transduction. / G. Eric Schaller, Shin-Han Shiu, Judith P. Armitage //Current Biology . –2011 . – V.21. – P.320–330. – DOI 10.1016/j.cub.2011.02.045
66. Thomas, V.C. Suicide and fratricide in bacterial biofilms. / Thomas VC, Hancock LE. // Int J ArtifOrgans. –2009. –V.32. –N 9. – P. 537-544.
67. Groicher, K.H. The Staphylococcus aureus lrgAB operon modulates murein hydrolase activity and penicillin tolerance. / K.H. Groicher, B.A. Firek, D.F. Fujimoto, K.W.Bayles // J Bacteriol.– 2000. –V.182. – N 7. – P. 1794-801.
68. Rice, K.C. The cidA murein hydrolase regulator contributes to DNA release and biofilm development in Staphylococcus aureus. / K.C. Rice, E.E. Mann, J.L. Endres, E.C. Weiss, J.E. Cassat, M.S. Smeltzer, K.W. Bayles // ProcNatlAcadSci U S A. –2007. –V.104. – P.8113–8118.
69. Ranjit, D.K. Staphylococcus aureus CidA and LrgA proteins exhibit holin-like properties. / D.K. Ranjit, J.L. Endres, K.W. Bayles // J Bacteriol.– 2011. –V.193. – P. 2468–2476.
70. Mann EE Modulation of eDNA release and degradation affects Staphylococcus aureus biofilm maturation. / E.E. Mann, K.C. Rice, B.R. Boles, J.L. Endres, D. Ranjit, L. Chandramohan, L.H. Tsang, M.S. Smeltzer, A.R. Horswill, K.W. Bayles // PLoS One. – 2009. –V.4. – P. e5822.
71. Patton, T.G. The role of proton motive force in expression of the Staphylococcus aureus cid and lrg operons. / T.G. Patton, S.J. Yang, K.W. Bayles // MolMicrobiol. – 2006. – V.59. – P.1395–1404.
72. Rice, K.C. Acetic acid induces expression of the Staphylococcus aureus cidABC and lrgAB murein hydrolase regulator operons. / K.C. Rice, J.B. Nelson, T.G. Patton, S.J. Yang, K.W. Bayles // J Bacteriol. – 2005. – V.187 . – P. 813–821.
73. Rice, K.C. Transcription of the Staphylococcus aureus cid and lrg murein hydrolase regulators is affected by sigma factor B. / K.C. Rice, T. Patton, S.J. Yang, A. Dumoulin, M. Bischoff, K.W. Bayles // J Bacteriol. – 2004. –V.186. – N 10. – P. 3029-3037.
74. Chen, C. Secreted proteases control autolysin-mediated biofilm growth of Staphylococcus aureus. / C. Chen, V. Krishnan, K. Macon, K. Manne, S.V. Narayana, O. Schneewind // J BiolChem.– 2013 . – V.288. – N 41 . – P. 29440-29452 .– doi: 10.1074/jbc.M113.502039.
75. Thomas, V.C. A fratricidal mechanism is responsible for eDNA release and contributes to biofilm development of Enterococcus faecalis. / VinaiChittezham Thomas, YasuakiHiromasa, Nathan Harms, Lance Thurlow, John Tomich, Lynn Hancock // MolMicrobiol. – 2009 . – V.72. – N 4. – P. 1022–1036 . – doi:10.1111/j.1365-2958.2009.06703.x.

76. Claverys, J.P. Competence-induced fratricide in streptococci. / J.P.Claverys, B.Martin, L.S.Håvarstein //MolMicrobiol. –2007. – V.64. – P. 1423–1433.
77. Kari, H.B. Properties and Biological Role of Streptococcal Fratricins. / Helene Berg Kari, Truls Johan Biørnstad, Ola Johnsborg, andLeivSigve // Appl Environ Microbiol. –2012. – V.78 . – N10 . – P. 3515–3522. – doi: 10.1128/AEM.00098-12
78. Johnston, C. Streptococcus pneumoniae, le transformiste. / C. Johnston, N. Campo, M.J. Bergé, P. Polard, J.P.Claverys // Trends Microbiol. . – 2014 . – V.22 . – N3 . – P. 113-119 . –doi: 10.1016/j.tim.2014.01.002.
79. Claverys, J.P. The genetic transformation machinery: composition, localization, and mechanism. / J.P. Claverys ,B. Martin, P.Polard// FEMS Microbiol Rev. – 2009 . – V.33. – N3. – P.643-56. – doi: 10.1111/j.1574-6976.2009.00164.x.
80. Berg, K.Properties and biological role of streptococcal fratricins. / Kari Helene Berg, Truls Johan Biørnstad, Ola Johnsborg, andLeivSigve // Appl Environ Microbiol. . – 2012 . – V.78. – N 10. – P.3515–3522. – doi: 10.1128/AEM.00098-12
81. Berg, K.H. LytF, a novel competence-regulated murein hydrolase in the genus Streptococcus. / K.H. Berg, H.S. Ohnstad, LS.Håvarstein // J Bacteriol. – 2012 . – V.194 . – N3. – P.627-635 . –doi: 10.1128/JB.06273-11.
82. Li, Y.H. Natural genetic transformation of Streptococcus mutans growing in biofilms. / Li YH, Lau PC, Lee JH, Ellen RP, Cvitkovitch DG. //J Bacteriol. . – 2001 . –V.183 . – P.897–908.
83. Martin, B. Expression and maintenance of ComD-ComE, the two-component signal-transduction system that controls competence of Streptococcus pneumoniae. / B.Martin, C. Granadel, N.Campo, V. Hénard, M. Prudhomme, J.P. Claverys//MolMicrobiol. – 2010 . – V.75 . – N6 . – P.1513-1528 . – doi: 10.1111/j.1365-2958.2010.07071.x.
84. Zhu, L.Deletion analysis of Streptococcus pneumoniae late competence genes distinguishes virulence determinants that are dependent or independent of competence induction. / L.Zhu, J.Lin, Z.Kuang, J.E.Vidal, G.W.Lau //Molecular Microbiology . – 2015 . – V.97 . – N1 . –P.151–165 . – doi: 10.1111/mmi.13016.
85. Morrison, D.A.Regulation of competence for genetic transformation in Streptococcus pneumoniae: a link between quorum sensing and DNA processing genes. / D.A.Morrison, M.S.Lee//Res Microbiol. . – 2000 . – V.151 . – N6 . – P. 445-51.
86. Claverys, J.P.Induction of competence regulons as a general response to stress in gram-positive bacteria. / J.P.Claverys, M.Prudhomme, B.Martin // Annu Rev Microbiol . – 2006 . – V.60 . – P. 451-75.
87. Da Re, S. Resistance acquisition via the bacterial SOS response: the inducive role of antibiotics. / S.Da Re, M.C.Ploy // Med Sci (Paris). – 2012 . – V.28. – N2. – P. 179-84. – doi: 10.1051/medsci/2012282016.
88. Claverys, J-P.Adaptation to the environment: Streptococcus pneumoniae, a paradigm for recombination-mediated genetic plasticity? / J-P Claverys, M Prudhomme, I.Mortier-Barriere, B. Martin //MolMicrobiol. – 2000 . – V.35 . – P.251–259.
89. Trappetti, C.LuxS Mediates Iron-Dependent Biofilm Formation, Competence, and Fratricide in Streptococcus pneumoniae . / Trappetti C, Potter AJ, Paton AW, Oggioni MR, Paton JC.Camilli A // Infection and Immunity. . – 2011 . – V.79 . – N11 . – P. 4550-4558 . – doi:10.1128/IAI.05644-11.
90. Kreth, J. Co-ordinated bacteriocin production and competence development: a possible mechanism for taking up DNA from neighbouring species. / Kreth J, Merritt J, Shi W, Qi F.//MolMicrobiol. . – 2005 . –V57 . – P.392–404.
91. Senadheera, D.B.Regulation of bacteriocin production and cell death by the VicRK signaling system in Streptococcus mutans. / D.B.Senadheera, M.Cordova, E.A.Ayala, L.E.Chávez de Paz, K.Singh, J.S.Downey, G.Svensäter, S.D.Goodman, D.G.Cvitkovitch //J Bacteriol. . – 2012 . – V.194 . – N 6 . – P.1307-1316 . – doi: 10.1128/JB.06071-11.
92. Perry, J.A.Peptide alarmonesignalling triggers an auto-active bacteriocin necessary for genetic competence. / Perry JA, Jones MB, Peterson SN, Cvitkovitch DG, Lévesque CM.//Molecular microbiology . – 2009 . –V.72 . – N4 . – P. 905-917 .– doi:10.1111/j.1365-2958.2009.06693.x.
93. Perry, J.A. Involvement of Streptococcus mutans regulator RR11 in oxidative stress response during biofilm growth and in the development of genetic competence. / J.A.Perry, C.M.Lévesque, P.Suntharaligam, R.W.Mair, M.Bu, R.T.Cline, S.N.Peterson, D.G.Cvitkovitch // LettApplMicrobiol. – 2008 . – V.47 . – N5 . – P.439-444 . – doi: 10.1111/j.1472-765X.2008.02455.x.
94. Perry, J.A. Peptide alarmone signaling triggers an auto-active bacteriocin necessary for genetic competence. / J.A. Perry, M.B. Jones, S.N. Peterson, D.G.Cvitkovitch, C.M.Levesque//Mol. Microbiol. – 2009 . – V.72 . – P.905–917.
95. Perry, J.A. Cell death in Streptococcus mutans biofilms: a link between CSP and extracellular DNA. / J.A.Perry, D.G.Cvitkovitch, C.M.Lévesque // FEMS MicrobiolLett. . –2009 . – V.299 . – N2 . – P.261-266 . – doi: 10.1111/j.1574-6968.2009.01758.x.
96. Barnes, A. M. Enterococcus faecalis Produces Abundant Extracellular Structures Containing DNA in the Absence of Cell Lysis during Early Biofilm Formation. / A.M.T. Barnes, K. S. Ballering, R.S.Leibman, C.L.Wells, G. M. Dunny //mBio. – 2012 . – V.3. – N 4. – P.e00193–e00212 . – doi:10.1128/mBio.00193-12
97. Zweig, M. Secreted single-stranded DNA is involved in the initial phase of biofilm formation by Neisseria gonorrhoeae. / M. Zweig, S. Schork, A.Koerdt, K.Siewering, C.Sternberg, K.Thormann, S.V. Albers, S. Molin, C. van der Does // Environ Microbiol. –2014 . – V.16. – N 4. – P.1040-1052 . – doi: 10.1111/1462-2920.12291.
98. Liao, S. Streptococcus mutans extracellular DNA is upregulated during growth in biofilms, actively released via membrane vesicles, and influenced by components of the protein secretion machinery. / S. Liao, M.I. Klein, K.P. Heim, Y. Fan, J.P. Bitoun, S.J. Ahn, R.A. Burne, H.

Koo, L.J. Brady, Z.T.Wen // J Bacteriol. – 2014. – V.196. – N 13. – P. 2355-2366. – doi: 10.1128/JB.01493-14.
99. Jack, A.A. Streptococcus gordoniicomCDE (competence) operon modulates biofilm formation with Candida albicans. / A.A. Jack, D.E. Daniels, M.A. Jepson, M.M. Vickerman, R.J. Lamont, H.F. Jenkinson, A.H. Nobbs // Microbiology. – 2015. – V.161. – Pt2 . – P.411-421 . – doi: 10.1099/mic.0.000010. Epub 2014
100. Bhatty, M. Enterococcus faecalis pCF10-encoded surface proteins PrgA, PrgB (aggregation substance) and PrgC contribute to plasmid transfer, biofilm formation and virulence. / M. Bhatty, M.R. Cruz, K.L. Frank, J.A. Gomez, F. Andrade, D.A. Garsin, G.M. Dunny, H.B. Kaplan, P.J.Christie // MolMicrobiol. – 2015 . – V.95. – N 4. – P.660-677 . –doi: 10.1111/mmi.12893.
101. Meibom, K.L. Chitin Induces Natural Competence in Vibrio cholera. / Karin L. Meibom, Melanie Blokesch, Nadia A. Dolganov, Cheng-Yen Wu, Gary K. Schoolnik // Science . – 2005 . – V. 310 . – N 5755 . – P. 1824-1827 . – DOI: 10.1126/science.1120096
102. Langereis, J.D. Novel concepts in nontypeableHaemophilusinfluenzae biofilm formation. / J.D. Langereis, P.W. Hermans //FEMS MicrobiolLett. – 2013. – V.346 . – N 2 . – P.81-89 . – doi: 10.1111/1574-6968.12203.
103. Hu, W. DNA builds and strengthens the extracellular matrix in Myxococcusxanthus biofilms by interacting with exopolysaccharides. / W. Hu, L. Li, S. Sharma, J. Wang, I. McHardy, R. Lux, Z. Yang, X. He, J.K. Gimzewski, Y. Li, W. Shi // PLoSOne. – 2012 . – V. 7 . – N12 . – P.e51905 .– doi: 10.1371/journal.pone.0051905.
104. Kaplan, J.B. Low levels of β-lactam antibiotics induce extracellular DNA release and biofilm formation in Staphylococcus aureus. / J.B. Kaplan, E.A. Izano, P. Gopal, M.T. Karwacki, S. Kim, J.L. Bose, K.W. Bayles, A.R.Horswill // MBio. – 2012 . – V.3. – N 4 . –P. e00198-e00212. – doi: 10.1128/mBio.00198-12.
105. Odenholt, I. Pharmacodynamiceffects of subinhibitory antibiotic concentrations. / I. Odenholt // Int. J. Antimicrob. Agents. – 2001 . – V.17. – P. 1–8.
106.Das, T. Pyocyanin Promotes Extracellular DNA Release in*Pseudomonas aeruginosa*. / T.Das, M.Manefield // PLoSOne.– 2012. – V 7. – N 10. – P.e46718. – doi: 10.1371/journal.pone.0046718
107. Das, T. Phenazine production enhances extracellular DNA release via hydrogen peroxide generation in Pseudomonas aeruginosa. / T.Das, M.Manefield // CommunIntegrBiol. – 2013 . – V6 . – N3 . – P. e23570 . – doi: 10.4161/cib.23570

EXTRACELLULAR DNA IN BACTERIAL BIOFILMS. PART I: ORIGIN

Krestetska S.L.

Significant number of chronic bacterial infections involves the biofilm formation, but regulation of this process is still far from being well understood. Some progress has been achieved since the reassessment of extracellular DNA (eDNA) functions in biofilm establishment and remodeling, including influence of this natural polymeric substance on mechanical stability and adhesiveness of extracellular polymeric matrix (EPM). As was shown eDNA can appear in EPM at different stages of biofilm development via different ways, including active secretion or assimilation from surrounding milieu, but the main source is widely considered to be induced cell death with subsequent lysis. Cell death induction as a kind of social behavior in prokaryotes seems to represents an essential part of the developmental program, clearly associated with a switch to a sessile community lifestyle and biofilm formation per se. Review is focused on mechanisms allowing controlled eDNA release, mainly on those underlying self- or hetero-destructive behavior in bacterial populations.

Keywords: bacterial biofilms, extracellular DNA, review