1. Introduction

Quorum is a Greek word. In the ancient Hellenic and Roman forums, it was necessary for the members of the senate to form at least a minimal majority in order for them to make a decision. The group of persons whose number was sufficient for them to be able to accept or reject an act was the quorum. In general, microbes have numerous genes which become activated only when the bacterial population owersteps a threshold concentration. The term used to describe this population size-dependent gene regulation is quorum sensing (QS). In spite of the ancient origins of the word "quorum", QS is a relatively new area of science. When Leuweenhoek first glimpsed inside the bacterial world, it was thought that microbes live in a blind and deaf world, where all cells live their own lives without any influence from others. The first signs of the collapse of this theory, was the discovery of the competence stimulating factor of *Streptococcus pneumoniae* (*S. pneumoniae*) in 1965. It was obserwed that this bacterium needs an extracellular peptide to reach the competent state, and this molecule can be produced only at a high population size density. This is very useful, because there is no reason for a bacterium to waste energy reaching the competent state if there are insufficient cells in the near environment. The first direct evidence and the birth of QS emerged 5 years after this interesting discovery. This was the light production of *Vibrio fischeri* (*V. fischery*). This bacterium can colonize the light organ of several species of deepwater animals, includeing squids and fish. In this special organ, all nutrients are given to reach a high cell density. The wall of this organ is usually very flexible and fish can press water out of it. When this happens, the cell density of the vibrios increases, and light is produced. The first-described QS system was the *V. fischeri* LuxI/LuxR system, which is a characteristic feature in other G-bacterial species too. After these initial discoveries, it seemed that QS is very rare and almost unique in the bacterial world, influencing merely a few and not too important bacterial features. Nowadays, this has completely changed. As more and more QS systems have been discovered, it has become clear that bacterial communication does not occur in only a few isolated cases. QS is an essential part of bacterial life. The bacterium, with its QS system, has leverage, which is why QS is widely prevalent in the microbial world. With communication systems, microbes can accommodate to their habitat like multicellular organisms, and can fight as a unit against environmental impacts, other bacteria or the host immune system. Group behaviour is probably the most developed bacterial lifestyle. In a complex multispecies biofilm, every bacterium knows its place, and what is the best to do for the community and for itself. Division of labour is an evident thing in multicellular eukaryotes, but in prokaryotic biofilms too. QS has a special role not only in prokaryotic-prokaryotic, but also in eukaryotic-prokaryotic communication. The human body contains 10 times more bacterial cells than human cells, and most bacteria generally live in full harmony with us. It is clear that, it is virtually impossible to maintain this symbiosis without communication. We have hormones which affect our flora, and our symbionts also deploy QS signals which have effects on our cells. QS is also a very important phenomenon for the pathogenic bacteria. Signal molecules contribute directly to pathogenesis via the organization of biofilms or the synchronized production of virulence factors. Antibiotic resistance is the most important problem in modern microbiology. Bactericidal or bacteriostatic compounds exert strong selective pressure on microbes. Because of the

irresponsible use of antibiotics, pathogenic bacteria nowadays often display resistance to at least a few medicines. The appearance of multidrug-resistant strains which tolerate the latest antibiotics too enhances this problem. There is an urgent need to discover new antimicrobial, antipathogenic and antivirulence drugs. QS inhibitors may be a good alternative to solve this situation.

1.1. QS

QS is a widely prevalent feature of both Gram-negative and Gram-positive bacteria. There are many different types of QS systems and signal molecules in the bacterial world, but each system relies on the same basics. Each bacterium synthesizes signal molecules in low amounts. When the population density is low, the number of signal molecules is also low, when the number of bacteria increases, the concentration of QS molecules increases. When the concentration of signal molecules exceeds a threshold concentration, a positive feedback starts, and the bacteria increase the production of signal molecules. At this very high concentration of QS molecules, they bind their receptors. The nature of the receptors can differ considerably in each case, but in the end of the process is the same: the initiation of target gene transcription.

1.2. QS in Gram-negative bacteria

1.2.1. LuxI/LuxR system of *V. fischeri*

The first described QS was the LuxI/LuxR system of *V fischeri*. Two proteins, LuxI and LuxR, control the synthesis of the enzyme luciferase. The bacterium synthesizes the signal molecules with the acyl homoserine lactone (AHL) synthase LuxI. When the AHL reaches the threshold concentration, it can bind to LuxR, the cytoplasmic receptor. The LuxR-AHL complex activates the transcription of the luciferase operon. The LuxR-AHL complex also induces the expression of luxI because it is encoded in the luciferase operon. This positive feedback leads to a flood of more and more AHL molecules to the environment, which can diffuse freely to the receptor and activate the transcription of the structure genes. The end of this process is the light production of the bacteria. LuxI/LuxR-type AHL-dependent QS systems are highly prevalent in G-bacteria. It mainly takes place in intraspecies communication too.

1.3. QS in Gram-positive bacteria

1.3.1. The QS system of *Staphylococcus aureus* (*S. aureus*)

S. aureus is a widely prevalent bacterium which causes numerous human diseases. It can nearly infect all kinds of tissues e.g. skin, bone, the lungs and the nervous system. It has great resistance to most antibiotics, and has evolved multiresistant strains, that tolerate all kinds of antibiotics. *S. aureus* strains use interesting QS-mediated strategies to cause diseases. At low cell density they express factors which promote attachment, biofilm formation and colonization, while at high cell density they produce proteases and toxins, which promote dissemination and infection. This density-dependent pathogenicity in *S. aureus* is regulated by the RNAIII molecule, the level of which is mediated through the AgrC/AgrA QS system. The AIP of *S. aureus* is encoded by agrD. The AgrD molecule is transported and modified with a thiolactone ring by the AgrB transporter. The AgrC receptor binds the AIP extracellularly,

this complex leads to the phosphorylation of AgrA and the phosphorylated AgrA then induces the expression of RNAIII. This regulatory RNA represes the expression of biofilm formation factors and promotes the expression of toxins and proteases. RNAIII also induces the expression of the agrBDCA locus, which increases the levels of AIPs.

Interestingly, these AIPs and therefore the *S. aureus*-es belong in 4 different groups. Each type of signal molecule competitively blocks the receptors of the others. The bacteria which colonize first, therefore kill or inhibit the colonization of the bacteria from the other groups.

1.4. QS signal molecules

QS is population size-dependent gene regulation, mediated by small signal molecules called AIs. The natures of these molecules are very different, but most belong in 3 groups: AHLs are mainly used by Gram-negative bacteria, autoinducer II (AI-2) is used by both Gram-negatives and Gram-positives, and AIPs are used by Gram-positives. Some bacteria use AIs which do not fit these groups, e.g. the PQS diffusible signal factor (DSF) or autoinducer III (AI-3).

1.5. Quorum quenching

Bacteria with different QS systems gain numerous benefits. They can monitor the density of their own population and those of other bacteria and eukaryotes too. They can coordinate and optimize their gene expression in a density-dependent manner. The cost of signal molecule production is very low and is usually associated with necessary cell metabolic pathways. For example, a bacterium does not gain leverage and waste much energy if it produces antibiotics when the cell number is not large enough to produce it in sufficient concentration. Hence it is necessary to produce signal molecules to optimize the production of difficult and expensive molecules. QS is essential in bacterial life, and many strategies have therefore been developed to disrupt it in bacterial-bacterial, and also prokaryotic-eukaryotic interactions. The mode of interaction with other QS systems can be very different, but the 3 attack points are the same: the synthesis of the signal molecule, the signal molecule and the signal detection.

2. Materials and Methods

2.1. Tricyclic compounds used: promethazine (Pipolphen, EGIS, Hungary), amitriptyline (Teperin, EGIS, Hungary), acridine orange (AO) (Reanal, Hungary), imipramine (Melipramin, EGIS, Hungary), desipramine, chlorprothixene (Truxal, Lundbeck, Denmark), promazine, diethazine (Parkazin, Rhone-Poulenc, France) and desertomycin. Stock solutions of these tricyclic compounds were prepared in distilled water at 25.0 mg/ml before use. Thioridazine (TZ) (Sigma, Madrid, Spain) was used as positive control for the efflux pump inhibition.

2.2. Other chemicals: 5-Fluorouracil (5-FU) (Sigma-Aldrich, Budapest, Hungary), Iodine deoxyuridine (IDU) Ethidium bromide (EB) (Sigma, Madrid, Spain), Dimethyl sulfoxide (DMSO)**,** N-Hexanoyl-DL-homoserine lactone (SIGMA, Budapest).

2.3. Trifluoromethyl ketones used: Eleven TFs (***1-9***, ***11*** and ***12***) were synthetized by reaction of the corresponding 2-methylbenzazoles with trifluoroacetic or chlorodifluoroacetic anhydride. Compound ***10*** was prepared by treatment of 2-lithiomethylbenzoxazole with ethyl

acetate. *N*-Hexanoyl-DL-homoserine lactone was purchased from Sigma (Budapest, Hungary). TFs were dissolved in, DMSO.

2.4. Essential oils used: Rose (*Rosa damascena* L., *Rosaceae*), lavender (*Lavandula angustifolia* L., *Labiatae*), chamomile (*Matricaria recutica* L., *Asteraceae*), orange (*Citrus sinensis* L., *Myrtaceae*), eucalyptus (*Eucalyptus globulus* L.,*Myrtaceae*), geranium (*Geranium robertianum* L., *Geraniaceae*), juniper (*Juniperus communis* L., *Cupressaceae*), citrus (Citrus lemon) and rosemary oils (*Rosmarinus officinalis* L., *Lamiaceae*) were purchased from Phoenix Pharma Ltd. (Hungary). The oils were either used directly (concentrated) or dissolved in DMSO to yield a dilution of 10% (v/v). The stock solution was either used directly or further diluted with 10% DMSO. Both AO and 5-FU were dissolved in distilled water.

2.5. Bacterial strains: *C. violaceum 026* (CV026) served as QS sensor. *C. violaceum* is a common bacterium which lives in soil and water. When *C. violaceum* reaches a high cell density, it produces a purple pigment, violacein (110). The CV026 sensor strain is a Tn*5* mutant which alone cannot synthesize AHLs; it produces the purple pigment only in the presence of externally added inducers. This strain has been used to detect a wide range of short-chain AHLs or QS inhibitors.

EZF 10-17 was isolated from a grapevine crown gall tumour. This strain induced pigment production by CV026 and proved to be efficient for the study of QS interactions.

Enterobacter cloaceae (*E.* cloaceae) 31298; a clinical wound isolate, was used as AHL producer.

E. coli wild-type AG100 [argE3 thi-1 rpsL xyl mtl (gal-uvrB) supE44] was employed for determination of the effects of TFs on the activity of the intrinsic efflux pump of this organism (116).

P. aureginosa 49010 was clinical strains isolated from the human trachea.

Strains used in the interference of QS amongst microbial species experients:

Fungi: *Candida krusei, Candida tropicalis and C. albicans*

Bacteria*: Achromobacter xylosoxidans 40502, Acinetobacter baumannii 32703, A. baumanni 42701, Bacillus cereus, Bacillus subtilis, Bacillus clausii, Bacillus megaterium PV 361, Bacillus megaterium MS 941, Bacillus megaterium 216, Staphylococcus epidermidis, S. aureus and E. coli* strains were isolated from extraintestinal infections numbered *5536, 10902, 10904, 11925, 14525, 14584, 18596, 19579, 19672, 24310, 24409, 24442, 33444, 36446* and *40312*.

2.6. Media

LB (Luria-Bertani) medium: containing yeast extract 5 g/l, trypton 10 g/l and NaCl 10 g/l

Modified LB agar (LB*) containing yeast extract 5 g, trypton 10 g, NaCl 10 g, K_2HPO_4 1 g, $MgSO_4.7H_2O$ 0.3 g and FeNaEDTA 36 mg in 1.0 l of distilled water, Potato dextrose agar (PDA), Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) purchased in powder form from Sigma (Madrid, Spain), Blood agar, complemented with sheep blood

Methods

2.7 QS tests: LB* was used for the experiments. The sensor strain CV026 and the AHL producer strains EZF 10-17, *E. cloaceae* 31298 or *P. aureginosa* 49010 were inoculated as parallel lines and incubated at room temperature (20 ^{o}C) for 24-48 h. QS inhibition was monitored by the agar diffusion method. Filter paper discs (7.0 mm in diameter, Whatmann 3MM) were impregnated with 10 µl of stock solutions of the compounds in distilled water or DMSO. The discs were placed between the parallel lines of sensor and AHL producer strains on the surface of nutrient agar. The plates were incubated at room temperature for a further 24-48 h and the interactions between the strains and compounds were evaluated as concerns the reduction in size of the zone of pigment production and the zone of growth inhibition of the affected strains, in mm. 5-FU and AO was applied as positive controls.

2.8 QS interference tests

QS modification experiments with *E. coli* strains: Suspensions of each *E. coli* strain were separately mixed with molten LB* agar medium. One hour later, parallel lines of the pair of CV026 sensor and Ezf 10-17, and *E. cloaceae* 31298 AHL-producing strains were inoculated, and incubation was performed at room temperature (20 ºC) for 24-48 h.

QS inhibition of bacterial strains: Each investigated strain was inoculated at right angles through the parallel lines of the pair of CV026 sensor and EZF 10-17 AHL-producing strains, and then incubated at room temperature for a further 24-48 h. LB* medum was used, for *Candida, Acinetobacter, Achromobacter, Bacillus, E. coli* and *Staphylococcus* species, etc. For *Streptococcus* species, blood agar was used, and the plates were pre-incubated for 5 h at 37 ºC, and further incubation being continued at room temperature. QS inhibition was revealed as a decreased level of violacein production by CV026.

2.9. Evaluation of complex formation between N-hexanoyl-DL-homoserine lactone and QS inhibitors: Biological method: 1 µl of a solution of 5, 10 or 25 ng of AHL was mixed with 10 µl of stock solution (25 mg/ml) of the potential QS inhibitor. Filter paper discs (7.0 mm in diameter, Whatmann 3MM) were impregnated with 11 µl of the mixture of QS inhibitor and AHL solution. The discs were placed upon the inoculation line of the CV026 sensor strain on the surface of nutrient agar medium. The plates were incubated at room temperature (18-22 °C) for a further 24-48 h, and the interactions between the AHL and the tricyclic compounds were evaluated in terms of the reduction in diameter of pigment production, and the reduced colour intensity.

2.10. Taxonomic identification of EZF 10-17 and analysis of its AHL production.

The V3 region of 16S rDNA from EZF 10-17 was amplified by using the forward primer (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer (5'-ATTACCGCGGCTGCTGG-3') and sequenced. Sequence data were compared and analysed by BLAST to the published 16S V3 sequences available in the database. The AHLs from the liquid culture of EZF 10-17 were extracted and concentrated by using acidified ethyl acetate liquid-liquid extraction. The purified AHLs were analysed by using thin-layer chromatography (TLC) overlaid with the *C. violaceum* CV026 biosensor strain.

2.11. Minimum inhibitory concentration (MIC) of each TF on CV026 and *E. coli* AG100. The MICs of TFs were determined by the broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.12. Assessment of the effects of each TF on the activities of the efflux pump systems of CV026 and *E. coli* AG100. The activities of the TFs on the real-time accumulation of EB were assessed by the automated EB method, using the Rotor-Gene 3000^{TM} thermocycler with real-time analysis software (Corbett Research, Sydney, NSW, Australia). Briefly, *E. coli* AG100 was cultured in MHB medium until the culture reached an optical density (OD) of 0.6 at 600 nm. The culture was then centrifuged at 13,000 rpm for 3 min, the pellets were resuspended in phosphate-buffered saline (PBS; pH 7.4), with a final concentration of 0.4% glucose, and the OD was adjusted to 0.6 at 600 nm. Aliquots of 45 μl of the cell suspension were distributed to 0.2 ml tubes. The TFs were individually added at concentrations equal to half their MIC against the strain in 5 μl volumes of their stock solutions, and finally 45 μl of EB to yield a final concentration of 1 mg/l (Sigma-Aldrich Química SA, Madrid, Spain) in PBS, with or without glucose, was added. The selection of the concentration of each TF at half its MIC was due to the empirical fact that at this concentration there is no significant effect on the viability of the organism. It is also important to note that prior to the experiments described, the maximum concentration of EB which was within the capacity of the bacterium to extrude was determined at least three times. For the wild-type *E. coli* AG100 reference and the CV026 strains employed in the study, these concentrations of EB were determined to be 1 and 0.5 mg/l, respectively. The tubes were placed into a Rotor-Gene 3000^{TM} thermocycler and the fluorescence was monitored on a real-time basis. From the real-time data, the activity of the TF, i.e. the relative final fluorescence (**RFF**) of the last time point (at 30 min) of the EB accumulation assay was calculated according to the formula:

$$\mathbf{RFF} = \frac{\mathbf{RF}_{\text{treated}} - \mathbf{RF}_{\text{untreated}}}{\mathbf{RF}_{\text{untreated}}}$$

where $\mathbf{RF}_{\text{treated}}$ is the relative fluorescence at the last time point of the EB retention curve in the presence of an inhibitor, and $\mathbf{RF}_{\text{untreated}}$ is the relative fluorescence at the last time point of the EB retention curve of the untreated control. The greater the difference between $\mathbf{RF}_{\text{treated}}$ and $\mathbf{RF}_{\text{untreated}}$, the greater the degree of EB accumulated, and therefore the greater the degree of inhibition of the efflux pump system of the bacterium promoted by the agent at that concentration. The **RFF** was then divided by the concentration of the TF that corresponded to half its MIC. This yielded a measure of the effect of each TF at a milligram level (specific activity) and therefore afforded comparison of each TF for activity against the efflux pump systems of the CV026 and *E. coli* AG100 strains. The experiments were repeated three times and the specific activity values presented are the averages of three independent assays. This method of analysis has been described previously. TZ an efflux pump inhibitor, served as a positive control.

3. Results

3.1 QS inhibition of essential oils The agar diffusion method provides a convenient and semiquantitative method with which to assess antibacterial activities and QS signal production in the presence of essential oils. Using this method, we tested 9 essential oils compared to the positive controls of AO and 5-FU. Most of these oils are known as inhibitors of bacterial growth. Our data demonstrate that several plant-derived essential oils not only inhibit bacterial growth, but also block QS regulation processes. As examples, although geranium oil was most effective as an inhibitor of the QS response of CV026 following induction by the AHL-producing strains *E. cloaceae* 31298 and EZF 10-17. Rose, lavender and rosemary oils also inhibited the colour development to varying degrees. QS was moderately inhibited by lavender, eucalyptus and citrus oils, while chamomile, orange and juniper oils were ineffective.

3.2. QS inhibition of tricyclic compounds

The mechanism of QS inhibition was studied by measuring the effects of the direct interactions between the AHL and QS inhibitors on violacein production in the bioassay. The most effective inhibitors were imipramine, promethazine, desipramine and amitriptyline.

3.3. Identification of EZF 10-17 and its AHLs

In our previous studies, the unidentified grapevine tumour isolate EZF 10-17 proved to be an inducer of violacein production by *C. violaceum* CV026. Thus, this pair of inducer/sensor strains was successfully used to study potential QS inhibitors. To identify EZF 10-17, we sequenced the V3 region of the 16S rDNA gene from its genome. On comparison of the sequence data with those found in the databases, this strain proved to be a member of the *Sphingomonadaceae* family. The signal production of EZF 10-17 was analysed by TLC overlaid with *C. violaceum* CV026. As compared with the standard AHLs, EZF 10-17 produces a strong signal that co-migrated with 3-oxo-C6 AHL. Additionally, weaker signals which seemed to be identical to C6 AHL, 3-oxo-C8 AHL and C8 AHL were also observed. These data support our earlier observations on the suitability of EZF 10-17 in QS assays with *C. violaceum* CV026.

3.4. QS and efflux pump inhibitor activity of TF compounds

TF *5* had the least inhibitory effect (a deep colour associated with the responder CV026) and TF *3* had the greatest inhibitory effects on the response of the CV026. The effect of the TFs on the QS system were clearly inhibitory. Whether the effect is due to the TF inhibiting the release of the QS signal or due to the inhibition of the response of the responding species cannot be distinguished from the above evaluation. The direct effects of each TF on the QS response by CV026 were determined with the use of discs impregnated with combinations of a constant amount of the AHL and differing amounts of TF. The presence of pure AHL in the disc led to the production of the deep purple colour associated with CV026. The presence of the TF that inhibited the production of colour in the QS assay described, when in combination with the AHL, inhibited the production of the purple colour by the responding CV026. These results clearly show that the TF has a powerful inhibitory effect on the QS responding strain. TFs have activity against the efflux pump of CV026. Briefly, the activity of the positive control TZ was 1.02. On comparison of the activity of each TF relative to the positive control, TFs-*2* and *3* prove to exhibit the greatest activities against the efflux pump system of CV026; TFs-*1, 4, 5* and *9* displayed significant activity; and TFs-*7, 8, 10, 11* and *12* had no activity.

The facts that the TFs inhibited the response of an environmental strain to a QS signal and the same TFs inhibited the efflux pump of the environmental responding strain themselves did not support clinical interest in the TFs for possible use in the therapy of a bacterial infection. TFs-*2* and *3* exerted the highest activities against the efflux pump system of *E. coli*. TFs-*1, 4, 5* and *9* were also very active since their inhibitory activities exceeded that of TZ, the efflux pump inhibitor that served as positive control.

3.5. Bacterial-bacterial interactions in QQ

Our results reflect *ex vivo* interactions, and exemplify various bacterial interactions on QS. We investigated 31 bacteria and 3 yeast strains for their ability to inhibit or modify QS, of which 2 bacterial genera, *Escherichia* and *Bacillus*, proved to be effective inhibitors. Of the 6 investigated bacillus strains, *B. cereus* was the best inhibitor, with a clear QS inhibitory effect, while *B. subtilis* and *B. clausii* inhibited QS moderately, and the 3 *B. megaterium* strains (PV361, MS941 and 216) did not exhibit any QS inhibitory activity. Surprisingly, 14 of the 15 investigated *Escherichia* clinical isolates were effective inhibitors, and only 1 had no inhibitory effect. Interestingly, phenothiazines enhanced the QS-inhibitory effect of the ineffective *E. coli* 19579 and the 2 *Bacillus* strains, which displayed moderate QS-inhibitory effects without phenothiazines.

4. Discussion

The discovery of QS has opened up new perspectives in modern microbiology. QS is necessary in many crucial bacterial features e.g. biofilm formation (127), virulence factor production (128), competence (1), sporulation (129), antibiotic production (130) and resistance (131). Without communication, the microbes are lose the ability to fight as a unit against the immune system, produce virulence factors or organize biofilms. On the whole, it is impossible for most the bacteria to cause infections without QS. On the other hand, the increasing level of antibiotic resistance is currenly the greatest problem in microbiology. Most bacteria tolerate at least a few antibiotics, and, unfortunately, numerous multiresistant strains have appeared, which have also adapted to the latest antibiotics. The investigation of QS is therefore of increasing significance. In the future, QS inhibitors may offer a new perspective in the fight against multiresistant infections and take over the function of decaying antibiotics. Numerous compounds have been investigated in this search of QS inhibitors. Three main categories, essential oils, tricyclic compounds, and TFs proved to be the best inhibitors. These compounds and bacterial-bacterial co-existence are discussed in the thesis.

4.5 The following of our results are considered novel:

The QSI activity of the investigated essential oils
The QSI activity of phenothiazines
The QSI activity of TFs, and the connection between efflux pump inhibitor activity and QSI activity of TFs
The AHL profile of and identification of EZF 10-17
The interaction of *E. coli* with AHL-based QS systems

5. Summary

QS is population size-dependent gene regulation, mediated by small signal molecules called AIs. The natures of these molecules are very different, but most belong in 3 groups: AHLs are mainly used by Gram-negative bacteria, autoinducer II (AI-2) is used by both Gram-negatives and Gram-positives, and AIPs are used by Gram-positives. Some bacteria use AIs which do not fit these groups, e.g. the PQS, DSF or AI-3. QS is a widely prevalent feature of both Gram-negative and Gram-positive bacteria. There are many different types of QS systems, but each system relies on the same basics. Each bacterium synthesizes signal molecules in low amounts. When the population density is low, the number of signal molecules is also low, when the number of bacteria increases, the concentration of QS molecules increases. When the concentration of signal molecules exceeds a threshold concentration, a positive feedback starts, and the bacteria increase the production of signal molecules. At this very high concentration of QS molecules, they bind their receptors. The nature of the receptors can differ considerably in each case, but in the end of the process is the same: the initiation of target gene transcription. QS is necessary in many crucial bacterial features e.g. biofilm formation (127), virulence factor production (128), competence (1), sporulation (128 Steiner 2012), antibiotic production (130) and resistance (131). Without communication, the microbes are lose the ability to fight as a unit against the immune system, produce virulence factors or organize biofilms. In the present thesis, my attention focused on in vitro models of QS to investigate QSI alternatives. Numerous compounds have been investigated in this search of QS inhibitors. Three main categories, essential oils, tricyclic compounds, and TFs proved to be the best inhibitors. The bacterial-bacterial co-existence also investigated. Our results have afforded some evidence of the complexity of bacterial-bacterial interactions. Of the tested isolates, *E. coli* strains proved to be the best inhibitors of the AHL-dependent QS, 14 of the 15 samples exhibiting an inhibitory effect. Antibiotic resistance is the most important problem in modern microbiology. Bactericidal or bacteriostatic compounds exert strong selective pressure on microbes. Because of the irresponsible use of antibiotics, pathogenic bacteria nowadays often display resistance to at least a few medicines. The appearance of multidrug-resistant strains which tolerate the latest antibiotics too enhances this problem. There is an urgent need to discover new antimicrobial, antipathogenic and antivirulence drugs. QS inhibitors may be a good alternative to solve this situation.

Összefoglalás

A „quorum” egy görög eredetű szó, igazán jó magyar megfelelője nincsen. Az ókori görög és római fórumokkal lehet összefüggésbe hozni. A döntéshozás ezen színterein, egy törvény elfogadásához vagy elvetéséhez létre kellett jönnie a quorumnak, azaz egy minimális többségnek ami a szavazás eredményességéhez szükséges. A quorum sensing, lényegében a mikrobák között sem zajlik másként, rengeteg olyan bakteriális gént ismerünk, amely csak bizonyos sejtkoncentráció felett aktiválódik. Számos különféle QS rendszert és jelmolekulát ismerünk, de a működési elvük minden esetben azonos alapokon nyugszik. A baktériumok egy alap szinten mindig termelnek jelmolekulákat. Alacsony mikrobaszámnál a szignálmolekulák száma is alacsony marad, és nem indukálnak változást a génkifejeződésben. A baktériumszám növelésével a jelmolekulák száma is növekszik, amint elérik a küszöbkoncentrációt kötődnek a receptoraikhoz. A receptor típusok és szignál transzdukciós

utak szintén nagyon változatosak, de végső soron minden esetben egyrészt a jelmolekulák termeléséért felelős gének, másrészt a struktúrgének transzkripcióját indukálják. A pozitív visszacsatolás hatására egyre több jelmolekula kerül a környezetbe, egyre több struktúrgén is íródik át, és bekövetkezik a baktériumpopuláció fenotipusbeli változása. Rendszerint a jelmolekulák szintézisének növelése együtt jár a receptorok szintézisének csökkenésével, így téve szabályozottá a reakciót. A szignál molekulák többsége 3 nagy csoportba sorolható: a Gram-pozitívokra jellemző autoinducer peptidekre, a Gram-negatívokra jellemző acyl homoszerin laktonokra és az interbakteriális kommukációban szerepet játszó autoinducer 2-re. Egyre több szignál molekulát ismerünk meg, amely nem sorolható be egyik csoportba sem, ilyenek például az AI-3, DSF, PQS és HHQ szignálok. Quorum sensing rendszerükkel az egysejtű élőlények is képesek bizonyos mértékben többsejtűként viselkedni. Az adott mikrobaközösségek egy egységként képesek organizálni biológiai folyamataikat. Napjaink egyik legnagyobb, minél sürgetőbb megoldást igénylő mikrobiológiai problémáját az egyre szélesebb körben terjedő antibiotikum rezisztencia jelenti. Ennek leküzdésére a különféle quorum sensing gátló szerek jelenthetnek alternatívát. A QS rendszerek a baktériumok legkülönfélébb tulajdonságait befolyásolják. Szerepük lehet az antibiotikum rezisztenciában, biofilm képződésben, a kompetencia kialakításában, virulencia faktorok termelésében, az antibiotikum termelésben, a biolumineszcenciában, a konjugációban, sporulációban, motilitásban és még számos egyéb tulajdonság kialakításában. Így egy hatásos QS blokkolóval meggátolhatjuk az antibiotikum rezisztencia terjedését, a biofilmek létrejöttét, vagy a virulencia faktorok termelését is. Ezáltal a kórokozók zöme elveszítené, vagy csökkentené az antibiotikum rezisztenciáját, virulenciáját és más QS mediált tulajdonságait. Munkám során célul tűztem ki, a quorum sensing gátlási lehetőségeinek vizsgálatát, különféle gátló anyagok keresését és tanulmányozását, a mikrobák egymás kommunikációs rendszereire való hatásának megfigyelését. Kísérleteim alapját a CV026 (*Chromobacterium violaeum* 026) szenzortörzs jelentette, mely rövid szénláncú AHL-ek jelenlétében lila pigmentet, violaceint termel. Segítségével több batériumtörzsnél is detektáltunk AHL termelést, melyek közül a *P.aeriginosa* 49010-et és az *E. cloaceae* 31298-at és az akkor még identifikálatlan EZF 10-17-et használtuk a további munkákhoz. Kísérleteimben elsőként különféle illóolajok QS-re kifejtett hatását vizsgáltam. Az irodalmakban már említett (fokhagyma, kakukkfű) kivonatokon kívül is számos esetben mutattam ki QS gátlást, így például a rózsa, citrom, levendula, gólyaorr és rozmaringolaj esetében is. Két vegyületcsoport az ABC transzporter gátló háromgyűrűs vegyületek és a proton pumpa gátló trifluorometil ketonok többsége esetében szintén erőteljes QS gátlás volt tapasztalható. A háromgyűrűs vegyületek közül az amitriptilin fejtette ki a legerősebb hatást az AHL termelő törzsek segítségével végzett kísérletek esetében. A C6 HSL jelmolekula és a háromgyűrűs vegyületek direkt interakcióján alapuló vizsgálatoknál pedig az imipramin gátolta legeredményesebben a QS-et. A vizsgált 12 protonpumpa gátló trifluorometil keton közül 6 bizonyult erős QS gátlónak. A trifluoro keton proton pumpa gátló TF vegyületek efflux pumpákra kifejtett hatását is vizsgáltuk. A legeredményesebb efflux pumpa gátlóknak ugyanazok a vegyületek bizonyultak, mint a legeredményesebb QS inhibitorok (TF-1, 2, 3, 4, 5, 9,). A különféle baktériumok egymás QS rendszereire való hatásának tanulmányozása során pedig a vizsgált 15 *E. coli* törzs közül 14 bizonyult hatásosnak. A jövőben a különféle QS gátló szerek óriási jelentőségre tehetnek szert a komplikált bakteriális fertőzések kezelésénél.

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