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PREGLEDNI RAD
REVIEW

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Međustanična komunikacija parodontopatogenih bakterija

Quorum Sensing of Periodontal Pathogens

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Sažetak
Pojam *quorum sensing* označava međustaničnu komunikaciju bakterija kojom koordiniraju gensku ekspresiju populacije sukladno njezinoj gustoći. Bakterije proizvode i ispuštaju u međustanični prostor male molekule, tzv. autoinduktore, čija koncentracija raste s povećanjem populacije. Postizanje koncentracije stimulatornog praga rezultira promjenom u genskoj ekspresiji. Gram-pozitivne i gram-negativne bakterije imaju različite *quorum sensing* sustave. Najbolje istražen,kanonski sustav jest LuxI/R-tip/acil homoserin laktonom posredovan *quorum sensing* sustavom gram-negativnih bakterija koje ga koriste uglavnom za komunikaciju unutar svoje vrste. Gram-pozitivne bakterije posjeduju *quorum sensing* sustav posredovan peptidima. Bakterije mogu komunicirati unutar svoje vrste, ali i s bakterijama drugih vrsta, što im omogućuje autoinduktor-2 *quorum sensing* sustav koji se zato i naziva univerzalnim bakterijskim jezikom. Parodontopatogene bakterije imaju AI-2 *quorum sensing* sustav. Poznato je da njime kontroliraju formaciju biofilmova, unos željeza, odgovor na stres i ekspresiju pojedinih faktora virulencije. Bolje razumijevanje mehanizama bakterijske komunikacije omogućit će utjecanje na *quorum sensing* inhibitorima u svrhu sprječavanja i kontrole bolesti.

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Uvod

Do prije nekoliko desetljeća i revolucionarnih otkrića u području molekularne mikrobiologije, bakterije su smatrane samodostatnim jednostaničnim organizmima (1). Sredinom 20. stoljeća nekoliko pionirskih istraživanja provedenih na morskim bakterijama pokazalo je da bakterije zapravo preferiraju međusobnu blizinu te tako broj bakterijskih stanica koje adheriraju na neku čvrstu površinu znatno premašuje broj planktonskih bakterijskih stanica u okružujućem tekućem mediju (2,3). Poslije je potvrđeno, a danas itekako poznato, da bakterije zapravo preferiraju život u složenim zajednicama vezanima za površine kojima je poslije nadjenut naziv *biofilm* (4).

Biofilm se može definirati kao agregacija jedne ili više različitih skupina mikroorganizama, uloženih u matriks koji sami proizvode i pričvršćen na neku čvrstu površinu. Biofilmovi su sveprisutni. Bakterije ih mogu formirati na najrazličitijim površinama – živima i neživima, u vlažnom okolišu, na medicinskoj opremi i živom tkivu, ali i u najekstremnijim prirodnim uvjetima. Recentna istraživanja pokazala su da su bakterijski biofilmovi pronađeni i u uvjetima ekstremne hladnoće antarktičkih mora, u termalnim vodama u temperaturnom rasponu od 35 do 50 stupnjeva Celzijevih i u uvjetima iznimne kiselosti, s visokim udjelima metala i manjkom nutrijenata (5 – 7).

Introduction

Until several decades ago and groundbreaking discoveries in the field of molecular microbiology, bacteria were believed to be independently functioning single-cell organisms, living a self-sufficient lifestyle (1). In the mid-20th century several pioneer studies conducted on marine bacteria showed that bacterial cells actually favor living in close proximity to such an extent that the number of the organisms adhering to a firm surface greatly surpasses the number of the free swimming, planktonic organisms in the surrounding liquid media (2, 3). It was further confirmed and is now a well-known fact that bacteria prefer living in complex surface-associated communities which were later named biofilms (4).

Biofilm can be defined as an aggregation of one or more groups of different microorganisms, embedded in a self-produced matrix and adhering to a firm surface. Biofilms are ubiquitous. Bacteria can form them on the greatest variety of surfaces, living or non-living, in humid natural conditions, on medical equipment and living tissue, but also in the most extreme living conditions. Recent studies have described bacterial biofilms found in the extreme subzero temperatures of the Antarctic seawaters, thermal waters ranging from 35 to 50 degrees Celsius and conditions of extreme acidity, high metal content and lack of nutrients (5-7).

Bakterijske kolonije čine tek 15 do 20 posto sveukupnog volumena biofilma, a ostatak tvori matriks u kojem su kolonije uložene, mješavina različitih prirodnih polimera, primarno polisaharida, ali i proteina, glikoproteina, glikolipida te također nukleinskih kiselina (8). Okružujući matriks ima itekako važnu ulogu u životu bakterijskih zajednica koje ga nastanjuju. On je svojevrsno utočište za kolonije i zaštita od katkad iznimnih promjena vanjskih okolišnih čimbenika, zbog čega je lakše razumjeti zašto bakterije preferiraju život u biofilmu, a ne u formi solitarnoga planktonskog organizma (9, 10).

Biofilmove može tvoriti populacija jedne vrste bakterija ili, što je uobičajenije, oni su zajednica sastavljena od mnoštva različitih međuovisnih bakterijskih vrsta. Vrijedno je spomenuti da čak i u biofilmovima koje tvori jedna bakterijska vrsta postoji fenotipska genetička heterogenost kao posljedica odgovora pojedinih bakterijskih stanica na lokalne mikrookolišne čimbenike (11). Biofilm dentalnoga plaka i onaj povezan s parodontnim bolestima među najbolje su opisanim biofilmovima sastavljenima od više bakterijskih vrsta. Kako ih bakterije formiraju na površinama koje se ne ljušte (zubi, fiksni protetski radovi), omogućen je nastanak doista stabilnih i kompleksnih bakterijskih zajednica.

Imajući na umu sve te informacije, postavlja se neizbježno pitanje: kojim se mehanizmima mogu koordinirati ponašanja ovakvih zajednica?

Kratka povijest *quorum sensing*a

Quorum sensing kao mehanizam bakterijske komunikacije prvi je put opisan prije nešto više od 30 godina kod morske bakterije *Aliivibrio fischeri* (12). Do tog se otkrića socijalna interakcija i suradnja smatrala odlikom viših razvijenih organizama. Simbiotski odnos bakterije *Aliivibrio fischeri* i havajske kratkorepe lignje – *Euprymna scolopes*, bio je ključno otkriće za razumijevanje kako funkcionira bakterijska komunikacija (13). Havajska kratkorepa lignja noćni je lovac koji, kako joj i ime sugerira, živi u havajskim plitkim priobalnim vodama. Njezin svjetlosni organ nastanjuju bioluminiscentne bakterije *Aliivibrio fischeri* koje stvaraju svjetlost tijekom noći i lignjinih aktivnih sati i tako kamufliraju svojeg domaćina. Naime, svjetlost mjeseca i zvijezda uobičajeno bi ocrtavala obris lignje i time je otkrivala grabežljivcima ispod nje. Svjetlost emitirana iz lignjina svjetlosnog organa koji se nalazi na njezinu donjem dijelu tijela, oponaša vanjsku noćnu svjetlost pa lignja nije uočljiva zato što ne stvara sjenu niti joj je vidljiva silueta. Zauzvrat se bakterije u lignjinu svjetlosnom organu hrane dostupnim šećerima i aminokiselinama. *Aliivibrio fischeri* inače je planktonska bakterija čija je koncentracija u morima gotovo nemjerljiva. Lignjina cirkadijalna pumpa ubacuje i izbacuje morsku vodu s bakterijama iz svjetlosnog organa i u njega. Svako jutro u zoru lignja izbaci oko 95 posto populacije vibrija koji su nastanjivali svjetlosni organ. Broj bakterijskih stanica danju u organu raste, a kada je navečer bakterijska populacija obnovljena, moguća je proizvodnja svjetlosti (12). Neobičan, ali ritmičan proces uključivanja i isključivanja svjetla potaknuo je pitanje kako bakterije znaju kada moraju *pritisnuti* prekidač.

Bacterial colonies make up approximately 15-20% of the biofilm volume while the rest is an EPS matrix in which the colonies are embedded, a mixture of different natural polymers, primarily polysaccharides, but also a variety of proteins, glycoproteins, glycolipids, and also nucleic acids (8). The surrounding matrix has many important roles in the lives of its inhabitants. It represents a sort of a safe haven for the bacterial colonies, acting as a ward against sometimes extreme changes of environmental conditions, making it easier to understand why bacteria prefer life in biofilms than that in the form of planktonic organisms (9, 10).

Biofilms can be formed from a single-species bacterial community or, which is more typical, represent a community derived from several different microbial species living an interdependent lifestyle. A fact worth mentioning is that even in the mono-species biofilms, phenotypic heterogeneity exists as a response of the individual bacterial cells to local microenvironment conditions (11). Dental plaque biofilm and biofilm in periodontal diseases are among the best described multi-species biofilms. What characterizes these biofilms is their formation on non-shedding surfaces (teeth, fixed prosthodontic appliances) which subsequently enables the formation of stable and complex bacterial communities.

Bearing all of the presented information on bacterial biofilm communities in mind, an inevitable question arises; how do these communities coordinate their behavior?

A glimpse in the history of quorum sensing

Quorum sensing as a form of bacterial communication was first described a little over 30 years ago in a bioluminescent marine bacteria, *Aliivibrio (Vibrio) fisheri* (12). Until then social cooperation was thought to be a distinctive feature of 'higher', developed organisms. Symbiotic relationship between *Aliivibrio fischeri* and Hawaiian bobtail squid, *Euprymna scolopes*, has been studied thoroughly and it was the key finding for understanding how bacterial communication works (13). Hawaiian bobtail squid is a nocturnal hunter living in, as its name suggests, clear Hawaiian shallow coastal waters. Its light organ houses bioluminescent bacteria, *Aliivibrio fischeri*, producing the right amount of light during the night, squid's active hours thus camouflaging their host. Namely, the light from the moon and the stars would normally make the silhouette of the squid stand out to predators below. By emitting a glow from its underside, the squid mimics ambient night light and virtually has no silhouette and creates no shadow. In turn, the bacteria in the squid's light organ feed on sugar and aminoacids. *Aliivibrio fischeri* is a planktonic bacterium and its quantities are virtually undetectable in ocean waters. The squid's circadial pump pumps in and out the water containing *Aliivibrio fischeri*. Every morning at dawn, the squid disposes of up to 95% of *Aliivibrio fischeri* from the light organ. Concentration of the bacterial population in the light organ increases during the day and by nighttime, and when the critical bacterial population is restored, the production of light is enabled (12). The unusual yet rhythmic process of turning the light on and off left researchers asking how bacteria know when it is the right time to start producing light and shutting it off.

Quorum sensing

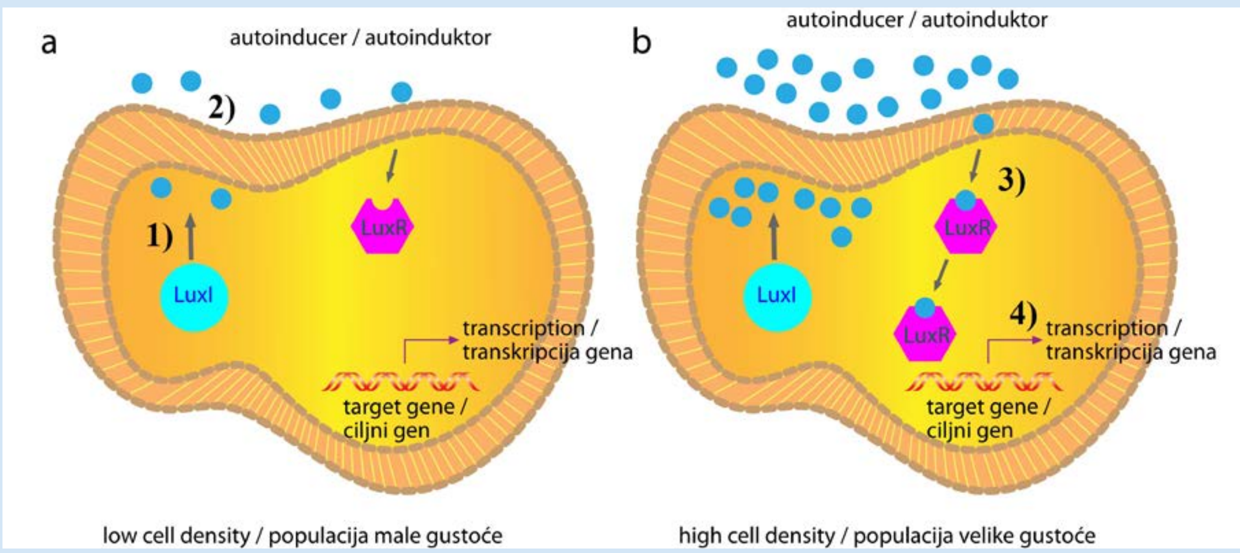
Kvorum je, prema definiciji, minimalan broj prisutnih članova neke skupine potrebnih da se prihvati određena odluka. Bakterijski kvorum podrazumijeva minimalnu, kritičnu koncentraciju bakterijskih stanica u populaciji potrebnih da se dogodi zajednička genska ekspresija i koordinirani odgovor na promjene u njihovu okolišu. *Quorum sensing* tako se može definirati kao regulacija genske ekspresije sukladna gustoći bakterijske populacije.

Bakterije proizvode i ispuštaju kemijske signalne molekule, tzv. autoinduktore. Izvanstanična koncentracija tih molekula raste kao funkcija gustoće bakterijskih stanica. Kada koncentracija tih signalnih molekula dosegne minimalni stimulatorni prag, bakterije usklađeno odgovaraju genskom ekspresijom i mijenjaju svoje ponašanje. *Quorum sensing* radi lakšeg razumijevanja možemo podijeliti u četiri koraka: 1) sinteza malih biokemijskih signalnih molekula unutar stanice, 2) otpuštanje signalnih molekula iz bakterijske stanice, bilo aktivnim transportom ili pasivno, 3) prepoznavanje signalnih molekula specifičnim receptorima, 4) promjene u genskoj regulaciji i transkripcija gena (slika 1.) (13 – 15).

Quorum sensing

Quorum, by its definition, is a minimum number of members of a certain assembly required to be present in order to make a certain decision. Bacterial quorum implies a minimum, critical concentration of bacterial cells in a population which leads to common, coordinated gene expression and coordinated response to changes in their environment. Quorum sensing can thus be defined as a regulation of gene expression in accordance with cell-population density.

Bacteria produce and secrete chemical signaling molecule, autoinducers. Extracellular concentration of these molecules increases as a function of bacterial cell density. When bacteria detect the minimal threshold stimulatory concentration of a certain autoinducer they act accordingly and alter their behavior in response to it. Quorum sensing can be divided into 4 steps: 1) intracellular synthesis of the signal molecules, 2) secretion of the molecules, either actively or passively, 3) detection of the signaling molecule and its binding to an inducer and 4) gene transcription activation (Figure 1) (13-15).



Slika 1. Mehanizam *quorum sensing* sustava – od sinteze signalne molekule, do transkripcije gena.
Figure 1 Quorum sensing mechanism – from signaling molecule production to gene transcription.

Quorum sensing sustavi

Quorum sensing sustav u gram-negativnim bakterijama

Quorum sensing sustav opisan u vrsti *Aliivibrio fischeri* najuobičajeniji je sustav i dosad je opisan u više od 70 bakterijskih vrsta. Dva proteina – LuxI (autoinduktor sintaza koja proizvodi acil-homoserin laktonski autoinduktor) i LuxR (autoinduktorski receptor koji je transkripcijski aktivator) nadziru gensku ekspresiju *Aliivibrio fischeri* (13). Ovaj tip sustava, reguliran Lux I/R homolozima i AHL autoinduktorskom molekulom, opisan je kod različitih bakterijskih vrsta koje ga najčešće upotrebljavaju za komunikaciju unutar iste vrste (15).

Quorum sensing systems

Quorum sensing in Gram-negative bacteria

The type of quorum sensing system as described in *Aliivibrio fischeri* is the most common quorum sensing system and has so far been observed in more than 70 bacterial species. Two proteins, LuxI (autoinducer synthase which produces acyl-homoserine lactone autoinducer) and LuxR (autoinducer receptor, which is the DNA-binding transcriptional activator) control the gene expression in *Aliivibrio fischeri* (13). This type of system, regulated by LuxI/R homologes described for different species and acyl homoserine lactone (AHL) as an autoinducer molecule, is mainly used for intra-species communication (16).

Quorum sensing u gram-pozitivnim bakterijama

Za razliku od gram-negativnih bakterija, gram-pozitivne bakterije služe se oligopeptidnim molekulama u ulozi autoinduktora, a koje se vežu za dvokomponentne membranski vezane receptorne histidin kinaze (14).

Autoinduktor -2 (AI-2) *quorum sensing* sustav

Gram-negativne i gram-pozitivne bakterije posjeduju *quorum sensing* sustave koji im omogućuju komunikaciju unutar svoje vrste, no život u kompleksnom biofilmu i heterogenoj bakterijskoj zajednici zahtijeva komunikaciju i posljedično koordinaciju ponašanja na zajedničkom jeziku. Autoinduktor-2 univerzalna je autoinduktorska molekula koja omogućuje komunikaciju između različitih bakterijskih vrsta, a prvi je put otkrivena i opisana u ulozi autoinduktora drugog *quorum sensing* sustava koji posjeduje morska bakterija *Vibrio harveyi* (13, 14). Gen *luxS Vibrio harveyi* kodira enzim sintazu koji proizvodi autoinduktor-2, a poslije su geni sličnih sekvencija također identificirani u drugim gram-negativnim i gram-pozitivnim bakterijama (17, 18).

Međustanična komunikacija i parodontni patogeni

Iako su istraživanja, provedena u svrhu otkrivanja tipa *quorum sensing* sustava i *quorum sensing*om regulirane genske ekspresije, uglavnom uključivala *Aggregatibacter actinomycetemcomitans* i *Porphyromonas gingivalis*, u nekoliko su radova identificirani geni koji pokazuju homolognost s *luxS* genom i kod drugih parodontnih patogena, *Fusobacterium nucleatum*, *Prevotella intermedia* i *Eikenella corrodens* (19, 20). Parodontni patogeni, kao i neki drugi istraživani oralni streptokoki, posjeduju dakle autoinduktor-2 *quorum sensing* sustav koji im omogućuje komunikaciju s drugim vrstama u kompleksnim bakterijskim zajednicama oralnoga biofilma (19, 21, 22). *Quorum sensing* sustav posredovan acil homoserin laktonskim autoinduktorom, sličan onome koji posjeduje *Aliivibrio fischeri,* nije dokazan u parodontnim patogenima (19).

Iako etiologija bakterija u parodontnim bolestima nije upitna, početak i napredak bolesti danas se povezuje s promjenama u profilu bakterijske populacije biofilma (eng. *population shift).* Parodontni patogeni povećano su prisutni u uzorcima uzetima s bolesnih mjesta, a inače čine tek neznatni dio u populaciji na zdravim mjestima (23, 24). Razvoj izrazite složenosti i promjene bakterijske populacije rezultat je komunikacije bakterija unutar svoje vrste i izvan nje.

Uloga međustanične komunikacije u razvoju biofilmova

Uloga *quorum sensing*a u razvoju biofilma najprije se proučavala na organizmima koji ne nastanjuju usnu šupljinu, bakterijskim zajednicama *Pseudomonas aeruginosa* i složenim zajednicama koje čine *Pseudomonas aeruginosa* i bakterije vrste *Burkholderia* (25). Komunikacija između tih dviju bakterija posredovana je acil homoserin laktonskim (AHL) *quorum sensing* sustavom koji im omogućuje koordiniranu ekspresiju faktora virulencije i formaciju biofilma (26, 27). Sposobnost *Aggregatibacter actinomycetemcomitans* da raste u biofilmu usko je vezana za autoinduktor-2 *quorum sensing*

Quorum sensing in Gram-positive bacteria

Unlike Gram-negative bacteria, Gram-positive bacteria use modified oligopeptides in the role of autoinducers which are detected by two-component membrane bound histidine kinase receptors (14).

Autoinducer-2 (AI-2) quorum sensing

While both Gram-negative and Gram-positive bacteria possess a certain quorum sensing system which enables them to communicate within their own species, to live in a complex biofilm, in a heterogenic community and it requires communication and behavior coordination in a common language. Autoinducer-2 represents a universal autoinducer molecule that enables inter-species communication and was first discovered as the autoinducer of the second quorum sensing system in the marine bacteria *Vibrio harveyi* (13, 14). Gene luxS of *Vibrio harveyi* was identified as the encoder of the synthase enzyme that produces autoinducer-2 while, later on, the genes exhibiting sequence similarity were also found in other Gram-Negative and Gram-Positive bacteria (17, 18).

Quorum sensing and the periodontal pathogens

Although studies conducted to characterize quorum sensing systems and quorum sensing coordinated gene expression in periodontal pathogens have mostly been limited to *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, several reports also identified luxS homologous genes in other pathogens, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Eikenella corrodens* (19, 20). Periodontal pathogens, as well as some other studied oral streptococci species, possess the AI-2 quorum sensing circuit, which enables them inter-species communication in complex bacterial communities of the oral biofilm (19, 21, 22). Acyl homoserine lactone (AHL)-mediated quorum sensing system, similar to the one of *Aliivibrio fischeri*, has not been found in periodontal pathogens so far (19).

Although microbial etiology in periodontitis is indisputable, disease onset and progression is now associated with population shift within the microbial biofilm community. Periodontal pathogens are overrepresented in samples taken from diseased places, whereas they represent a significantly small portion of the total species in healthy sites (23, 24). The increased complexity and shifts in the microbial community are the result of species association and intra- and inter-species communication.

The role of quorum sensing in biofilm development

The role of quorum sensing in biofilm formation was first studied on non-oral organisms, single-species *Pseudomonas aeruginosa* community and also biofilm communities comprised of *Pseudomonas aeruginosa* and *Burkholderia* species (25). The communication between these bacteria is mediated through AHL signaling quorum sensing systems allowing them to coordinate virulence expression and biofilm formation (26, 27). The ability of *Aggregatibacter actinomycetemcomitans* to grow in biofilm is closely linked to AI-2 quorum sensing system. A luxS gene mutant (no AI-2 synthase) can form a mature biofilm, yet the biofilm contains notasustav. Genetski *luxS* mutant (nema autoinduktor-2 sintaze) može formirati zreli biofilm, no on sadržava značajno manju biomasu. Ako se u medij za rast doda egzogeni autoinduktor-2 ili plazmidi koji nose funkcionalni *luxS* gen, omogućeno je normalno formiranje biofilma (28). Identificirana su dva periplazmička receptora za autoinduktor-2 – LsrB i RbsB te je uočeno, ako se inaktivira jedan ili oba, formirani biofilm bit će manji, odnosno njegova će formacija biti potpuno onemogućena (29). Predmet je znanstvene rasprave i daljnjih istraživanja reagira li doista *Aggregatibacter actinomycetemcomitans* na raspon različitih koncentracija autoinduktora-2, s obzirom na to da prije spomenuti periplazmički receptori pokazuju različitu kinetiku interakcije s autoinduktorom. Čini se da RbsB receptor ima veći afinitet za molekule autoinduktora, što doista sugerira da je *Aggregatibacter actinomycetemcomitans* sposoban odgovoriti i na veće i na manje koncentracije autoinduktora i tako napredovati u bakterijskim populacijama veće i manje stanične gustoće (29).

Povezanost *quorum sensinga* i unosa željeza

Željezo ima esencijalnu ulogu u mnoštvu različitih bakterijskih funkcija, s obzirom na to da utječe na staničnu strukturu, intermedijarni metabolizam, sekundarni metabolizam, enzimatsku aktivnost i mnoge druge (30). Autoinduktorom-2 posredovan *quorum sensing* sustav usko je povezan s bakterijskim unosom željeza dokazanim u *Aggregatibacter actinomycetemcomitans* i *Porphyromonas gingivalis*. *Aggregatibacter actinomycetemcomitans* željezo pribavlja preko kelatora (enterobaktin – nalik na siderofore) ili od proteina domaćina (transferina i hemoglobina). U mutantnim sojevima smanjena je ekspresija gena koji kodiraju receptore za hem, transferin i hemoglobin, što smanjuje pribavljanje željeza od proteina domaćina. Istodobno se u tim mutantnim sojevima primjećuje da, premda postoji mutacija *luxS* gena, nema produkcije LuxS proteina (enzima sintaze) i produkcije autoinduktora-2, nastaje pojačana ekspresija gena koji kodiraju proteine poput enterobaktinskih receptora, a omogućuju pribavljanje željeza kelacijom. Može se zaključiti kako u populaciji veće stanične gustoće, u kojoj je koncentracija autoinduktora-2 veća, bakterija željezo pribavlja od izvora domaćina, nasuprot čemu se oslanja na kelatore u stanjima male stanične gustoće i niske koncentracije autoinduktora-2 (31, 32).

Porphyromonas gingivalis željezo pribavlja iz hemina (porfirin koji sadržava željezo), za što se koristi specifičnim receptorima vanjske membrane, lipoproteinima i proteazama (33). Također je kontroliran autoinduktor-2 posredovanim *quorum sensing*om, što uključuje neovisnu regulaciju različitih mehanizama unosa. Autoinduktor-2 može tako pozitivno ili negativno regulirati ekspresiju različitih gena koji kodiraju receptore membrane, ovisno o količini dostupnog hemina. Također je pronađena veza između unosa željeza i virulentnosti bakterije, pri čemu manjak željeza uzrokuje smanjenu virulenciju (34).

Quorum sensing i regulacija drugih funkcija

Regulacija gena uključenih u funkcije vezane za stres *Porphyromonas gingivalis* također je pod kontrolom autoinduk-

bly less biomass. If exogenous autoinducer-2 or a functional luxS gene carrying plasmid is added in the growth medium, normal growth of the biofilm is restored (28). Two periplasmic receptors for autoinducer-2, LsrB and RbsB, were also identified and it was observed that if one or both receptors are inactivated, biofilm growth was either reduced, or completely disabled (29). The fact that *Aggregatibacter actinomycetemcomitans* also responds to a broad range of autoinducer-2 concentration, is a subject of scientific discussion and further research since the aforementioned periplasmic receptors show different kinetics of interaction with the autoinducer. It seems that RbsB interacts with the autoinducer-2 at higher affinity than LsrB, suggesting that *Aggregatibacter actinomycetemcomitans* is able to act upon both lower and higher autoinducer-2 concentrations and thus thrive in biofilms with both low and high cell density (29).

Quorum sensing and bacterial iron-uptake

Iron has an essential role in wide variety of bacterial functions since it influences cell composition, intermediary metabolism, secondary metabolism, enzyme activity and many other functions (30). Autoinducer-2 mediated quorum sensing system is also intimately linked to bacterial iron acquisition which was studied on both *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. The *Aggregatibacter actinomycetemcomitans* can acquire iron from either iron-scavenging chelators (enterobactin-like siderophore) or host cell sources (transferrin and hemoglobin). Autoinducer-2 mediated quorum sensing system has a role in adapting bacteria's iron uptake, depending on the bacterial cell density in the biofilm and autoinducer-2 concentration. Mutant strains exhibit reduced expression of genes coding the receptors for hem, transferrin and hemoglobin, thus reducing iron-uptake from the host cells. Simultaneously, despite the luxS mutation and consequently no production of synthase enzyme and autoinducer-2 respectively, expression of several other genes encoding siderophore receptors is upregulated, inducing a shift towards iron uptake from chelators. This suggests that in a high density cell population, with higher concentration of autoinducer-2, bacteria will obtain iron from host-cell sources, whereas it will turn to iron-scavenging chelators in low autoinducer-2 conditions (31, 32).

Porphyromonas gingivalis obtains iron from hemin (iron-containing porphyrin) by employing specific outer membrane receptors, lipoproteins and proteases (33). It is also controlled by an autoinducer-2 mediated signaling which involves independent regulation of distinct uptake mechanisms. Autoinducer-2 positively or negatively regulates different genes that encode membrane receptors, depending on the available hemin content. There is also a link between iron uptake and virulence in *Porphyromonas gingivalis*, iron starvation resulting in reduced virulence (34).

Quorum sensing and other functions

AI-2 quorum sensing regulation of stress-related genes in *Porphyromonas gingivalis* has also been studied. It was shown tor-2 *quorum sensing*a. Tako LuxS uključen u preživljavanje *Porphyromonas gingivalis* regulacijom odgovara na promjene okolišnih uvjeta domaćina, poput izlaganja povišenoj temperaturi, otpornosti na djelovanje vodikova peroksida i promjenama u pH pojačanim izražavanjem stresnih proteina (35).

Usporedba *luxS* mutanta i soja divljeg tipa *Aggregatibacter actinomycetemcomitans* upozorila je i potvrdila o autoinduktoru-2 ovisnu regulaciju gena koji kodiraju proteine vanjske membrane, enzime, transkripcijske regulatore i komponentne fimbrija. Ekspresija bakterijina najmoćnijeg faktora virulencije – leukotoksina, smanjuje se trostruko nakon inaktivacije *luxS* gena (31).

Quorum sensing inhibitori (gasitelji međustanične komunikacije)

Kako se moderno društvo nalazi na rubu ulaska u eru potpune bakterijske rezistencije na antibiotike, sve je veće zanimanje za razvoj lijekova koji inhibiraju *quorum sensing*. Prekidanjem bakterijske komunikacije pogođeni su kritični koraci u formaciji biofilma i ekspresiji faktora virulencije (36, 37). Ono što se svakako mora uzeti u obzir jest raznolikost *quorum sensing* sustava i strukturna različitost autoinduktorskih molekula. Dosad su otkriveni pojedini prirodni spojevi koji imaju inhibitorno djelovanje te su sintetizirane potencijalne inhibitorne molekule temeljene na poznatoj strukturi autoinduktora. Spojevi iz češnjaka, ekstrakta vanilije, papra i halogenirani furanoni koje proizvodi morska alga *Delisea pulchra* pokazali su *quorum sensing* inhibitorna svojstva (37 – 44). Istraživanja su također pokazala da dodatak spojeva sličnih ribozi u kulturu *Aggregatibacter actinomycetemcomitans* negativno utječu na formaciju biofilma, s obzirom na to da receptori autoinduktor-2 iskazuju određenu homolognost receptorima koji vežu ribozu, zbog čega riboza djeluje kao receptorni antagonist (45). Dokazano je i da halogenirani furanoni, konkretno furanoni s bromom, negativno djeluju na formaciju biofilma *Porphyromonas gingivalis* bez utjecaja na rast bakterijske populacije (46). Utjecaj na autoinduktor-2 *quorum sensing* sustave parodontnih patogena kao oblik terapeutske intervencije, također je svojevrsni izazov s obzirom na to da komenzali domaćina, od kojih on može imati i koristi, također se koriste tim istim sustavima u uspostavljanju svoje populacije (47).

Zaključak

*Quorum sensing*om regulirana genska ekspresija i koordinacija ponašanja bakterijske zajednice pokazuje nam da bakterije nisu *samotnjački* organizmi kakvima ih je smatrala klasična mikrobiologija. Komunikacija unutar vlastite bakterijske vrste i izvan nje ključ je u formaciji biofilmova koji su danas sve veći problem u zdravstvu jer su izvor kroničnih i perzistentnih infekcija. Iako *jezike* kojima bakterije komuniciraju još uvijek u cijelosti, ne razumijemo, važnost istraživanja ovog područja itekako je prepoznata.

Izjava

Autori opovrgavaju bilo kakav sukob interesa.

that LuxS might be involved in promoting survival of *Porphyromonas gingivalis* in the host by regulating its response to host-induced stresses such as high temperature exposure, resistance to hydrogen peroxide, and changes in the pH by elevated expression of stress proteins (35).

A comparison of the luxS mutant and wild type strains of *Aggregatibacter actinomycetemcomitans* showed and confirmed autoinducer-2-dependant regulation of genes encoding proteins of the outer membrane, enzymes, transcriptional regulators and fimbriae components. Expression of bacteria's most powerful virulence factor, leukotoxin, reduces trifold after luxS gene inactivation (31).

Quorum sensing inhibitory drugs

Since modern civilization is on the brink of entering a complete antibiotic resistance era, development of quorum sensing inhibitory drugs is sparking more and more interest. By terminating bacterial communication critical aspects of biofilm formation or expression of virulence factors would be affected (36, 37). A variety of quorum sensing systems and structural diversity of autoinducers must also be taken into account. So far, several naturally occurring quorum sensing inhibitory compounds have been discovered and synthetic derivatives based on the known structure of quorum sensing inducers produced. Components of garlic, vanilla extract, pepper and halogenated furanones produced by the marine alga *Delisea pulchra* showed quorum sensing inhibitory properties (37-44). Studies have shown that addition of ribose derivatives to culture medium negatively affected the formation of *Aggregatibacter actinomycetemcomitans* biofilm, as autoinducer-2 receptors exhibit homology to known ribose binding proteins, with the ribose acting as a receptor antagonist on autoinducer-2 receptors (45). As one study showed, brominated furanones also influence negatively the biofilm formation of *Porphyromonas gingivalis*, without affecting the bacterial growth (46). Affecting the autoinducer-2 quorum sensing circuits of the periodontal pathogens as a therapeutic intervention also represents a challenge as other oral bacteria, including the host's beneficial commensals; use these circuits to establish their communities (47).

Conclusion

Quorum sensing regulated gene expression and behavior coordination of the bacterial community points to the fact that bacteria are not solitary organisms as they were once thought of by traditional microbiology. Intra- and inter-species communication is the key in biofilm formation which represents an ever rising problem in healthcare, being the cause of persistent and chronic infections. Although bacterial language is not yet understood in its entirety, the importance of research in this field has been recognized.

Transparency declaration

The authors deny any conflicts of interest.

Abstract

The term 'quorum sensing' describes intercellular bacterial communication which regulates bacterial gene expression according to population cell density. Bacteria produce and secrete small molecules, named autoinducers, into the intercellular space. The concentration of these molecules increases as a function of population cell density. Once the concentration of the stimulatory threshold is reached, alteration in gene expression occurs. Gram-positive and Gram-negative bacteria possess different types of quorum sensing systems. Canonical LuxI/R-type/acyl homoserine lactone mediated quorum sensing system is the best studied quorum sensing circuit and is described in Gram-negative bacteria which employ it for inter-species communication mostly. Gram-positive bacteria possess a peptide-mediated quorum sensing system. Bacteria can communicate within their own species (intra-species) but also between species (inter-species), for which they employ an autoinducer-2 quorum sensing system which is called the universal language of the bacteria. Periodontal pathogenic bacteria possess AI-2 quorum sensing systems. It is known that they use it for regulation of biofilm formation, iron uptake, stress response and virulence factor expression. A better understanding of bacterial communication mechanisms will allow the targeting of quorum sensing with quorum sensing inhibitors to prevent and control disease.

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