Bioactive Natural Products: An Overview, with Particular Emphasis on Those Possessing Potential to Inhibit Microbial Quorum Sensing

Vijay Kothari, Pooja Patel, and Chinmayi Joshi

Abstract Bioactive natural products have formed the core of most ancient systems of healthcare and medicine. Crude natural preparations have been used for relief in a variety of infections and disease conditions. This review starts with a general description of the bioactive natural products, followed by the information on natural products being used for dealing with infectious microorganisms. In the latter section, much emphasis has been on the natural products capable of disrupting microbial communication, i.e., quorum sensing. Quorum sensing inhibitors are being expected to emerge as an important class of novel therapeutic agents in the future. Few other issues, important while performing lab experiments with natural products, are also touched upon.

Keywords Bioactive natural products • Quorum sensing • Quorum sensing inhibitors

1 Prelude

Since the start of this century, there has been an increasing interest among researchers in exploring the variety of biological activities possessed by different natural products (NPs). Though natural products (largely secondary metabolites) from both terrestrial and marine origin are being investigated, much of the work has focused on natural products of plant origin. Plant preparations have formed the core of most of the ancient systems of medicine. For example, one of the most ancient systems of medicine/healthcare—*Ayurved*—has been practiced widely in India and neighboring countries like Sri Lanka (Chopra and Doiphode 2002). *Atharvaved* (around 1200 BC), *Charaka Samhita*, and *Sushruta Samhita* (100–500 BC) are the underlying classics containing detailed descriptions of over 700 herbs (Dash and Sharma 2001). Descriptions of the use of natural substances for medicinal purposes

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can be located in texts as old as 78 A.D.; for example, *De Materia Medica*, written by Dioscorides, mentions thousands of medicinal plants (Tyler et al. 1988). In ancient times, human lifestyle was such that for every need they had to look into the nature as a source. Today, even when the mankind has developed the skill for synthesizing different molecules in the chemistry lab, we look into the nature to find new classes (i.e., novel structures) of bioactive molecules. Many of the natural secondary metabolites are large molecules with complex structures, and it is not always possible to synthesize them chemically. Few others are first extracted from some natural source, and identified as a *lead* molecule, following which that structure can serve as scaffold for synthetic products. In any case, screening natural products for the desired bioactivity remains an attractive option. In this article, we start with a short description of the bioactive natural products and then focus particularly on the natural products capable of interfering with microbial quorum sensing. Toward the end of the article, we describe some of the issues important for a natural product researcher.

NPs are the substances found in nature, i.e., synthesized by a living organism. These NPs can have one or more pharmacological or biological activities (Koehn and Carter 2005). Among these NPs, primary metabolites usually have some essential role in a cell/organism that produces them, whereas secondary metabolites generally are used by the producing organisms to perform accessory (but important) functions such as controlling natural relationships, particularly those related to defense against predation, competition for resources, interspecies communication for mating and hunting, etc. Owing to their interesting and potentially useful properties, secondary metabolites can prove to be beneficial to humans. NP can be used as therapeutic agents for managing conditions such as cancer, inflammation, bacterial infections, etc. (Bhatnagar and Kim 2010; Lv et al. 2011; Gyawali and Ibrahim 2012). Table 1 lists some of the reported therapeutic uses of certain NP. NP research holds its value as one of the most thriving sources of drugs, while offering a wide range of structural diversities and biological activities. Hitherto, only a fraction of the world's biodiversity has been investigated for biological activity, and a larger lot remains to be explored. Additionally NP research can help building the bridge between traditional wisdom and modern medicine. The active interest of international research community in NP research is evident from the search results obtained using "natural product" as a keyword. Such a search, for example, in "Google Scholar" yields more than 2.8 million results; in DOAJ, this retrieves 16 journals and more than 2,400 articles. A year-wise search performed in PubMed, using the same keyword, shows the rise in count from 2 in 1958 to >1300 in 2015. Parallel to the increase in number of participating researchers, quite a few databases (Table 2) have come into existence providing a lot of useful information relevant to natural products.

Table 1 Examples of natural products reported for various biological activities

Compound/ product	Source	Reported activity	Reference
Artemisinin	*Artemisia annua*	Antimalarial	Tu (2011)
Paclitaxel	*Taxus brevifolia*	Anticancer	Priyadarshini and Keerthi (2012)
Axisonitrile	Marine sponge *Axinella cannabina*	Antimalarial, antituberculosis, antibacterial	Perdicaris et al. (2013)
Quinine	*Cinchona pubescens*	Antimalarial	Achan et al. (2011)
Vinblastine, Vincristine	*Catharanthus roseus*	Anticancer	Sain and Sharma (2013)
Curcumin	*Curcuma longa*	Antimicrobial	Tyagi et al. (2015)
Quercetin	Found in multiple plants, e.g., *Malus domestica*, *Syzygium cumini*, etc.	Anti-biofilm	Lee et al. (2011) Kothari et al. (2011)
Pentaphyte P-5®	*Ficus benghalensis*, *Ficus religiosa*, *Ficus racemosa*, *Ficus lacor*, *Albizia lebbeck*	Anti-inflammatory, antiasthmatic, antibacterial	http://www.palepmrf.com/Pentaphyte_P5.html

Table 2 Natural product databases

Database	Content of database	Relevant weblink
Natural Health Products Ingredients Database	Medicinal and nonmedicinal ingredients	http://www.hc-sc.gc.ca
Natural Medicines Comprehensive Database	Natural product effectiveness, drug interaction, clinical information on complementary, alternative and integrative therapies	http://naturaldatabase.therapeuticresearch.com
Super Natural II	A database of natural products comprising >325,000 natural compounds, including information on the corresponding 2D structures, physicochemical properties, predicted toxicity class, and vendors	http://bioinf-applied.charite.de/supernatural_new
Natural Products Alert	Organism, pharmacology, compound, and author-based queries	https://www.napralert.org
Universal Natural Products Database	Chemical name, CAS registry number, molecular weight and formula, international chemical identifier, and molecular input line entry specification	http://pkuxxj.pku.edu.cn/UNPD/index.php
Links to the 64 Databases For Natural Products	Structures, physical characteristics, formula, author information	http://depth-first.com/articles/2011/10/12/sixty-four-free-chemistry-databases/

2 Natural Products for Dealing with Naughty Microbes

Among the variety of biological activities being looked for in the NP, one of the most common is the antimicrobial (more recently, antivirulence, too) activity. As pathogenic microorganisms have been troubling the mankind since the prehistoric times, there has been a well-practiced tradition of employing antimicrobial NP to deal with infections. Essential oils and other plant preparations have been reported to contain a large variety of bioactive secondary metabolites (Tiwari et al. 2009). Phytochemicals are being extensively studied as promising human disease-controlling agents and/or as functional food ingredients. A variety of plant metabolites with antimicrobial properties have been documented to be effective against pathogenic and spoilage microbes (Ngwoke et al. 2011). Plants as a source of natural antimicrobials have been recognized for centuries by ancient civilizations; however, over the last three decades or so, this is being increasingly confirmed using the tools of modern science (Aires et al. 2009; Gyawali and Ibrahim 2012). Animals have also evolved different antimicrobial substances/defense mechanisms over the long process of evolution. Many of the antimicrobial peptides inherent to animals help the producing host while dealing with the invasion by pathogenic microbes (Hoskin and Ramamoorthy 2008).

Animals and plants are the major hosts for the pathogenic microbes, and hence they can be naturally expected to produce a variety of antimicrobial substances as a part of their defense strategy. In addition to this, antimicrobial substances are produced by microorganisms too, for a variety of ecological purposes. In fact, most of the currently used antibiotics have come from bacteria and fungi. Metabolites such as penicillins, cephalosporins, tetracyclines, aminoglycosides, chloramphenicol, macrolides, etc. are good examples of effectively used antibiotics derived from bacteria or fungi (Demain 1999). Food industry has also exploited the ability of different microbes to produce various antimicrobial metabolites such as different organic acids, hydrogen peroxide, ethanol, diacetyl, bacteriocins, etc., for preservation and/or flavor purpose (Nes and Johnsborg 2004).

Over the very long period of their existence on earth, microbial populations had encountered in nature a wide range of naturally occurring antibiotic substances, and for becoming more fit for survival, they developed multiple resistance mechanisms (Hancock 2007).This rise of drug resistance limits the effectiveness of any of the available antimicrobials put into therapeutic use and makes it imperative for us to keep finding new antimicrobials. Another problematic dimension of this issue is the ability of pathogenic microbes to form biofilms, which can be much more (up to few hundred times) antibiotic resistant than their planktonic counterparts. Biofilm formation is one of the many traits of pathogenic microbes whose regulation is related to *quorum sensing* (QS). QS refers to the phenomenon whereby microbes communicate among themselves, within and across populations. QS-associated microbial behavior is often of high relevance from a human perspective (Hense and Schuster 2015). This is executed via small diffusible molecules and directs most members of the given microbial population to exert a common behavior.

Gram-negative bacteria employ autoinducers, viz., *N*-acyl homoserine lactones (AHLs), to coordinate gene expression in a population density-dependent fashion (Molina et al. 2003), whereas gram-positive bacteria make use of autoinducer peptides to achieve the same. When a single bacterium secretes autoinducers (AI) into the surrounding, their concentration is too less to be detected. However, when enough bacteria are present, AI concentration reaches a threshold level allowing the bacteria to sense a critical biomass and, in response, to activate or repress the target genes associated with functions like sporulation, bioluminescence, antibiotic production and resistance, biofilm formation, pathogen/host interaction, virulence factor release, etc. (Adonizio et al. 2008; Rutherford and Bassler 2012; Kalia 2014; Hense and Schuster 2015; Lixa et al. 2015).

The fact that biofilm formation and expression of several other virulence factors is linked to QS (Fig. 1) raises new hopes for the discovery and development of anti-pathogenic (i.e., antivirulence/anti-infective) drugs capable of interfering with the bacterial communication system, without necessarily inducing lethal effects (Song and Wen 2013). The anti-infective compounds are expected to exert lesser selection pressure on the target pathogens (Rasko and Sperandio 2010; Breah and Michael 2013), than that exerted by conventional antimicrobial agents acting either as microbicidal or microbiostatic agents, i.e., affecting the pathogen growth in a direct fashion. However, QS inhibitors (QSI) should not be thought as evolution-proof drugs (Allen et al. 2014). Reports of resistance to QSI have already appeared (Kalia 2013; Grandclément et al. 2015).

QSI may exert their disrupting effect by inhibiting synthesis of the signal molecule, binding with the signal molecule and thus not allowing it to reach the compatible receptor, or binding itself with the receptor and not allowing the actual signal to occupy the binding site on the signal receptor (Kalia et al. 2014; Kalia and

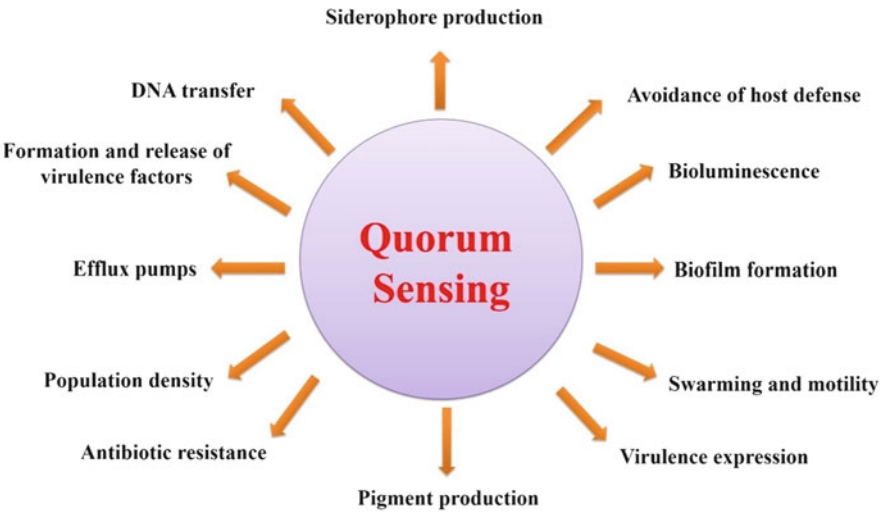

Fig. 1 A multitude of QS-regulated functions among microorganisms

Kumar 2015a). Natural as well as synthetic preparations with QS inhibitory potential are being extensively studied. Among natural entities, several plant compounds have been reported to be capable of acting as QSI (Table 3). Plants, such as carrots, chili, garlic, tomato, soybean, vanilla, pea, etc., have been shown to possess compounds having anti-QS activity (Zhu et al. 2011). Various species of marine algae, fungi, lichens, animals, honeybees, etc., are also reported to produce anti-QS compounds (Zahin et al. 2010; Lazar et al. 2013; Martín-Rodríguez et al. 2014).

As QS is put to use by multiple pathogens (e.g., *Enterococcus faecalis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and those belonging to the genera *Helicobacter*, *Neisseria*, *Porphyromonas*, *Proteus*, *Salmonella*, etc.) for regulation of virulence expression (George and Muir 2007; Bhardwaj et al. 2014; Kalia 2014); the QS machinery is being viewed as a very attractive target for drug design (Kalia et al. 2014). A limited number of QSI may prove effective against a multitude of pathogens, as there are many parallels among pathogenic microbes with respect to the components/mechanisms of their QS circuit. QS inhibitory compounds are thought to emerge as a new type of antimicrobial agents with possible applications in different fields, including human and veterinary medicine, agriculture, and aquaculture. Commercial interests associated with these fields are massive, as evident from a good number of biotechnology firms, which emerged on the scene in the near past, aiming specifically at developing anti-QS formulations [for instance, QSI Pharma A/S (Denmark); Quorex Pharmaceuticals Inc., Carlsbad (USA); 4SC AG (Germany)] (Hentzer and Givskov 2003).

2.1 Selection of the Model Bacterium for Screening of Possible QSI Property

Researchers, while screening their test substances for possible in vitro QS inhibitory property, usually employ one or more bacteria as the model test organism, and then they investigate the effect of their test substances on one or more QS-regulated phenotypes in the selected test bacteria. Though there are quite a few QS-associated traits, pigment production is one, which can be measured relatively easily. Production of pigment in many bacteria (e.g., *Pseudomonas aeruginosa*, *Chromobacterium violaceum*, *Serratia marcescens*, *S. aureus*, etc.) is known to be associated with QS (Table 4). However, while working with colored organisms, experiments may be tricky in some way. Particularly while quantifying the bacterial growth photometrically, the experimenter must ensure that there is no interference due to light absorption by the pigment. Most pigments are likely to absorb significantly at the wavelengths commonly used (e.g., 625 or 660 nm) for measuring OD of bacterial cultures. To overcome this problem, one must prepare the absorption spectrum of the pigment produced by the test organism and should avoid quantifying microbial growth at any wavelength where pigment absorbs to any notable

Table 3 Phytochemicals reported to possess anti-quorum sensing property

Principal compound responsible for anti-QS property	Source plant	Effective against	Reference
Epigallocatechin gallate	*Camellia sinensis* L.	*S. aureus*	Blanco et al. (2005)
Gingerol	*Zingiber officinale*	*P. aeruginosa*	Kim et al. (2015)
Ellagic acid	*Terminalia chebula Retz.*	*P. aeruginosa*	Sarabhai et al. (2013)
Pyrogallol	*Punica granatum*	*Vibrio harveyi*	Sangeetha and Vijayalakshmi (2011) and Brackman et al. (2008)
Urolithin A and B	*Punica granatum*	*Yersinia enterocolitica*	Truchado et al. (2012)
Methyl eugenol	*Cuminum cyminum*	*C. violaceum*, *P. aeruginosa*, *S. marcescens*	Packiavathy et al. (2012)
Gallic acid	Found in many plants, e.g., grapes	*Salmonella typhimurium*, *Citrobacter freundii*, *Proteus mirabilis*, *S. aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *E. coli*, *P. aeruginosa*	Boussoualim et al. (2014)
Quercetin	*Guiera senegalensis*	*E. coli*	Djifaby et al. (2012)
Vanillin	*Vanilla planifolia*	*C. violaceum*	Choo et al. (2006)
Naringenin	*Citrus sinensis*	*P. aeruginosa*	Vandeputte et al. (2011)
Taxifolin	*Combretum albiflorum*	*P. aeruginosa*	Vandeputte et al. (2011)
Cinnamolide-valdiviolide	*Drimys winteri*	*C. violaceum*	Carcamo et al. (2014)
Iberin	*Armoracia rusticana*	*P. aeruginosa*	Jakobsen et al. (2012a)
Erucin	*Brassica oleracea*	*P. aeruginosa*	Ganin et al. (2013)
Ajoene	*Allium sativum*	*P. aeruginosa*	Jakobsen et al. (2012b)
Allicin	*Allium sativum*	*S. aureus*	Leng et al. (2011)
Caffeine	*Coffee arabica*	*E. coli*, *P. aeruginosa*	Norizan et al. (2013)

Table 4 QS signaling molecules and QS-associated phenotypes in some pigmented bacteria

Bacterium	QS system of the organism	Autoinducer(s)	Phenotype (s) controlled	References
P. aeruginosa	*LasI*/*LasR*	*N*-(3-Oxododecanoyl)-homoserine lactone	Biofilm formation, virulence factors expression, pyocyanin production, bioluminescence, sporulation, and mating	Zahin et al. (2010), Jimenez et al. (2012), Nazzaro et al. (2013) and Aswathanarayan and Rai (2014)
	RhlI/*RhlR*	*N*-(Butyryl)-homoserine lactone		
S. aureus	LuxS/AI-2	Autoinducing peptide (AIP1-AIP4)	Cross-signaling between strains and species, biofilm formation, virulence factor expression, staphyloxanthin production	Zhao et al. (2010) and Gordon et al. (2013)
S. marcescens	SpnIR	*N*-3-Oxohexanoyl-homoserine lactone (3-oxo-C6-HSL), *N*-hexanoyl-homoserine lactone, *N*-heptanoyl-homoserine lactone, and *N*-octanoyl-homoserine lactone	Flagellum-independent population surface migration (sliding); synthesis of biosurfactant, prodigiosin, and nuclease	Wei et al. (2006) and Lutfi et al. (2014)
C. violaceum	CviI/R	*N*-hexanoyl homoserine lactone (C6-AHL)	Violacein production, exoprotease, aggregation, biofilm formation, swarming motility	Vasavi et al. (2013) and Juarez et al. (2013)

extent. For example, *C. violaceum* is among the most widely used bacteria in QS-related experiments, and it produces the violet pigment violacein. To avoid any notable interference from violacein, bacterial growth in this case can be quantified at 764 nm (Gallardo et al. 2014), as violacein does not absorb at this wavelength. Similarly, appropriate wavelengths need to be selected while working with other pigmented bacteria.

Though screening for QSI can initially be performed using any of the suitable test bacterium, any QSI can be of some real value only when it is shown to be capable of inhibiting QS in multiple bacteria. This is to say that an ideal QSI should exert a broad spectrum of activity by being capable of interfering with the QS machinery in gram-positive as well as gram-negative bacteria. The most effective approach will be to show multiple QS-associated traits (in each of the test organisms) to get affected upon exposure to the test product.

2.2 *Possible Workflow While Mining NP for Potential QSI*

Once a test product has been demonstrated to possess a broad-spectrum in vitro capacity to inhibit quorum sensing, the next logical step can be to investigate whether this capacity can be demonstrated in vivo. For achieving the latter, availability of a suitable model host is essential. Though animal models are in use since many years, ethical issues are associated with their use. The nematode *Caenorhabditis elegans* has emerged in recent years as an attractive model host for infectious microorganisms (Ewbank and Zugasti 2011), at least for initial in vivo studies.

Following the confirmation of in vitro and in vivo activity, the next step of investigation can be to find out the mode of action of the potential QSI. For this one can take the in silico approach, if the phytochemical profile of the test plant extract is known, wherein structures of different constituent metabolites of the active extract can be docked against the possible bacterial target(s), e.g., the QS signal and/or the signal receptor protein (CviR in *C. violaceum*, as an example). Performing the in vitro experiments with and without exogenous supply of QS signal can provide useful indication on whether the potential QSI is a *signal-supply inhibitor* or a *signal-response inhibitor*. This information is of obvious utility while selecting target proteins during molecular docking exercise. Performing *wet lab* experiments with pure compounds can be of additional value. In silico exercise can run in parallel with the in vitro or in vivo experiments (Fig. 2).

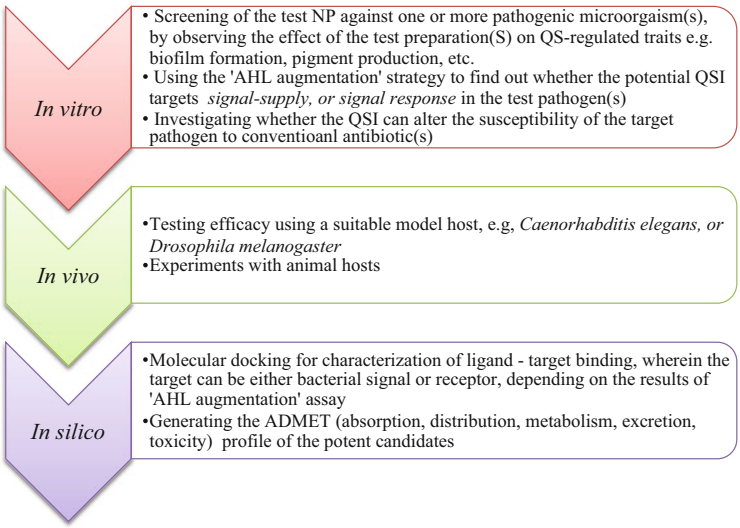

Fig. 2 An indicative list of experiments to be performed while mining NP for potential QS inhibitors

3 Applied Aspects of the QS Research

QSI seem to have varied applications in different fields including medicine (Joshi et al. 2010; Kalia and Kumar 2015b). QS research of course adds a lot to our knowledge about the fundamental aspects of microbial communication and regulation of the population behavior. Magnitude of the applied aspect of QS research is also evident from a good number of patents being filed in this area (Table 5).

3.1 QSI in Medicine

As an alternative/augmentation to the currently practiced conventional antibiotic therapy, QSI are being viewed with great hopes. In order to be therapeutically relevant, a QSI need not be 100% effective, as disturbing the QS machinery of the given pathogen even partially can reduce its virulence significantly, which in turn can offer the host immune system a better chance of winning over the pathogen. Further QSI may enhance antibiotic susceptibility of the given pathogen, making the conventional antibiotic(s) more effective at lesser concentration. QSI may act in synergy with the routinely applied antibiotics. The term "synergy" refers to the fact that the effect of combined treatment is more than the sum of each component's individual effects. Certain components in a plant extract can improve the therapeutic effect of the chemotherapeutic agents (Cooney 2011). In certain cases, one herb can enhance the effect of another, if given simultaneously (Spinella 2002). As an example, we may consider the *Panchvalkal* preparations described in *Ayurved*. These are mixtures of extracts of bark from different plants. Such preparations have been prescribed in *Ayurvedic* texts for relief in microbial infections. One such commercially available product Pentaphyte P5® is being investigated by us for its QS inhibitory potential. Our yet unpublished findings suggest that this product (listed in Table 1) can reduce QS-regulated violacein synthesis in the bacterium *C. violaceum*. It could also enhance (~10%) the susceptibility of this bacterium to the antibiotic streptomycin. It is appealing to consider the combined use of antibiotics with anti-QS strategies, since QSI by disrupting bacterial signal production/reception can reduce antimicrobial resistance (e.g., by reducing drug efflux) or discourage transition to physiological states that enhance persistence (e.g., biofilms). Many such reports describing the benefit of using QSI in combination with antibiotics have accumulated in literature (Rasmussen et al. 2005; Brackman et al. 2009, 2011), which show the combination approach to be more effective against pathogens like *P. aeruginosa*, *Burkholderia* spp., *S. aureus*, etc. QSI compounds were also shown to improve survival probabilities in invertebrate infection models and to decrease bacterial load in mouse pulmonary tissues (Brackman et al. 2009). A rise in the antibiotic susceptibility of the test bacterial strains was attributed to the synergistic activity of quercetin (Venkadesaperumal

Table 5 Some examples of the patents related to QS research[a]

Sr. no.	Patent title	Inventor(s)	Reference no. and date of publication
1	Development of zinc oxide nanoparticles at varied incubation periods for regulating anti-quorum sensing	Khan Mohd Farhan, Ansari Akhter H	IN2232DE2015 (A),2015-08-14
2	Small-molecule antagonists of bacterial quorum sensing receptors	Bassler Bonnie L, Swem Lee R	US2015306067 (A1), 2015-10-29
3	Bacterial quorum sensing inhibitor and antibacterial application thereof	Yu Wengong, Gong Qianhong	CN104784160 (A), 2015-07-22
4	Modulation of bacterial quorum sensing with synthetic ligands	Blackwell Helen E, Geske Grant D	US2015080349 (A1), 2015-03-19
5	Antibody-mediated disruption of quorum sensing in bacteria	Kim D Janda, Gunnar F Kaufmann	JP2014221774 (A), 2014-11-27
6	Quorum sensing inhibitors	Givskov Michael, Yang Liang	WO2014142748 (A1), 2014-09-18
7	Use of ellagitannins as inhibitors of bacterial quorum sensing	Mathee Kalai, Adonizio Allison L	US2013317094 (A1), 2013-11-28
8	Detecting antigens such as bacterial quorum sensing proteins	Bell Charleson S, Giorgio Todd D	WO2013170229 (A1), 2013-11-14
9	Bacterial quorum sensing biosensor	Sayre Richard T, Rajamani Sathish	US2012122115 (A1), 2012-05-17
10	Synthetic analogs of bacterial quorum sensors	Iyer Rashi, Ganguly Kumkum	US2012071430 (A1),2012-03-22;US8350061 (B2) 2013-01-08

[a]This table was generated by performing a search using the keyword "quorum sensing" on the website of European Patent Office: https://www.epo.org/searching.html. This search yielded >340 results, of which few examples are listed here

et al. 2015). Such investigations may pave the way for novel treatment options for dealing with "difficult-to-eradicate" bacterial infections.

3.2 QSI in Aquaculture and Agriculture

In commercial aquaculture, bacterial infections are one of the most critical problems. Vibriosis is known to cause heavy mortality in almost all types of aquacultured organisms (Defoirdt et al. 2007). Natural and synthetic brominated furanones were shown to protect brine shrimps (*Artemia franciscana*) from pathogenic isolates of *Vibrio* (*V. harveyi*, *V. campbellii*, and *V. parahaemolyticus*) through the disruption of AI-2-based QS (Defoirdt et al. 2006).

In agriculture, nonpathogenic bacteria capable of disrupting QS of the phytopathogenic bacteria can be used as biocontrol agents (Dong et al. 2004; Uroz et al. 2008). QS-regulated virulence in plant pathogens, including the soft rot associated with *Pectobacterium* spp., was shown to be disrupted by some QSI (Faure and Dessaux 2007).

3.3 QSI as Anti-biofouling Agents

Biofouling can be defined as the attachment of one or more organisms to a surface in contact with water. This phenomenon causes serious technological and economic problems in various fields or processes such as naval transportation, aquaculture, petroleum industries, medical devices, bioreactors or water distribution networks, and wastewater plants (Fitridge et al. 2012; Harding and Reynolds 2014). Marine organisms constitute a good source of antifouling molecules. *Flustra foliacea*, a marine colonial animal of the Bryozoa phylum, produces a set of ten brominated alkaloids, two of which exhibit QSI activity (Peters et al. 2003). In glass plate assays, kojic acid, an oxo-pyrone, prevented biofouling (Dobretsov et al. 2011). *Piper betle* extracts were indicated as anti-QS agent to mitigate membrane biofouling (Siddiqui et al. 2012).

4 Issues While Experimenting with NP

Natural products, particularly crude extracts, being undefined preparations pose certain challenging issues, while investigating them for different biologically relevant activities. Some of the important aspects of natural product research, which researchers should be conscious about, include:

- Batch-to-batch variation
- Selection of the most appropriate extraction method
- Appropriate "controls" in all experimental sets (particularly the "abiotic control" while dealing with colored extracts in a study involving photometric measurements) (Chaudhary et al. 2014; Wadhwani et al. 2009)
- Low solubility in the assay medium
- Existence of the phenomenon of "synergy," making it difficult to get a clue about mode of action
- Lack of globally accepted authentic guidelines regarding protocols for assaying NP and their therapeutic uses

Few suggestions for troubleshooting with NP issues can be found in Kothari (2014).

5 Conclusions

Research on bioactive natural products is being intensively practiced across the globe. NPs with antimicrobial and/or anti-infective potential are getting more and more attention in the background of the threatening problem of antibiotic resistance among pathogenic microbes. Particularly the NP with QS inhibitory potential are being viewed with high optimism, as QS regulates a notable portion of the microbial genome, including that associated with their virulence. QSI are expected not to persuade bacteria toward rapid development of resistance. They may be used alone or in combination with conventional microbiostatic/microbicidal agents. It is believed that QSI can help the host immune system by reducing the expression of virulence traits, as well as potentiate the effect of antibiotic therapy by making the target pathogen population more susceptible. Though many reports on QSI potential of NP are appearing, the real challenge will be to develop these active NP as usable therapeutic agents. We also need to develop some insight into how the normal human microbiota may respond to the QS inhibitory natural products, if employed as therapeutic agents. NP research is an interesting area, but having its own complications. However, there are enough reasons to believe that the future will see a good number of NPs entering the list of approved therapeutic formulations. A structured approach of research in this area will help us to explain the scientific basis of many of the traditional medicinal practices, for example, the use of pomegranate peel for relief in sore throat, or applying coffee powder on wounds. Natural product researchers can play a crucial role in bridging the gap between ancient and modern systems of medicine.

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