Open Journal of Pharmacology

Science Publishers

Review Article

OPEN ACCESS

Inhibitors of efflux pumps of Gram-negative bacteria inhibit Quorum Sensing

Leonard Amaral[1,2*], *Joseph Molnar*[2,3]

[1] Grupo de Micobacterias, Unidade de Microbacterilogia, Centro de Malaria e Doenças Tropicais (CMDT), Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal
[2] Cost Action BM0701 (ATENS) of the European Commission/European Science Foundation
[3] Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

Corresponding Author & Address:
Leonard Amaral[*]
Unidade de Microbacterilogia, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa Rua Junqueira 100, 1349-008 Lisbon, Portugal; Tel +3513652600; Fax +351 213632105; Email: lamaral@ihmt.unl.pt

Published: 16th July, 2012 Accepted: 16th July, 2012
Received: 11th April, 2012 Revised: 8th June, 2012

Open Journal of Pharmacology, 2012, **2**-2

Keywords: Quorum Sensing, QS signal, acylated hydroxyl lactone (AHL), efflux pumps, Proton Motive Force (PMF), inhibitors of efflux pumps, inhibitors of QS systems, phenothiazines, Trifluormethyl Ketones (TFs), plants sources for QS inhibitors

ABSTRACT

Quorum Sensing (QS) systems of bacteria consist of a producer of the QS signal and the responder. The generation of a QS signal provides the means by which a population can behave in a concerted manner such as swarming, swimming and secretion of biofilm, etc. Because concerted bahaviour bestows protection to the bacterial species, and hence factors involved in the severity of an infection such as virulence are products of QS systems, compounds that inhibit the QS system have significant clinical relevance. Recent evidence suggests that the secretion of QS signals takes place via the efflux pump system of the producer of the signal. Interestingly, compounds such as phenothiazines and trifluoromethyl ketones (TFs) that inhibit proton motive force (PMF) activities such as swarming and swimming also inhibit the PMF dependent efflux pump systems of bacteria and their QS systems. This review discusses the relationship between the efflux pump, the QS system and the compounds that affect both. Lastly, suggestions are made regarding classes of compounds that have been shown to inhibit PMF dependent efflux pumps and the need to evaluate them for QS inhibitory properties.

INTRODUCTION

The role of efflux pumps in multi-drug resistance

Efflux pumps of bacteria provide protection from noxious agents that are present in the environment in which they exist. Noxious agents may be naturally occurring compounds present in environments outside and within the human. With respect to infectious disease, the universe of external environments may be aqueous reservoirs where compounds are added to reduce or even eliminate infectious agents, the home where

attempts to reduce the bacterial load present in the kitchen, bathroom, bedroom, play areas, swimming pool, etc., facilities used by children (child care, schools, etc) and on occasion, the property (area of septic systems, etc.) are often needed, and transportation facilities (cruise ships) that may require fumigation or disinfectants. However, although the aforementioned sites are important for reducing infectious disease, it is the environmental sites of health care areas where the problem of potential infectious disease is far more difficult to control. At these sites, patients presenting with an infectious disease may infect others even when no longer present, leaving behind bacterial loads in nasal discharges, saliva and other body fluids. Transmission of bacteria to patients in a health care facility may be from contaminated surfaces and microdroplets of expired air and certainly from health care workers who are attending the patient as well as those who are ancillary to health care such as orderlies who transport the patient, maids who change soiled bed linen, etc. A large proportion of patients that enter health care facilities for non-infectious pathology acquire a bacterial infection [1-3]. These nosocomial infections pose serious threats to the patient since it is highly probable that the infectious agent has its origins in a patient that receives antibiotic therapy. Due to a variety of reasons which will be discussed, therapy of the bacterial infection places the bacterium under stress and the bacterium responds by invoking a series of mechanisms that render it resistant to one or more antibiotics-hence it is multi-drug resistant (MDR). MDR infections are problematic for therapy and as of today, nosocomial infections such as those produced by methicillin resistant *Staphylococcus aureus* are more prevalent in most hospitals world-wide than methicillin susceptible strains of *Staphylococcus aureus* (MSSA) [4, 5]. Consequently, in order to prevent nosocomial infections, hospitals devote much energy towards maintaining as sterile environment as possible, and to accomplish this disinfectants are used in great quantities. Agents used to prevent infectious disease in the house and its property, in the health care facilities as well as agents used to treat bacterial infections, to the bacterium, they are "one of the same", namely, noxious agents that threaten their survival, and their response to these agents in part involves the over-expression of genes that regulate and code for efflux pumps that recognise such agents and extrude them to the environment before they reach their intended targets [6-10]. Because over-expressed efflux pumps render antibiotic therapy problematic, an intense search for agents that inhibit specific efflux pumps of specific bacteria has been conducted during the past decade [9].

The role of Quorum Sensing (QS) in infection

Communication between bacteria of the same strain or species and between species contributes to their survival [11-13]. Communication involves the secretion of signals that invoke a specific response from the responder [11-13].This communication process is termed Quorum sensing (QS). When it takes place between strains of the same species, communication is directed towards the reduction of population growth and hence, reducing the possibility of exceeding the nutritional support of the environment [13]. Other signals may involve a population response that involves the secretion of bioactive molecules that inhibit the replication of a competing population species [14-16] or even kill [biocidins) [17-21] or promote a swarming effect that recruits members of the same species to migrate to a specific location [22-24] similar to swarming by insects subsequent to signals indicating site of food [example bees). Other responses involving "community action" are also invoked and these result in secretion of materials that will protect the bacterium from external danger. These materials, termed biofilm, encase the bacteria at distances from each other [25-29] and within the matrix of this biofilm are channels used for further communication [30]. Biofilms are produced in the wild, at sites such as surfaces of rocks which maintain the bacterial population *in situ* [31] and are also produced at sites of the human colonised by infecting bacteria [32, 33]. Biofilms are also problematic with prosthetic devices in that subsequent to their placement, an infection may take place and the surfaces of the device are suitable for the production and maintenance of biofilm which has bacteria embedded [34]. Agents that inhibit the QS response of the infecting bacterium are obviously important and hence, the search for such agents that inhibit the QS system and biofilm formation has been in effect for the past two decades [11-13].

Relationship between Efflux Pumps, QS and Biofilm secretion

There is a relationship between efflux pumps (EP), QS and biofilm (BF) secretion which has come to the forefront only recently [13]. Control of this relationship is critical for successful therapy of MDR bacterial infections which have become rather commonplace. It is the intent of this review to identify agents which may serve to interfere with the complex system of EP-QS-BF interaction.

Bacterial Efflux pumps (EPS)

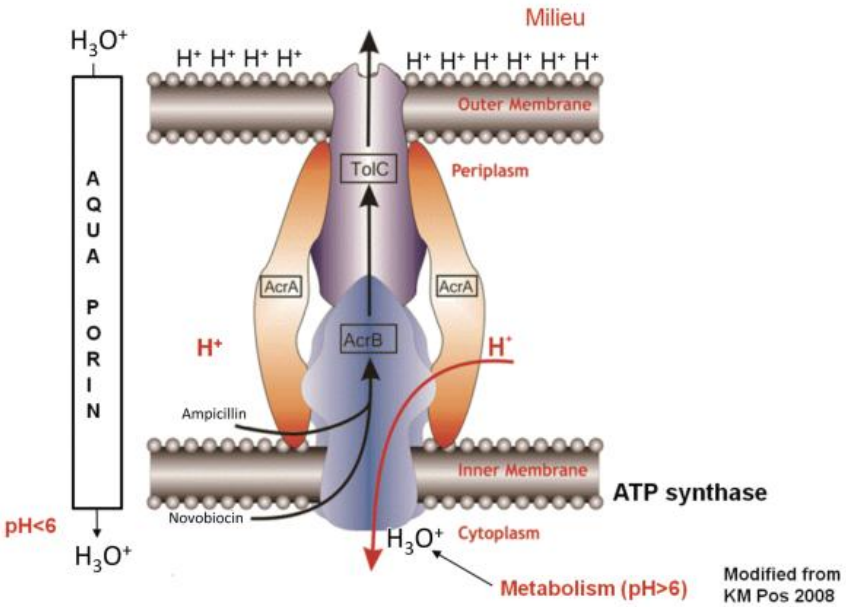
Bacterial efflux pumps (EPs) are proteins that are localised and imbedded in the plasma membrane of the bacterium and whose function is to recognise noxious agents that have penetrated the protective cell wall of the organism and have reached the periplasm or cytoplasm, and extrude the agents prior to their reaching their intended targets [6-10]. However, they also recognise toxic compounds produced from their own metabolic processes and hence perform excretory functions as well [35, 36]. In other words, they are transporters of noxious compounds from within the bacterial cell to the external environment. With the possible exception of excretory functions, efflux pumps utilize sources of energy for their function inasmuch as they transport compounds against a concentration gradient. In general there are two types of efflux pumps that are distinguished with respect to the immediate source of energy that is utilized for export functions: ABC transporters [37-40] and proton motive force dependent transporters [6-10, 35, 36, 41]. ABC transporters consist of two domains-one that is embedded in the plasma membrane and the other is on the medial side of the plasma membrane. Their structure may consist of 6 or 12 helical sequences parts of which face the medial side of the cytoplasmic membrane. For ABC transporters that consist of 6 helices, there are two types of binding sites; one for the agent (substrate) that is to be transported and another for the binding of ATP. Subsequent to the recognition of the noxious agent and its binding, ATP is bound and hydrolysed and the energy liberated affords a conformational change of the transporter that promotes the movement of the noxious agent to the environment [37-42]. The precise structural changes that take place in the transporter that lead to the extrusion of the noxious agent as well as the means by which the transporter recognises structurally unrelated compounds is not yet completely understood. Proton motive force (PMF) dependent transporters obtain their energy for function from the proton motive force. The proton motive force is the result of cellular metabolism which yields protons that are not used for coupling with molecular oxygen and which are exported to the surface of the cell [43-45] where they are distributed and bound to components of the protective lipopolysaccharide layer that covers the cell and constitutes a part of the outer cell wall of Gram-negative [46] and the cell wall of Gram-positive bacteria [47]. The larger the concentration of protons (hydronium ions) on the surface of the cell with respect to their lower concentration on the medial side of the cytoplasmic membrane creates an electrochemical gradient that is termed the proton motive force (PMF) [48]. In addition, the binding of these hydronium ions promotes a pH on the surface of the cell that is two to three units lower than the pH of the bulk milieu [43-45]. Because hydronium ions cannot penetrate the cell wall or the membrane, they may re-enter the cell only through channels such as porins in general [49, 50]. The movement of these hydromium ions from the surface of the cell to the periplasm or cytoplasm is predicated upon systems that use the PMF as source of energy-namely the resistance nodulation division (RND) family of transporters. However, before this movement of hydronium ions is discussed, the structure and mechanism of the main efflux pump of *Escherichia coli* needs addressing.

E. coli has a multiplicity of efflux pumps that may exceed 30 in number [51]. However, the main efflux pump of this organism is the AcrAB-TolC efflux pump [52, 53] which when deleted, its function is replaced by the AcrEF-TolC efflux pump [51]. Both efflux pumps are members of the resistance nodulation division family of transporters [51] and consist of three proteins: the transporter AcrB coded by the gene *acrB* and is intimately attached to the plasma membrane; two fusion proteins AcrA coded by the gene *acrA* that flank the AcrB transporter and are believed to assist the movement of a substrate through the AcrB transporter [35]; and, TolC which is also part of other tri-unit efflux pumps of the organism [35], is contiguous with the AcrB transporter and provides a conduit for the extrusion of the

substrate [38]. The structure of the tripartite AcrAB-TolC efflux pump is in the plasma membrane is not yet defined. The AcrAB-TolC efflux pump has been studied for three decades and has been shown to extrude a large variety of unrelated compounds with widely different structures [35]. Although the means for the recognition of the substrate to be extruded appears to involve a pocket within the transporter, it appears to be defined by a phenyalanine residue [54]. Nevertheless, studies employing fluorochromes recognised by the AcrB transporter indicate that the binding and release of the substrate are pH dependent [55]. At low pH the dissociation of the substrate is high and at high pH it is very slow. In a physiological environment of ca. pH 7, if the dissociation of the substrate is slow or not at all, then the effectiveness of the pump to extrude a noxious agent would be nullified. However, since the pump functions at this pH, conditions that result in the dissociation of the substrate needed for continuous pump action must involve a decrease of the pH of the internal cavity of the pump to which the substrate is bound. It has been postulated that the lowering of the pH takes place by the generation of hydronium ions from metabolism [6] which pass from the cytoplasmic side of the plasma membrane through the transporter. At lower pH, there is no need for the generation of metabolically derived hydronium ions since these ions can be diverted by the PMF from the surface of the cell to the periplasm via porins. Whether hydronium ions are to be generated from the hydrolysis of ATP at high pH or used for the synthesis of ATP at low pH is a special function of ATP synthase [56-58].

Nevertheless, a model of the RND AcrAB-TolC efflux pump of Gram-negatives and suggested hypothesis for its mechanism of action is presented by Figure 1.

Figure 1. Model of the AcrAB-TolC efflux pump of a Gram-negative bacterium.



Hypothesis. At near neutral pH, Hydronium ions from hydrolysis of ATP by ATP synthase pass through the AcrB transporter, reduce the pH to a point that causes the release of the substrate. When the hydronium ions reach the surface of the cell they are distributed over that surface and bind to lipopolysaccharides and basic amino acids. When there is a need for hydronium ions for activity of the efflux pump and the pH is lower than neutral, and the hydrolysis of ATP is not favoured, hydronium ions from the surface of cell via the PMF mobilize through the Aqua porins and reach the transporter where they are pushed through the transporter by the peristaltic action caused by the fusion proteins. Substrates bound to the transporter dissociate when the pH is reduced by the flow of hydronium ions and are carried out by the flow of water.

Inhibitors of bacterial efflux pumps

The compound phenylalanine-arginine-β-naphthylamide (MC-207,110), otherwise known as PAβN , was created by Lomovskaya et al. [59] and reported to significantly decrease intrinsic and acquired resistance of *Pseudomona. aeruginosa* to fluoroquinolones [59]. Because acquired resistance

was related to the over-expression of the organism's main efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN, PAβN rapidly became known as an inhibitor of efflux pumps whenever resistance to given antibiotics is increased as a consequence of over-expression of efflux pumps . PAβN therefore was claimed to inhibit the efflux pumps of *Escherichia coli* [60, 61], *Enterobacter aerogenes* [61], *Acinetobacter baumannii* [62], *Brucella melitensis* [63], *Salmonella enterica* serovar Typhimurium [64, 65], *Yersinia pestis* [66] and all encountered Gram-negatives [6, 7, 8, 9, 35, 39, 65-71]. However, PAβN is not an inhibitor of an efflux pump but rather a competitor of substrates used by the given efflux pump [72-74] and recent evidence has suggested to indicate that it is also a permeabilising agent [75]. If we use the term "efflux pump inhibitor" (EPI) to mean a compound that affects the efflux activity of an efflux pump, then PAβN and other compounds that affect the activity of an efflux pump could be called an EPI. In this respect, phenothiazines [6, 7, 9, 41, 51, 73, 74, 76-83] and hydantoin compounds [84] are EPI's since they reduce the activity of intrinsic- and over-expressed efflux pumps. However, it must be noted that with respect to the phenothiazines chlorpromazine (CPZ) and thioridazine (TZ), they express their anti-efflux pump activity by inhibiting the binding of Ca2+ to enzymes involved in metabolism [85]. It should also be noted that a chelating agent such as EDTA also can inhibit the activity of an efflux pump by denying Ca2+ to energy systems dependent upon this cation for activity [85]. How hydantoins exert their inhibitory activity against the efflux pump system of Gram-negatives is not yet known. However, because they are also powerful inhibitors of the human ABC transporter Pgp-1 [86-88], they probably have an effect on an ABC transporter of the Gram-negative bacterium that recognises the ethidium bromide substrate used in the efflux pump assay.

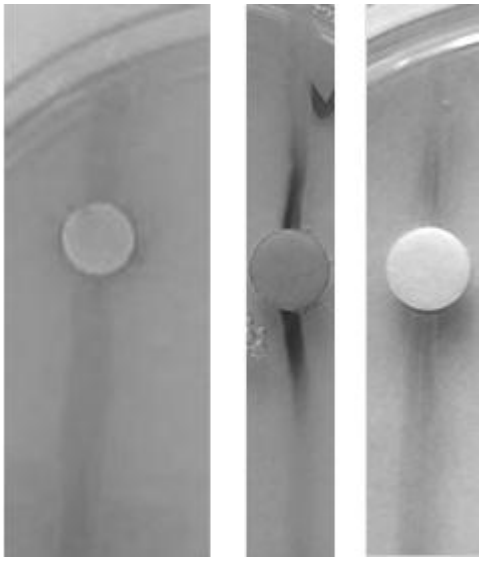
Phenothiazines are known to inhibit the motility of bacteria [89-90] and act synergistically with an inhibitor of the proton motive force (PMF) to inhibit motility [91]. Triflouromethyl ketones (TFs) are known to be inhibitors of motility by inhibiting access to the energy provided by the PMF for flagellae movement [90-95] and because phenothiazines also affect the PMF [6], the synergistic combination of a phenothiazine with TFs suggested that TFs might also have activity against PMF dependent efflux pumps of a Gram-negative bacterium such as *Escherichia coli*. Moreover, because TFs have similar structures to those of omeprasol, lansopresol, etc. all of which are strong inhibitors of the PMF, evaluation of 12 TFS for activity against the efflux pump of *Escherichia coli* provided evidence that indeed, some TFS were similarly powerful inhibitors of the PMF dependent efflux pump-supposedly, the main efflux pump AcrAB-Tol [13].

Inhibitors of the QS of bacteria

Inhibitors of a bacterial QS system must be distinguished from their activity on the producer of the signal, the responder to a QS signal or both. Quorum sensing inhibitors (QSIs) are compounds that specifically block QS systems without affecting bacterial growth. The QS system of bacteria that has received the greatest attention is the acylated homoserine lactone (AHL) system which produces and secretes AHL [96-113]. AHL acts as a communication molecule which regulates the behavior of the members of the bacterial population. Concerted behaviour such as swarming [99], production of surfactant which facililates movement on surfaces [99] and virulence factors by *Pseudomonas aeruginosa* [97], production and secretion of biofilms [104, 105] are examples among a growing list of concerted behaviours [100-103]. Obviously, the regulation of bacterial behavior and population density by AHLs suggests activity at the genetic level. The AHLs density-dependent regulatory systems rely on two proteins, an AHL synthase, most commonly a member of the LuxI family of proteins, and an AHL receptor protein belonging to the LuxR family of transcriptional regulators. Low population density cells produce a basal level of AHL that is dependent on an AHL synthase. With increase of population density, AHL accumulates in the medium and when it reaches a critical threshold concentration, the AHL molecule binds to its cognate receptor. The binding of AHL to its receptor promotes induction or repression of AHL-regulated genes. The genes which are regulated are responsible for a large number of functions such as bioluminescence [106], plasmid conjugal transfer [107], biofilm formation [104, 105], motility [101, 105], antibiotic biosynthesis [108-113], production of virulence factors [96], etc. It should be noted that *in vitro* experiments utilizing the cell free culture medium for purposes of studying the presence of QS signal

molecules, are difficult to carry out because of technical problems such as the very low concentration of these QS signal molecules present in the media, the instability of the QS signal molecule, and with attempts to concentrate the medium such as with lyophilisation, selective filtration, etc., the components of the concentrate cause formation of complexes that tie up or interfere with the QS signal molecule. Nevertheless, these difficulties can be overcome with selective design of the experimental protocol. An example of such as system is provided by Figure 2. In this example, a compound (a trifluoromethyl ketone) that is being studied for an effect on the responder of the QS signal, is impregnated onto a paper disk that contains the QS signal (acylated hydroxyl lactone-AHL) and is then placed directly on the agar streaked responder environmental bacterium CV026. As noted by Figure 2, the TF employed inhibits the production of colour that is generated by exposure to the QS signal AHL.

Figure 2. The effect of a trifluoromethyl ketone (TF) on the response of CV026 to the QS signal acylated hydroxyl lactone (AHL).



Legends:
A: Control disk with MM4 (20µg) alone (no colour).
B: Control disk with AHL (10ng) alone (deep purple coloration)
C: Disk with MM4 (20µg/disc) and AHL (10 ng) (very light purple coloration)

The inhibition of the QS system of pathogenic bacteria is a universal goal of drug discovery [114, 115]. Because the AHL system of Gram-negatives has been extensively studied and because in general, Gram-negatives due to their more complex cell envelope have greater resistance to current antibiotics as opposed to Gram-positives, the review of QS inhibitors will be mostly on pathogenic Gram-negatives.

Perhaps the best known inhibitors of the AHL QS system are homologues of AHL. N-butanoyl-L-homoserine lactone (C4-HSL) and N-hex anoyl-L-homoserine lactone (HHL) are produced in cultures of *Serratia liquefaciens* at a ratio of 10:1, respectively [116]. These analogues of AHL serve as autoinducers of swarming [116]. However, analogues of C4-HSL such as N-(3-oxododecanoyl)-l-homoserine lactone (3-oxo-C(12)-HSL) produced by *Pseudomonas aeruginosa* that do not affect cell growth of its own population as well as that of *Proteus mirabilis*, *Escherichia coli*, *Alcaligenes faecalis* and *Stenotrophomonas maltophilia*, inhibit completely the growth of Legionella pneumophila as well as the formation of its biofilm [117]. Among compounds that inhibit production and secretion of AHL are sulphur-containing AHL-analogues such as N-(heptylsulfanylacetyl)-L-homoserine lactone (HepS-AHL) which reduces production of protease by *Aeromonas salmonicida* [96]. Protease production is a virulence factor which when its production and release are blocked, render the bacterium less virulent [96].

Although it is obvious that analogues of QS signals such as AHLs may readily inhibit secretion and synthesis of QS signals, there may be a plethora of compounds that can similarly serve as inhibitors of QS signals, either generally or specifically. Among the general inhibitors of QS are phentothiazines [12, 118]. Because phenothiazines inhibit many energy dependent systems of bacteria such as motility [89, 90, 95], and these phenothiazines also inhibit efflux pumps of bacteria [6, 7, 9, 41, 51, 73, 74, 76-83], there seems to be a correlation between an active efflux pump system and a functional QS system. That this assumption is correct, recent evidence has been provided showing that the efflux pumps of the AHL responding environmental *Chromobacterium violaceum* (CV026) bacterium and that of *E. coli* are inhibited by the phenothiazine thioridazine (TZ) [12]. Because TZ is known to inhibit genes that regulate and code for efflux pumps of bacteria [41, 119, 120], it is possible that the inhibition of the responding CV0126 bacterium to AHLs [12] involves the inhibition of genes that code and regulate the efflux pump of the responder which is assumed to

recognise the AHL signal as an noxious agent and hence would extrude it to the environment [12]. Moreover, given the results that show that the phenothiazine structural scaffold is the component of the phenothiazine derivative that is responsible for the inhibition of QS [118], attention should be given to phenothiazines as parental compounds for derivatives with anti-QS properties. It should be noted that the secretion of biofilm takes place via the efflux pumps system of the bacterium [121, 122]. Consequently, the inhibition of an efflux pump should manifest itself as an inhibitor of the QS component responsible for biofilm formation.

During the past two decades drug development and discovery has focused on plants as sources of bioactive compounds [123-141]. However, as noted by the reviews cited, most of the focus has been on discovery of compounds from plant sources that have bioactivity in human pathology. Nevertheless, because of the successes encountered with compounds from plant sources and due to the ever increasing problem of multi-drug resistance that emanates from over-expressed efflux pumps [6-9], plants as sources of antimicrobial compound have during recent years assumed an important part in drug development and discovery [67, 142-158]. In particular, since the discovery of berberine a powerful inhibitor of bacterial efflux pumps [159], plants have become sources of inhibitors of efflux pumps [160-164]. Given that efflux pumps and the QS of bacteria have an intimate relationship as described in this review, attention has been focused on plants for potential sources of inhibitors of efflux pumps and QS systems. Essential oils from Columbian plants have yielded a large number of compounds that inhibit the QS system of responding bacteria such as limonene-carvone , the citral (geranial-neral) (isolated from Lippia alba), α-pinene (from Ocotea sp.), β-pinene (from Swinglea glutinosa), cineol (from Elettaria cardamomun), α-zingiberene (from Zingiber officinale) and pulegone (from Minthostachys mollis) [165]. Several other essential oils, in particular were shown to present promising inhibitory properties for the short chain AHL quorum sensing (QS) system in *Escherichia coli* containing the biosensor plasmid pJBA132, in particular Lippia alba. Citral was the only essential oil that presented some activity for the long chain AHL QS system in Pseudomonas putida containing the plasmid pRK-C12 [165]. Other essential oils crom rose, geranium, lavender and rosemary oils were shown to have potent inhibitory activity against the QS system of bacteria whereas essential oils from Eucalyptus and citrus oils presented moderate activity and those from chamomile, orange and juniper oils were ineffective [11]. Essential oil from Syzygium aromaticum (Clove) has also been shown to inhibit the QS systems of hromobacterium violaceum (CV12472 and CVO26) and *Pseudomonas aeruginosa* (PAO1) whereas essential oils from cinnamon, lavender and peppermint had moderate activity [166].

To our knowledge, no other work has been conducted for studies of essential oils as potential sources of inhibitors of the QS system. Moreover, given that an essential oil has now been shown to inhibit the efflux pump of antibiotic resistant Gram-negative bacteria [167], essential oils shown to inhibit the QS systems of bacteria are good candidates for evaluation for inhibitory activity against efflux pumps of multi-drug resistant Gram-negative bacteria.

The relationship between efflux pumps and the QS of bacteria seems well established. Given that efflux pumps of Gram-negative bacteria that bestow multi-drug resistant are dependent upon the PMF for activity [6, 8, 168-170], and since TFs inhibit the efflux pump of bacteria [13] supposedly by the inhibition of the PMF energy source [6, 8, 13], TFs were evaluated for activity against the QS sytem of bacteria [13]. As expected, the TFs that had the greatest specific activity against the efflux pump were the most active as inhibitors of the QS response [13]. These latter studies serve to support the intimate connection between efflux pumps and the QS system of bacteria. Consequently, compounds that affect both are good candidates for inhibitory properties against the secretion of biofilm matrix which is dependent upon the QS system [31-34] and the efflux pump for secretion of the biofilm [121, 122].

CONCLUDING REMARKS

The essence of this review is to correlate the relationship of the efflux pump system to the QS system of bacteria via the use of compounds that inhibit both systems. Simply put, inhibitors of the efflux pump system also, when studied, inhibit the QS system as well. Because the PMF dependent

efflux pump system of Gram-negatives that is over-expressed is responsible for the multi-drug phenotype of the bacterium, compounds that affect the PMF of the bacterium are candidates that will inhibit the activity of the pump. Consequently, this inhibition will inhibit the secretion of biofilm, and because biofilm is a deterrent to the action of antibiotics, compounds that affect the efflux pump system are promising candidates for clinical evaluation. The effect of compounds that inhibit the efflux pump on the QS system is at this time not completely understood. However, if we consider that QS signals directed at another bacterial species constitutes a noxious agent, then the extrusion of the QS signal by an efflux pump may be significantly affected by the inhibitor of the pump. With respect to concerted action by a bacterial population such as swarming that is brought about by a QS signal, the inhibitory qualities of an anti-QS compound may lie in the inhibition of the secretion of the QS signal via the efflux pump itself. Consequently, we should distinguish the effect of a compound on the producer of the QS signal versus the response of the same or different species of bacteria. At this time, little work has actually been reported concerning the QS of bacteria and the effect of compounds from different sources. Regardless, future studies are suggested to include a wider gamut of activities when studying the QS system of bacteria. Therefore, within one study, it may be very significant that any compound deemed worthy of consideration for QS study, be studied for effects on the producer of the QS signal, the responder to the QS signal, the efflux pump of the producer of the signal and the efflux pump of the responder. In this manner, a more complete understanding of how a compound can mediate its effects at the antibacterial level can be more fully understood.

ACKNOWLEDGEMENTS

We thank to Zoltán Varga for his contribution to the QS research and Mrs Aniko Vigyikán for technical assistance. L. Amaral was supported by BCC grant SFRH/BCC/51099/2010 provided by the Fundação para a Ciência e a Tecnologia (FCT) of Portugal and PTDC/SAU-FCF/102807/2008 provided by the UPMM This work was supported by the Foundation of Cancer Research Szeged, Szeged,Hungary , COST Action BM0701 (ATENS) of the European Commission/European Science Foundation, by EU-FSE/FEDER-PTDC/BIA-MIC/105509/2008 and EU-FSE/FEDERPTDC/SAU-FCF/102807/2008 from the Fundação para a Ciência e a Tecnologia (FCT) of Portugal.

CONFLICT OF INTERESTS

There is no conflict of interest.

REFERENCES

[1] Harrop JS, Styliaras JC, Ooi YC, Radcliff KE, Vaccaro AR, Wu C. Contributing factors to surgical site infections. *J Am Acad Orthop Surg.* **2012;** 20: 94-101.

[2] Tezer H, Canpolat FE, Dilmen U. Invasive fungal infections during the neonatal period: diagnosis, treatment and prophylaxis. *Expert Opin Pharmacother.* **2012;** 13: 193-205.

[3] Cipolla D, Giuffrè M, Mammina C, Corsello G. Prevention of nosocomial infections and surveillance of emerging resistances in NICU. *J Matern Fetal Neonatal Med.* **2011;** 24: 23-6.

[4] Calfee DP. The epidemiology, treatment, and prevention of transmission of methicillin-resistant *Staphylococcus aureus*. *J Infus Nurs.* **2011;** 34: 359-64.

[5] Rivera AM, Boucher HW. Current concepts in antimicrobial therapy against select gram-positive organisms: methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci. *Mayo Clin Proc.* **2011;** 86: 1230-43.

[6] Amaral L, Fanning S, Pagès JM. Efflux pumps of gram-negative bacteria: genetic responses to stress and the modulation of their activity by pH, inhibitors, and phenothiazines. *Adv Enzymol Relat Areas Mol Biol.* **2011;** 77: 61-108.

[7] Pagès JM, Amaral L, Fanning S. An Original Deal for New Molecule: Reversal of Efflux Pump Activity, A Rational Strategy to Combat Gram-Negative Resistant Bacteria. *Curr Med Chem.* **2011;** 18: 2969-80.

[8] Amaral L, Martins A, Molnar J, Kristiansen JE, Martins M,Viveiros M, Rodrigues L, Spengler G, Couto I, Ramos J, Dastidar S, Fanning S, McCusker M, Pages JM. Phenothiazines, Bacterial Efflux Pumps and Targeting the Macrophage for Enhanced Killing of Intracellular XDRTB. *In Vivo.* **2010;** 24: 409-24.

[9] Pagès JM, Amaral L. Mechanisms of Drug Efflux and Strategies to Combat Them, challenging the efflux pump of Gram negative bacteria. *Biochimica Biophysica Acta.* **2009;** 1794: 826-35.

[10] Amaral L, Spengler, G, Viveiros M, Rodrigues L, Martins A, Couto I, Martins M, Fanning S, Jean-Marie Pages and Molnar J. Assessment and comparison of efflux pumps of cancer cells and MDR bacteria under physiological conditions by a real-time semi-automated method. *Anticancer Res.* **2008;** 28: 3193-4.

[11] Szabó MA, Varga GZ, Hohmann J, Schelz Z, Szegedi E,

Amaral L, Molnár J. Inhibition of quorum sensing-regulation by plant extracts and Essential oils. *Phytother Res.* **2010;** 24: 782-6.

[12] Varga ZG, Szabó MA, Hohmann J, Schelz Z, Szegedi E, Amaral L, and Molnár J. Effect of phenothiazines and related compounds on quorum sensing regulated functions. *Lett Drug Discovery Design.* **2011;** 8: 133-7.

[13] Varga ZG, Armada A, Cerca P, Amaral L, Subki MA, Savka MA, Szegedi E, Kawase M, Motohashi N, Molnár J. Inhibition of quorum sensing and efflux pump system by trifluoromethyl ketone proton pump inhibitors. *In Vivo.* **2012;** 26: 277-85.

[14] Guillier L, Stahl V, Hezard B, Notz E, Briandet R. Modelling the competitive growth between Listeria monocytogenes and biofilm microflora of smear cheesewooden shelves. *Int J Food Microbiol.* **2008;** 128: 51-7.

[15] Komitopoulou E, Bainton NJ, Adams MR. Premature Salmonella Typhimurium growth inhibition in competition with other Gram-negative organisms is redox potential regulated via RpoS induction. *J Appl Microbiol.* **2004;** 97: 964-72.

[16] Yoon H, Klinzing G, Blanch HW. Competition for mixed substrates by icrobialpopulations. *Biotechnol Bioeng.* **1977;** 19: 1193-210.

[17] Stewart CR, Burnside DM, Cianciotto NP. The surfactant of Legionella pneumophila Is secreted in a TolC-dependent manner and is antagonistic toward other Legionella species. *J Bacteriol.* **2011;** 193: 5971-84.

[18] Klein GL, Soum-Soutéra E, Guede Z, Bazire A, Compère C, Dufour A. The anti-biofilm activity secreted by a marine Pseudoalteromonas strain. *Biofouling.* **2011;** 27: 931-40.

[19] Zakaria II, Rahman RN, Salleh AB, Basri M. Bacteriocin release protein-mediated secretory expression of recombinant chalcone synthase in *Escherichia coli*. *Appl Biochem Biotechnol.* **2011;** 165: 737-47.

[20] Zhang J, Yang Y, Teng D, Tian Z, Wang S, Wang J. Expression of plectasin in Pichia pastoris and its characterization as a new antimicrobial peptide against Staphyloccocus and Streptococcus. *Protein Expr Purif.* **2011;** 78: 189-96.

[21] Tiaden A, Hilbi H. α-Hydroxyketone Synthesis and Sensing by Legionella and Vibrio. *Sensors (Basel).* **2012;** 12: 2899-919.

[22] Zhu P, Li M. Recent progresses on AI-2 bacterial quorum sensing inhibitors. *Curr Med Chem.* **2012;** 19: 174-86.

[23] Venturi V, Bertani I, Kerényi A, Netotea S, Pongor S. Co-swarming and local collapse: quorum sensing conveys resilience to bacterial communities by localizing cheater mutants in *Pseudomonas aeruginosa*. *PLoS One.* **2010;** 5: e9998.

[24] Netotea S, Bertani I, Steindler L, Kerényi A, Venturi V, Pongor S. A simplemodel for the early events of quorum sensing in *Pseudomonas aeruginosa*: modeling bacterial swarming as the movement of an "activation zone". *Biol Direct.* **2009;** 4: 6.

[25] Lazar V. Quorum sensing in biofilms--how to destroy the bacterial citadels or their cohesion/power? *Anaerobe.* **2011;** 17: 280-5.

[26] Lazăr V, Chifiriuc MC. Architecture and physiology of microbial biofilms. *Roum Arch Microbiol Immunol.* **2010;** 69: 95-107.

[27] Bjarnsholt T, Tolker-Nielsen T, Høiby N, Givskov M. Interference of *Pseudomonas aeruginosa* signalling and biofilm formation for infection control. *Expert Rev Mol Med.* **2010;** 12: e11.

[28] Njoroge J, Sperandio V. Jamming bacterial communication: new approaches for the treatment of infectious diseases. *EMBO Mol Med.* **2009;** 1: 201-10.

[29] Rahman P, Dusane D, Zinjarde S, Venugopalan V, McLean R, Weber M. Quorum sensing: implications on rhamnolipid biosurfactant production. *Biotechnol Genet Eng Rev.* **2010;** 27: 159-84.

[30] Neu TR, Manz B, Volke F, Dynes JJ, Hitchcock AP, Lawrence JR. Advanced imaging techniques for assessment of structure, composition and function in biofilm systems. *FEMS Microbiol Ecol.* **2010;** 72: 1-21.

[31] Lear G, Anderson MJ, Smith JP, Boxen K, Lewis GD. Spatial and temporal heterogeneity of the bacterial communities in stream epilithic biofilms. *FEMS Microbiol Ecol.* **2008;** 65: 463-73.

[32] Filloux A, Vallet I. [Biofilm: set-up and organization of a bacterial community]. *Med Sci (Paris).* **2003;** 19: 77-83.

[33] Bortolaia C, Sbordone L. [Biofilms of the oral cavity. Formation, development and involvement in the onset of diseases related to bacterial plaque increase]. *Minerva Stomatol.* **2002;** 51: 187-92.

[34] Vergidis P, Patel R. Novel approaches to the diagnosis, prevention, and treatment of medical device-associated infections. *Infect Dis Clin North Am.* **2012;** 26: 173-86.

[35] Nikaido H. Structure and mechanism of RND-type multidrug efflux pumps. *Adv Enzymol Relat Areas Mol Biol.* **2011;** 77: 1-60.

[36] Li XZ, Nikaido H. Efflux-mediated drug resistance in bacteria: an update. *Drugs.* **2009;** 69: 1555-623.

[37] Moitra K, Silverton L, Limpert K, Im K, Dean M. Moving out: from sterol transport to drug resistance - the ABCG subfamily of efflux pumps. *Drug Metabol Drug Interact.* **2011;** 26: 105-11.

[38] Lorca GL, Barabote RD, Zlotopolski V, Tran C, Winnen B, Hvorup RN, Stonestrom AJ, Nguyen E, Huang LW, Kim DS, Saier MH Jr. Transport capabilities of eleven gram-positive bacteria: comparative genomic analyses. *Biochim Biophys Acta.* **2007;** 1768: 1342-66.

[39] Lewis K. In search of natural substrates and inhibitors of MDR pumps. *J Mol Microbiol Biotechnol.* **2001;** 3: 247-54.

[40] Marshall NJ, Piddock LJ. Antibacterial efflux systems. *Microbiologia.* **1997;** 13: 285-300.

[41] Spengler G, Rodrigues L, Martins M, McCusker M, Cerca P, Machado L, Costa SS, Dokou, Couto I, Viveiros M, Fanning S, Martins A, Molnar J and Amaral L. Genetic Response of *Salmonella enterica* serovar Typhimurium to Thioridazine rendering the organism resistant to the agent. *Int J Antimicrobial Agents.* **2012;** 39: 16-21.

[42] Bhattacharjee H, Zhou T, Li J, Gatti DL, Walmsley AR, Rosen BP. Structure-function relationships in an anion-translocating ATPase. *Biochem Soc Trans.* **2000;** 28: 520-6.

[43] Mulkidjanian AY. Proton in the well and through the desolvation barrier. *Biochim Biophys Acta.* **2006;** 1757: 415-27.

[44] Mulkidjanian AY, Heberle J, Cherepanov DA. Protons @ interfaces: implications for biological energy conversion. *Biochim Biophys Acta.* **2006;** 1757: 913-30.

[45] Mulkidjanian AY, Cherepanov DA, Heberle J, Junge W. Proton transfer dynamics at membrane/water interface and mechanism of biological energy conversion. *Biochemistry (Mosc).* **2005;** 70: 251-6.

[46] Roberts IS. The biochemistry and genetics of capsular polysaccharide production in bacteria. *Annu Rev Microbiol.* **1996;** 50: 285-315.

[47] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev.* **2003;** 67: 593-656.

[48] Prebble JN. Successful theory development in biology: a consideration of the theories of oxidative phosphorylation proposed by Davies and Krebs, Williams and Mitchell. *Biosci Rep.* **1996;** 16: 207-15.

[49] Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol.* **2008;** 6: 893-903.

[50] Achouak W, Heulin T, Pagès JM. Multiple facets of bacterial porins. *FEMS Microbiol Lett.* **2001;** 199: 1-7.

[51] Viveiros M, Jesus A, Brito M, Leandro C, Martins M, Ordway D, Molnar AM, Molnar J, Amaral L. Inducement and reversal of tetracycline resistance in *Escherichia coli* K-12 and the expression of proton gradient dependent multidrug efflux pump genes. *Antimicrob Agents Chemother.* **2005;** 49: 3578-82.

[52] Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol Microbiol.* **1995;** 16: 45-55.

[53] Okusu H, Ma D, Nikaido H. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. *J Bacteriol.* **1996;** 178: 306-8.

[54] Eicher T, Cha HJ, Seeger MA, Brandstätter L, El-Delik J, Bohnert JA, Kern WV, Verrey F, Grütter MG, Diederichs K, Pos KM. Transport of drugs by the multidrug transporter AcrB involves an access and a deep binding pocket that are separated by a switch-loop. *Proc Natl Acad Sci U S A.* **2012;** 109: 5687-92.

[55] Su CC, Yu EW. Ligand-transporter interaction in the AcrB multidrug efflux pump determined by fluorescence polarization assay. *FEBS Lett.* **2007;** 581: 4972-6.

[56] Padan E, Bibi E, Ito M, Krulwich TA. Alkaline pH homeostasis in bacteria: new insights. *Biochim Biophys Acta.* **2005;** 1717: 67-88.

[57] Feniouk BA, Junge W. Regulation of the F0F1-ATP synthase: the conformation of subunit epsilon might be determined by directionality of subunit gamma rotation. *FEBS Lett.* **2005;** 579: 5114-8.

[58] Walker JE, Saraste M, Gay NJ. The unc operon. Nucleotide sequence, regulation and structure of ATP-synthase. *Biochim Biophys Acta.* **1984;** 768: 164-200.

[59] Lomovskaya O, Warren MS, Lee A, Galazzo J, Fronko R, Lee M, Blais J, Cho D, Chamberland S, Renau T, Leger R, Hecker S, Watkins W, Hoshino K, Ishida H, Lee VJ. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrob Agents Chemother.* **2001;** 45: 105-16.

[60] Chollet R, Chevalier J, Bryskier A, Pagès JM. The AcrAB-TolC pump is involved in macrolide resistance but not in telithromycin efflux in *Enterobacter aerogenes* and *Escherichia coli*. *Antimicrob Agents Chemother.* **2004;** 48: 3621-4.

[61] Huguet F, Melet A, Alves de Sousa R, Lieutaud A, Chevalier J, Maigre L, Deschamps P, Tomas A, Leulliot N, Pages JM, Artaud I. Hydroxamic Acids as Potent Inhibitors of Fe(II) and Mn(II) E. coli Methionine Aminopeptidase: Biological Activities and X-ray Structures of Oxazole Hydroxamate-EcMetAP-Mn Complexes. *ChemMedChem.* **2012;** 7: 1020-30.

[62] Pannek S, Higgins PG, Steinke P, Jonas D, Akova M, Bohnert JA, Seifert H, Kern WV. Multidrug efflux inhibition in *Acinetobacter baumannii*: comparison between 1-(1-naphthylmethyl)-piperazine and phenyl-arginine-beta-naphthylamide. *J Antimicrob Chemother.* **2006;** 57: 970-4.

[63] Ravanel N, Gestin B, Maurin M. *In vitro* selection of fluoroquinolone resistance in *Brucella melitensis*. *Int J Antimicrob Agents.* **2009;** 34: 76-81.

[64] Coldham NG, Webber M, Woodward MJ, Piddock LJ. A 96-well plate fluorescence assay for assessment of cellular permeability and active efflux in *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. *J Antimicrob Chemother.* **2010;** 65: 1655-63.

[65] Karczmarczyk M, Martins M, Quinn T, Leonard N, Fanning S. Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. *Appl Environ Microbiol.* **2011;** 77: 7113-20.

[66] Lister IM, Raftery C, Mecsas J, Levy SB. *Yersinia pestis* AcrAB-TolC in antibiotic resistance and virulence. *Antimicrob Agents Chemother.* **2012;** 56: 1120-3.

[67] Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, Bolla JM. Geraniol restores antibiotic activities against multidrug-resistant isolates from gram-negative species. *Antimicrob Agents Chemother.* **2009;** 53: 2209-11.

[68] Schumacher A, Steinke P, Bohnert JA, Akova M, Jonas D, Kern WV. Effect of 1-(1-naphthylmethyl)-piperazine, a novel putative efflux pump inhibitor, on antimicrobial drug susceptibility in clinical isolates of Enterobacteriaceae other than *Escherichia coli*. *J Antimicrob Chemother.* **2006;** 57: 344-8.

[69] Routh MD, Zalucki Y, Su CC, Zhang Q, Shafer WM, Yu EW. Efflux pumps of the resistance-nodulation-division family: a perspective of their structure, function, and regulation in gram-negative bacteria. *Adv Enzymol Relat Areas Mol Biol.* **2011;** 77: 109-46.

[70] Vila J, Fàbrega A, Roca I, Hernández A, Martínez JL. Efflux pumps as an important mechanism for quinolone resistance. *Adv Enzymol Relat Areas Mol Biol.* **2011;** 77: 167-235.

[71] Bolla JM, Alibert-Franco S, Handzlik J, Chevalier J, Mahamoud A, Boyer G, Kieć-Kononowicz K, Pagès JM. Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. *FEBS Lett.* **2011;** 585: 1682-90.

[72] Lomovskaya O, Zgurskaya HI, Totrov M, Watkins WJ. Waltzing transporters and 'the dance macabre' between humans and bacteria. *Nat Rev Drug Discov.* **2007;** 6: 56-65.

[73] Viveiros M, Dupont M, Rodrigues L, Couto I, Davin-Regli A, Martins M, Pagès JM, Amaral L. Antibiotic stress, genetic response and altered permeability of *E. coli*.

PLoS One. **2007;** 2: e365.

[74] Martins A, Spengler G, Rodrigues L, Viveiros M, Ramos J, Martins M, Couto I, Fanning S, Pagès JM, Bolla JM, Molnar J, Amaral L. pH Modulation of efflux pump activity of multi-drug resistant *Escherichia coli*: protection during its passage and eventual colonization of the colon. *PLoS One.* **2009;** 4: e6656.

[75] Davin-Regli A, Chollet R, Bredin J, Chevalier J, Lepine F, Pagès JM. Enterobacter gergoviae and the prevalence of efflux in parabens resistance. *J Antimicrob Chemother.* **2006;** 57: 757-60.

[76] Amaral L, Viveiros M, Molnar J. Antimicrobial activity of phenothiazines. *In Vivo.* **2004;** 18: 725-31.

[77] Marquez B. Bacterial efflux systems and efflux pumps inhibitors. *Biochimie.* **2005;** 87: 1137-47.

[78] Chan YY, Ong YM, Chua KL. Synergistic interaction between phenothiazines and antimicrobial agents against Burkholderia pseudomallei. *Antimicrob Agents Chemother.* **2007;** 51: 623-30.

[79] Lehtinen J, Lilius EM. Promethazine renders *Escherichia coli* susceptible to penicillin G: real-time measurement of bacterial susceptibility by fluoro-luminometry. *Int J Antimicrob Agents.* **2007;** 30: 44-51.

[80] Tegos GP, Masago K, Aziz F, Higginbotham A, Stermitz FR, Hamblin MR. Inhibitors of bacterial multidrug efflux pumps potentiate antimicrobial photoinactivation. *Antimicrob Agents Chemother.* **2008;** 52: 3202-9.

[81] Coutinho HD, Costa JG, Lima EO, Falcão-Silva VS, Siqueira-Júnior JP. *In vitro* interference of Hyptis martiusii Benth. & chlorpromazine against an aminoglycoside-resistant *Escherichia coli*. *Indian J Med Res.* **2009;** 129: 566-8.

[82] Takács D, Cerca P, Martins A, Riedl Z, Hajós G, Molnár J, Viveiros M, Couto I,Amaral L. Evaluation of forty new phenothiazine derivatives for activity against intrinsic efflux pump systems of reference *Escherichia coli*, Salmonella Enteritidis, Enterococcus faecalis and *Staphylococcus aureus* strains. *In Vivo.* **2011;** 25: 719-24.

[83] Bhardwaj AK, Mohanty P. Bacterial Efflux Pumps Involved in Multidrug Resistance and their Inhibitors: Rejuvinating the Antimicrobial Chemotherapy. *Recent Pat Antiinfect Drug Discov.* **2012;** 7: 73-89.

[84] Machado L, Spengler G, Evaristo M, Handzlik J, Molnár J, Viveiros M, Kiec-Kononowicz K, Amaral L. Biological activity of twenty-three hydantoin derivatives on intrinsic efflux pump system of *Salmonella enterica* serovar Enteritidis NCTC 13349. *In Vivo.* **2011;** 25: 769-72.

[85] Martins A, Machado L, Costa S, Cerca P, Spengler G, Viveiros M, Amaral L. Role of calcium in the efflux system of *Escherichia coli*. *Int J Antimicrob Agents.* **2011;** 37: 410-4.

[86] Martins A, Dymek A, Handzlik J, Spengler G, Armada A, Molnar J, Kiéc-Kononowicz K, Amaral L. Activity of fourteen new hydantoin compounds on the human ABCB1 efflux pump. *In Vivo.* **2012;** 26: 293-7.

[87] Spengler G, Evaristo M, Handzlik J, Serly J, Molnár J, Viveiros M, Kiéc-Kononowicz K, Amaral L. Biological activity of hydantoin derivatives on P-glycoprotein (ABCB1) of mouse lymphoma cells. *Anticancer Res.* **2010;** 30: 4867-71.

[88] Spengler G, Handzlik J, Ocsovszki I, Viveiros M, Kiec-Kononowicz K, Molnar J, Amaral L. Modulation of multidrug efflux pump activity by new hydantoin derivatives on colon adenocarcinoma cells without inducing apoptosis. *Anticancer Res.* **2011;** 31: 3285-8.

[89] Molnár J, Ren J, Kristiansen JE, Nakamura MJ. Effects of some tricyclic psychopharmacons and structurally related compounds on motility of Proteus vulgaris. *Antonie Van Leeuwenhoek.* **1992;** 62: 319-20.

[90] Ren JK, Petöfi S, Molnár J. Mechanisms of antimotility action of tricyclic compounds in Proteus vulgaris. *Acta Microbiol Hung.* **1993;** 40: 369-377.

[91] Spengler G, Molnár A, Klausz G, Mándi Y, Kawase M, Motohashi N, Molnár J. The antimotility action of a trifluoromethyl ketone on some gram-negative bacteria. *Acta Microbiol Immunol Hung.* **2004;** 51: 351-8.

[92] Molnár A, Wolfart K, Kawase M, Motohashi N, Molnár J. Effect of a trifluoromethyl ketone on the motility of proton pump-deleted mutant of *Escherichia coli* strain and its wild-type. *In Vivo.* **2004;** 18: 505-7.

[93] Wolfart K, Molnar A, Kawase M, Motohashi N, Molnar J. Effects of trifluoromethyl ketones on the motility of Proteus vulgaris. *Biol Pharm Bull.* **2004;** 27: 1462-4.

[94] Spengler G, Molnar A, Klausz G, Mandi Y, Kawase M, Motohashi N, Molnar J. Inhibitory action of a new proton pump inhibitor, trifluoromethyl ketone derivative, against the motility of clarithromycin-susceptible and-resistant Helicobacter pylori. *Int J Antimicrob Agents.* **2004;** 23: 631-3.

[95] Kawase M, Motohashi N, Sakagami H, Kanamoto T, Nakashima H, Ferenczy L, Wolfard K, Miskolci C, Molnár J. Antimicrobial activity of trifluoromethyl ketones and their synergism with promethazine. *Int J Antimicrob Agents.* **2001;** 18: 161-5.

[96] Smith D, Wang JH, Swatton JE, Davenport P, Price B, Mikkelsen H, Stickland H, Nishikawa K, Gardiol N, Spring DR, Welch M. Variations on a theme: diverse N-acyl homoserine lactone-mediated quorum sensing mechanisms in gram-negative bacteria. *Sci Prog.* **2006;** 89: 167-211.

[97] Juhas M, Eberl L, Tümmler B. Quorum sensing: the power of cooperation in the world of Pseudomonas. *Environ Microbiol.* **2005;** 7: 459-71.

[98] Rasch M, Kastbjerg VG, Bruhn JB, Dalsgaard I, Givskov M, Gram L. Quorum sensing signals are produced by *Aeromonas salmonicida* and quorum sensing inhibitors can reduce production of a potential virulence factor. *Dis Aquat Organ.* **2007;** 78: 105-13.

[99] Heurlier K, Dénervaud V, Haas D. Impact of quorum sensing on fitness of *Pseudomonas aeruginosa*. *Int J Med Microbiol.* **2006;** 296: 93-102.

[100] Wei JR, Lai HC. N-acylhomoserine lactone-dependent cell-to-cell communication and social behavior in the genus Serratia. *Int J Med Microbiol.* **2006;** 296: 117-24.

[101] Daniels R, Vanderleyden J, Michiels J. Quorum sensing and swarming migration in bacteria. *FEMS Microbiol Rev.* **2004;** 28: 261-89.

[102] Di Cagno R, De Angelis M, Calasso M, Gobbetti M. Proteomics of the bacterial cross-talk by quorum sensing. *J Proteomics.* **2011;** 74: 19-34.

[103] Van Houdt R, Givskov M, Michiels CW. Quorum sensing in Serratia. *FEMS Microbiol Rev.* **2007;** 31: 407-24.

[104] Zavil'gel'skiĭ GB, Manukhov IV. ["Quorum sensing", or

how bacteria "talk" to each other]. *Mol Biol (Mosk).* **2001;** 35: 268-77.

[105] Eberl L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst Appl Microbiol.* **1999;** 22: 493-506.

[106] Flodgaard LR, Dalgaard P, Andersen JB, Nielsen KF, Givskov M, Gram L. Nonbioluminescent strains of Photobacterium phosphoreum produce the cell-to-cell communication signal N-(3-Hydroxyoctanoyl)homoserine lactone. *Appl Environ Microbiol.* **2005;** 71: 2113-20.

[107] Atkinson S, Sockett RE, Cámara M, Williams P. Quorum sensing and the lifestyle of Yersinia. *Curr Issues Mol Biol.* **2006;** 8: 1-10.

[108] Berger M, Neumann A, Schulz S, Simon M, Brinkhoff T. Tropodithietic acid production in Phaeobacter gallaeciensis is regulated by N-acyl homoserine lactone-mediated quorum sensing. *J Bacteriol.* **2011;** 193: 6576-85.

[109] Lee Y, Yeom J, Kim J, Jung J, Jeon CO, Park W. Phenotypic and physiological alterations by heterologous acylhomoserine lactone synthase expression in Pseudomonas putida. *Microbiology.* **2010;** 156: 3762-72.

[110] Kim JH, Lee SC, Kyeong HH, Kim HS. A genetic circuit system based on quorum sensing signaling for directed evolution of quorum-quenching enzymes. *Chembiochem.* **2010;** 11: 1748-53.

[111] Weeks JN, Galindo CL, Drake KL, Adams GL, Garner HR, Ficht TA. *Brucella melitensis* VjbR and C12-HSL regulons: contributions of the N-dodecanoyl homoserine lactone signaling molecule and LuxR homologue VjbR to gene expression. *BMC Microbiol.* **2010;** 10: 167.

[112] Liu X, Bimerew M, Ma Y, Müller H, Ovadis M, Eberl L, Berg G, Chernin L. Quorum-sensing signaling is required for production of the antibiotic pyrrolnitrin in a rhizospheric biocontrol strain of Serratia plymuthica. *FEMS Microbiol Lett.* **2007;** 270: 299-305.

[113] Zahin M, Hasan S, Aqil F, Khan MS, Husain FM, Ahmad I. Screening of certain medicinal plants from India for their anti-quorum sensing activity. *Indian J Exp Biol.* **2010;** 48: 1219-24.

[114] Vattem DA, Mihalik K, Crixell SH, McLean RJ. Dietary phytochemicals as quorum sensing inhibitors. *Fitoterapia.* **2007;** 78: 302-10.

[115] Eberl L, Winson MK, Sternberg C, Stewart GS, Christiansen G, Chhabra SR, Bycroft B, Williams P, Molin S, Givskov M. Involvement of N-acyl-L-hormoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Mol Microbiol.* **1996;** 20: 127-36.

[116] Kimura S, Tateda K, Ishii Y, Horikawa M, Miyairi S, Gotoh N, Ishiguro M, Yamaguchi K. *Pseudomonas aeruginosa* Las quorum sensing autoinducer suppresses growth and biofilm production in Legionella species. *Microbiology.* **2009;** 155: 1934-9.

[117] Khalilzadeh P, Lajoie B, El Hage S, Furiga A, Baziard G, Berge M, Roques C. Growth inhibition of adherent *Pseudomonas aeruginosa* by an N-butanoyl-L-homoserine lactone analog. *Can J Microbiol.* **2010;** 56: 317-25.

[118] Ni N, Choudhary G, Li M, Wang B. A new phenothiazine structural scaffold as inhibitors of bacterial quorum sensing in Vibrio harveyi. *Biochem Biophys Res Commun.* **2009;** 382: 153-6.

[119] Dutta NK, Mehra S, Kaushal D. A Mycobacterium tuberculosis sigma factor network responds to cell-envelope damage by the promising anti-mycobacterial thioridazine. *PLoS One.* **2010;** 5: e10069.

[120] Dutta NK, Mazumdar K, Dastidar SG, Karakousis PC, Amaral L. New patentable use of an old neuroleptic compound thioridazine to combat tuberculosis: a gene regulation perspective. *Recent Pat Antiinfect Drug Discov.* **2011;** 6: 128-38.

[121] Gillis RJ, White KG, Choi KH, Wagner VE, Schweizer HP, Iglewski BH. Molecular basis of azithromycin-resistant *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother.* **2005;** 49: 3858-67.

[122] Pearson JP, Van Delden C, Iglewski BH. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J Bacteriol.* **1999;** 181: 1203-10.

[123] Ramesha BT, Gertsch J, Ravikanth G, Priti V, Ganeshaiah KN, Uma Shaanker R. Biodiversity and chemodiversity: future perspectives in bioprospecting. *Curr Drug Targets.* **2011;** 12: 1515-30.

[124] Martins S, Mussatto SI, Martínez-Avila G, Montañez-Saenz J, Aguilar CN, Teixeira JA. Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. *Biotechnol Adv.* **2011;** 29: 365-73.

[125] Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and analysis of polyphenols: recent trends. *Crit Rev Biotechnol.* **2011;** 31: 227-49.

[126] Liu Q, Chen L, Hu L, Guo Y, Shen X. Small molecules from natural sources, targeting signaling pathways in diabetes. *Biochim Biophys Acta.* **2010;** 1799: 854-65.

[127] Villa FA, Gerwick L. Marine natural product drug discovery: Leads for treatment of inflammation, cancer, infections, and neurological disorders. *Immunopharmacol Immunotoxicol.* **2010;** 32: 228-37.

[128] Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF. Revealing the power of the natural red pigment lycopene. *Molecules.* **2010;** 15: 959-87.

[129] Furukawa H. [Search of novel bioactive natural products from plant sources--novel structures and biological activities--]. *Yakugaku Zasshi.* **2009;** 129: 1155-75.

[130] Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. *Med Res Rev.* **2009;** 29: 767-820.

[131] Sok DE, Cui HS, Kim MR. Isolation and bioactivities of furfuran type lignin compounds from edible plants. *Recent Pat Food Nutr Agric.* **2009;** 1: 87-95.

[132] Itokawa H, Morris-Natschke SL, Akiyama T, Lee KH. Plant-derived natural product research aimed at new drug discovery. *J Nat Med.* **2008;** 62: 263-80.

[133] Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. Chemical and biological characterisation of nutraceutical compounds of broccoli. *J Pharm Biomed Anal.* **2006;** 41: 1508-22.

[134] Baker DD, Alvi KA. Small-molecule natural products: new structures, new activities. *Curr Opin Biotechnol.* **2004;** 15: 576-83.

[135] Hazra B, Das Sarma M, Sanyal U. Separation methods of quinonoid constituents of plants used in Oriental traditional medicines. *J Chromatogr B Analyt Technol*

Biomed Life Sci. **2004;** 812: 259-75.

[136] Rao BN. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pac J Clin Nutr.* **2003;** 12: 9-22.

[137] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med.* **2002;** 113 Suppl 9B: 71S-88S.

[138] Setchell KD. Soy isoflavones--benefits and risks from nature's selective estrogen receptor modulators (SERMs). *J Am Coll Nutr.* **2001;** 20: 354S-62S; discussion 381S-3S.

[139] Rao AV, Gurfinkel DM. The bioactivity of saponins: triterpenoid and steroidal glycosides. *Drug Metabol Drug Interact.* **2000;** 17: 211-35.

[140] Cragg GM, Newman DJ. Discovery and development of antineoplastic agents from natural sources. *Cancer Invest.* **1999;** 17: 153-63.

[141] Grabley S, Thiericke R. Bioactive agents from natural sources: trends in discovery and application. *Adv Biochem Eng Biotechnol.* **1999;** 64: 101-54.

[142] Juneja VK, Dwivedi HP, Yan X. Novel natural food antimicrobials. *Annu Rev Food Sci Technol.* **2012;** 3: 381-403.

[143] Nematollahi A, Aminimoghadamfarouj N, Wiart C. Reviews on 1,4-naphthoquinones from Diospyros L. *J Asian Nat Prod Res.* **2012;** 14: 80-8.

[144] Cocquyt K, Cos P, Herdewijn P, Maes L, Van den Steen PE, Laekeman G. Ajuga emota Benth.: from ethnopharmacology to phytomedical perspective in the treatment of malaria. *Phytomedicine.* **2011;** 18: 1229-37.

[145] Gilca M, Gaman L, Panait E, Stoian I, Atanasiu V. Chelidonium majus—an integrative review: traditional knowledge versus modern findings. *Forsch Komplementmed.* **2010;** 17: 241-8.

[146] Wang H, Cui Y, Zhao C. Flavonoids of the genus Iris (Iridaceae). *Mini Rev Med Chem.* **2010;** 10: 643-61.

[147] Batista R, Silva Ade J Jr, de Oliveira AB. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. *Molecules.* **2009;** 14: 3037-72.

[148] Macías FA, Marín D, Oliveros-Bastidas A, Molinillo JM. Rediscovering the bioactivity and ecological role of 1,4-benzoxazinones. *Nat Prod Rep.* **2009;** 26: 478-89.

[149] Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ. application of natural antimicrobials for food preservation. *J Agric Food Chem.* **2009;** 57: 5987-6000.

[150] Bedoya LM, Bermejo P, Abad MJ. Anti-infectious activity in the Cistaceae family in the Iberian Peninsula. *Mini Rev Med Chem.* **2009;** 9: 519-25.

[151] Kaur K, Jain M, Kaur T, Jain R. Antimalarials from nature. *Bioorg Med Chem.* **2009;** 17: 3229-56.

[152] Aponte JC, Vaisberg AJ, Rojas R, Sauvain M, Lewis WH, Lamas G, Sarasara C, Gilman RH, Hammond GB. A multipronged approach to the study of Peruvian ethnomedicinal plants: a legacy of the ICBG-Peru Project. *J Nat Prod.* **2009;** 72: 524-56.

[153] Naithani R, Huma LC, Holland LE, Shukla D, McCormick DL, Mehta RG, Moriarty RM. Antiviral activity of phytochemicals: a comprehensive review. *Mini Rev Med Chem.* **2008;** 8: 1106-33.

[154] Fu G, Pang H, Wong YH. Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics. *Curr Med Chem.* **2008;** 15: 2592-613.

[155] Itokawa H, Morris-Natschke SL, Akiyama T, Lee KH. Plant-derived natural product research aimed at new drug discovery. *J Nat Med.* **2008;** 62: 263-80.

[156] Fiore C, Eisenhut M, Krausse R, Ragazzi E, Pellati D, Armanini D, Bielenberg J. Antiviral effects of Glycyrrhiza species. *Phytother Res.* **2008;** 22: 141-8.

[157] Copp BR, Pearce AN. Natural product growth inhibitors of Mycobacterium tuberculosis. *Nat Prod Rep.* **2007;** 24: 278-97.

[158] Stavri M, Piddock LJ, Gibbons S. Bacterial efflux pump inhibitors from natural sources. *J Antimicrob Chemother.* **2007;** 59: 1247-60.

[159] Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. *Proc Natl Acad Sci U S A.* **2000;** 97: 1433-7.

[160] Martins A, Vasas A, Viveiros M, Molnár J, Hohmann J, Amaral L. Antibacterial properties of compounds isolated from Carpobrotus edulis. *Int J Antimicrob Agents.* **2011;** 37: 438-44.

[161] Ramalhete C, Spengler G, Martins A, Martins M, Viveiros M, Mulhovo S, Ferreira MJ, Amaral L. Inhibition of efflux pumps in methicillin-resistant *Staphylococcus aureus* and Enterococcus faecalis resistant strains by triterpenoids from Momordica balsamina. *Int J Antimicrob Agents.* **2011;** 37: 70-4.

[162] Martins A, Vasas A, Schelz Z, Viveiros M, Molnár J, Hohmann J, Amaral L. Constituents of Carpobrotus edulis inhibit P-glycoprotein of MDR1-transfected mouse lymphoma cells. *Anticancer Res.* **2010;** 30: 829-35.

[163] Spengler G, Ramalhete C, Martins M, Martins A, Serly J, Viveiros M, Molnár J, Duarte N, Mulhovo S, Ferreira MJ, Amaral L. Evaluation of cucurbitane-typetriterpenoids from Momordica balsamina on P-glycoprotein (ABCB1) by flow cytometry and real-time fluorometry. *Anticancer Res.* **2009;** 29: 3989-93.

[164] Ordway D, Hohmann J, Viveiros M, Viveiros A, Molnar J, Leandro C, Arroz MJ, Gracio MA, Amaral L. Carpobrotus edulis methanol extract inhibits the MDR efflux pumps, enhances killing of phagocytosed S. aureus and promotes immune modulation. *Phytother Res.* **2003;** 17: 512-9.

[165] Jaramillo-Colorado B, Olivero-Verbel J, Stashenko EE, Wagner-Döbler I, Kunze B. Anti-quorum sensing activity of essential oils from Colombian plants. *Nat Prod Res.* **2012;** 26: 1075-86.

[166] Khan MS, Zahin M, Hasan S, Husain FM, Ahmad I. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett Appl Microbiol.* **2009;** 49: 354-60.

[167] Fadli M, Chevalier J, Saad A, Mezrioui NE, Hassani L, Pages JM. Essential oils from Moroccan plants as potential chemosensitisers restoring antibiotic activity in resistant Gram-negative bacteria. *Int J Antimicrob Agents.* **2011;** 38: 325-30.

[168] Murakami S. [Molecular mechanism of multi-drug resistance]. *Nihon Rinsho.* **2008;** 66: 193-203.

[169] Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol.* **2002;** 92 Suppl: 65S-71S.

[170] Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux 168-pumps. *Clin Infect Dis.* **1998;** 27 Suppl 1: S32-41.