Pallaval Veera Bramhachari *Editor*

Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry

Springer

Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry

Pallaval Veera Bramhachari
Editor

Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry

Springer

Editor
Pallaval Veera Bramhachari
Department of Biotechnology
Krishna University
Machilipatnam, Andhra Pradesh, India

ISBN 978-981-32-9408-0 ISBN 978-981-32-9409-7 (eBook)
https://doi.org/10.1007/978-981-32-9409-7

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

In the last decade, progress on the knowledge of bacterial quorum sensing and biofilm formation has been advanced exponentially. Bacteria have intriguing and diverse social lives. It is a unique phenomenon where microbes communicate and synchronize their behavior by the accumulation of (AHL) signaling molecules. A reaction occurs when AHL accumulates to an adequate concentration. They exhibit coordinated group behaviors regulated by QS systems that detect the density of other bacteria around them. Explicitly, QS is the chemical communication process wherein bacteria coordinate changes in their collective behavior in response to population density. The regulation of social behavior in several bacteria is fundamental to QS phenomenon in medicine, food industry, and agriculture including biofilm formation and the expression of virulence in dreadful pathogens to symbiotic relationships.

A contemporary challenge in the field is to comprehend how QS works in scenarios that mimic real host environments. The current research suggests that it must accumulate into large colonies or aggregate into "biofilms." To do this, it uses a microbial trick called QS, where chemical signals are employed by the bacterium to gather and sense a critical mass of cells and then act in harmony to exert virulence and pathogenesis, which in human patients and animals can manifest itself in the form of infectious diseases. When working as a group, they initiate typical behaviors different from those observed in an individual cell. They have the ability to take on more complex tasks, and many pathogens use QS to initiate certain specific group behaviors. Through QS and biofilm formation, the bacterium resists to most antibiotics, is seemingly immune to several antibiotics, chemicals, and can survive extreme environments at ease. Interestingly, the QS compounds are shown to thwart stubborn bacterial pathogen's social propensity.

QS regulates interactions both in signal-producing organisms and between different species present in surrounding medium, disease-causing and useful microbes, and higher organisms (symbiosis, growth promotion, pathogenicity). This Book "Implication of Quorum Sensing and Biofilm Formation in Medicine, Food Industry and Agriculture" brings a variety of chapters reporting the quorum sensing and biofilm formation and their importance in agriculture (in the areas of plant-microbe interactions in connection with pathogenicity (plant growth promotion, biocontrol), ecology (behavior of microbes), medicine (colonization and causing disease to

animal hosts), food industry (food production, preservation, and spoilage), aquatic industry, and industrial plant biofouling).

Our understanding of QS mechanisms currently restricts applications for quorum sensing. Though there has been progress made in the use of QS, more understanding of quorum functionality is necessary before the control of this tool can be completely raised. However, the full-scale management of the bacterial quorum circuit in a biotechnological application is still an unconvinced goal. These strategies are only in a preliminary phase and several questions on the exact nature of biofilms both in terms of implicated microbes and composition of the extracellular matrix remain, but undoubtedly this field bears high hopes for future applications. However, more of such QS compounds remain to be found and next-generation agents may then be ready to tackle the tricky microbes that have rapidly evolved resistance in medicine, food industry, and agriculture.

Primarily microbiologists discovered QS quite unconnectedly, not relating it to biofilm formation. Later on, it was revealed that QS is a molecular system based on regulation and expression of RNA genes, whereas biofilm formation is the quantitative community network for the microbes. The knowledge and information about quorum sensing and biofilm have skyrocketed since then. Rapid advances in molecular biology, biochemistry, and genetics have revolutionized the study of QS in microbes and enhanced the understanding of intra- and inter-species and inter-kingdom communications among microbial communities and eukaryotes. Additionally, the advent of next-generation sequencing technologies and bioinformatics has offered a number of revolutionary new insights into the QS research in microbes.

We now have an in-depth knowledge of how bacteria employ QS signals to communicate with each other and to coordinate their activities. In recent years there have been extraordinary advances in the recent understanding of genetics, genomics, biochemistry, and signal diversity of QS. The world has started to understand the connections between QS and bacterial social propensity. This foundation places us at the beginning of a new era in which researchers will be able to work toward new medicines to treat devastating infectious diseases and use bacteria to understand the biology of sociality. The application of QS as a target for the development of novel anti-infective agents is the major activity in providing "quality of life enhancement" from the public funding of research.

We strongly believe that this book would provide enough insights into the amazing world of microbial QS. The present book is an attempt to compile the novel information available on recent advancements on various functional aspects of QS systems in different gram-positive and gram-negative organisms. It is essential reading for the novice and expert in the field of QS including researchers, industrialists, as well as students. With these objectives in mind, the content of this book has been arranged in a logical progression from fundamental to more advanced concepts. We hope that this book stimulates your creativity and wish you success in your experiments.

These observations, and our involvement through the years in quorum sensing research led us to the conclusion that such a book is timely. Accordingly, this book provides the reader with the latest research advances with insights into the

implications of QS and biofilm formation in medicine, food industry, and agriculture. This book is written by a team of experts who represent the best in the field of microbiology and cell biology. We strongly affirm that the topics presented here will stimulate future innovative research studies. We successfully compiled our creative and thoughtful work with genuine concern and painstaking effort of many well-wishers whose names are not mentioned, but they are still in our hearts. So, the reward is surely worth their efforts. I want to dedicate this book to my mother S. Jayaprada (late). I and contributing authors hope from the bottom of our hearts that this book will be a good guidebook and compass for research studies in bacterial quorum sensing. Bon voyage, all!

Machilipatnam, Andhra Pradesh, India Pallaval Veera Bramhachari

Acknowledgments

My sincere thanks are extended to all the academicians and scientists who have contributed chapters and happily agreed to share their work on "Implication of Quorum Sensing and Biofilm Formation in Medicine, Food Industry and Agriculture" in this volume.

This book is a stunning reflection of the seriousness with which several scientific minds are dedicated to the scientific community. I am extremely thankful to the contributors for paying continuous attention to my request and showing faith in my competencies and capabilities. I shall always remain highly obliged to all of them forever. These words cannot justify the worthiness of their efforts. We appreciate the excellent work of the authors and coauthors who were invited to contribute chapters in this book. The credit for making this book a reality goes to them. We as coeditors and the review team for the chapters especially appreciate sharing expertise with the contributors. Each chapter is informative and written as a stand-alone, so the reader can begin anywhere in the book depending upon his/her interests and needs.

At the same time, I also express my deepest gratitude to my family members especially my wife (Ramadevi Ramaswamy) and my kids (Ruthvik and Jayati) for their kind support which has prompted me to complete the assignment on time. I am also thankful to the Department of Biotechnology, Krishna University, for the support. I must also especially acknowledge the constant support of my student A. M. V. N. Prathyusha throughout the preparation of this book. I am equally thankful to the Springer Nature Publishing Group for their full cooperation during the peer review and production of the volume.

I am thankful to my beloved teachers and mentors, for their constant support and motivation at all stages of the process.

Department of Biotechnology
Krishna University
Machilipatnam, Andhra Pradesh, India

Pallaval Veera Bramhachari

About the Book

This book illustrates the importance and applications of quorum sensing and its critical roles in regulating diverse cellular functions in microbes, including virulence, pathogenesis, controlled gene expression systems, antibiotic resistance, and biofilm formation in medicine, food industry, and agriculture. Microbes can coordinate population behavior with small molecules called autoinducers (AHL) which serve as a signal of cellular population density, triggering new patterns of gene expression for mounting virulence and pathogenesis. Therefore these microbes have the competence to coordinate and regulate explicit sets of genes by sensing and communicating among themselves utilizing a variety of signals. A diverse range of bacterial behaviors, which have a noteworthy impact upon a wide range of fields including medicine, agriculture, and industry, are regulated by QS systems. Therefore, the ability to modulate quorum sensing systems using small molecules could have tremendous real-world implications. Unsurprisingly, for that reason, this field has garnered noteworthy interest in recent years; a range of potent activators and inhibitors of quorum sensing systems have been developed, endowed with an expansive set of chemical tools to investigate and manipulate this complex signaling process. Nonetheless, these intricate quorum communications raise numerous fundamental questions that are increasingly attracting the attention of scientists in the food industry, agriculture, and medicine. Thus, understanding the microbial QS applications with an outcome has essential implications which may eventually endow with innovative novel communication networks. This book covers the emerging concepts of quorum sensing-controlled gene expression systems in bacteria, algae, plants, and other eukaryotes and inter-kingdom molecular cross-talk. The chapters describe the recent advancements in various functional aspects of QS systems and their applications, namely, survival behavior, antibiotic resistance, and application of quorum sensing inhibitors to counter antibiotic resistance. Finally, the book also elucidates a comprehensive yet representative description of basic and applied aspects of quorum sensing inhibitors and a large number of challenges associated with medicine, food industry, and agriculture.

Contents

Editor and Contributors

About the Editor

Dr. Pallaval Veera Bramhachari is currently a Senior Faculty member of the Department of Biotechnology at Krishna University, Machilipatnam, India. He has earlier served as Postdoctoral Research Scientist at the Bacterial Pathogenesis Laboratory, Queensland Institute of Medical Research (QIMR-Berghofoer Research Center), Brisbane, Australia, and DBT Postdoctoral Fellow at the Department of Microbiology and Cell Biology, Indian Institute of Science (IISc), Bangalore. He completed his Ph.D. in Microbiology from Goa University, India. His major research interests are the investigation on the applied research in microbiology and cell biology including biochemical mechanism of bacterial-biofilm formations, structure-function relationship of bacterial rhamnolipids, and basic and translational cancer research. He has published more than 125 research articles including 3 international books in prestigious peer-reviewed international Scopus-indexed journals and presented 47 abstracts at various national and international conferences. He has served in the Editorial Board member and as Reviewer for a number of national and international journals. He is a member of many international scientific societies and organizations, most importantly, Indian Science Congress and Society of Biological Chemists, India. He successfully completed two major research projects. He was awarded a Travel scholarship from QIMR Australia in 2007 for attending 4th Indo-Australian Biotechnology Conference at Brisbane, Australia and awarded with young scientist travel fellowship (2007) from the DST,

Govt. of India for attending XVII Lancefield International Symposium at Porto Heli–Greece-2008. Dr. Bramhachari was conferred with various prestigious awards, notably, Science Education Research Board (SERB), Government of India-Young Scientist award (2011) with a Research Project, Nominated as Associate Fellow of Andhra Pradesh Academy of Sciences (APAS)-2016, MASTER TRAINER: Andhra Pradesh, English Communications Skills Project. British Council & APSCHE 2017, Andhra Pradesh State Best Scientist award-2017, Dr.V. Ramalingaswamy Memorial award for Biomedical Sciences-2019. He also obtained two Indian patents in 2017. He has more than 13 years of teaching and research experience at the university level.

Contributors

Tanveer Ahmad Department of Biotechnology, School of Life Sciences, Pondicherry University, Puducherry, India

V. K. Shakeel Ahmed Department of Orthodontics, Ragas Dental College and Hospital, Chennai, India

Harish Annavarapu Department of Food and Nutritional Sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

K. Archana Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India
Kalam Biotech Pvt. Ltd, Hyderabad, India

Mudasir Bashir Department of Food Science and Technology, School of Life Sciences, Pondicherry University, Puducherry, India

Hameeda Bee Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India
Kalam Biotech Pvt. Ltd, Hyderabad, India

Chanda Vikrant Berde Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

Vikrant B. Berde Department of Zoology, Arts, Commerce and Science College, Lanja, Maharashtra, India

Prudhvi Lal Bhukya Hepatitis Division, National Institute of Virology, Pune, Maharashtra, India
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Pallaval Veera Bramhachari Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Surekha Challa Department of Biochemistry and Bioinformatics, Institute of Science, Gandhi Institute of Technology and Management (GITAM) (Deemed-to-be University), Visakhapatnam, Andhra Pradesh, India

Titash Dutta Department of Biochemistry and Bioinformatics, Institute of Science, Gandhi Institute of Technology and Management (GITAM) (Deemed-to-be University), Visakhapatnam, Andhra Pradesh, India

Kishore Kumar Godisela SRR Government Arts & Science College, Karimnagar, Telangana, India
Government Degree & PG College for Men, Adilabad, Telangana, India

Saqib Hassan Department of Medicine, Imperial College London, London, UK

Mani Jayaprakashvel Department of Marine Biotechnology, AMET deemed to be University, Kanathur, Chennai, Tamil Nadu, India

G. Seghal Kiran Department of Food Science and Technology, School of Life Sciences, Pondicherry University, Puducherry, India

Chandrajit Lahiri Department of Biological Sciences, Sunway University, Petaling Jaya, Selangor, Malaysia

Lukuo Lee Department of Biology, Colgate University, Hamilton, NY, USA

Max Majireck Department of Chemistry, Hamilton College, Clinton, NY, USA

Veda Manerikar Department of Microbiology, Goa University, Taleigao Plateau, Goa, India

Rajinikanth Mohan Department of Biology, Colgate University, Hamilton, NY, USA

Milind Naik Department of Microbiology, Goa University, Taleigao Plateau, Goa, India

Renuka Nawadkar Department of Pathogenesis and Cellular Response, National Centre for Cell Science, Pune, Maharashtra, India

Prerna Pathak Jawaharlal Nehru University, New Delhi, India

Shrikant Pawar Yale Center for Genome Analysis, Yale University, New Haven, CT, USA

Angela E. Peter Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India

Meghanath Prabhu Department of Microbiology, Goa University, Taleigao Plateau, Goa, India

A. M. V. N. Prathyusha Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Sathish Rajamani Division of Molecular Translational Sciences, US Army Medical Research Institute of Infectious Diseases, General Dynamics Information Technology, Fort Detrick, MD, USA

B. Ganga Rao College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

P. Ravinder Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India
Kalam Biotech Pvt. Ltd, Hyderabad, India

Prachiti P. Rawool Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

L. Prasanna Anjaneya Reddy Department of Lipid Science, Central Food Technological Research Institute (CFTRI), Mysuru, India

L. Veeranjaneya Reddy Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

Neelapu Nageswara Rao Reddy Department of Biochemistry and Bioinformatics, Institute of Science, Gandhi Institute of Technology and Management (GITAM) (Deemed-to-be University), Visakhapatnam, Andhra Pradesh, India

Sagar P. Salvi Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

B. V. Sandeep Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India

K. Sathi Reddy Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India
Kalam Biotech Pvt. Ltd, Hyderabad, India

Joseph Selvin Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India

Ganugula Mohana Sheela Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

Emma Smith Department of Biology, Colgate University, Hamilton, NY, USA

Ramesh Subramani School of Biological and Chemical Sciences, Faculty of Science, Technology & Environment, The University of the South Pacific, Suva, Republic of Fiji

Pola Sudhakar Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India

Y. J. Wee Department of Food Science & Technology, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea

M. Yahya Khan Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India
Kalam Biotech Pvt. Ltd, Hyderabad, India

N. M. Yugandhar Department of Chemical Engineering, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abbreviations

ABC	ATP binding cassette family
ACP	Acyl carrier protein
acyl-ACP	Acyl-acyl carrier protein
agr	Accessory gene regulator
AHL	Acyl homoserine lactone
AI	Autoinducer
Als	Agglutinin-like sequence
AME	Aminoglycoside modifying enzymes
AMR	Antimicrobial resistance
APC	Antigen presenting cells
ATP	ATP binding cascade
BHL	Butanoyl homoserine lactone
C4-HSL	N-Butanoyl-L-homoserine lactone
C4-HSL	N-Butanoyl-L-homoserine lactone
C6-HSL	N-Hexanoyl-L-homoserine lactone
C7-HSL	N-Heptanoyl-L-homoserine lactone
C8-HSL	N-Octanoyl-L-homoserine lactone
cag PAI	Cytotoxin-associated gene pathogenicity island
CAI-1	Cholera AI-1
Cat	Catalase
CAT	Chloramphenicol acetyltransferases
C-HA	Collagen-hydroxyapatite
CIP	Ciprofloxacin
CqsA	CAI-1 AI synthase
CVC	Central venous catheters
DC	Dendritic cells
DKP	Diketopiperazine
DOX	Dioxygenase
DPD	4,5-Dihydroxy-2,3-pentanedione
DSF	Diffusible signal factor
DSF	Diffusible signal factor
DSF	Diffusible signal factors
ECM	Extracellular matrix
EO	Essential oils

EPS	Extracellular polymeric substances
ESO	Ephemeral spoilage organism
FAAH	Fatty acid amide hydrolase
FOS	Food Safety and Zoonoses
FusB, FusC	Fusidic acid resistance proteins
GABA	γ-Amino butyric acid
GFN	Global Foodborne Infections Network
GLV	Green leaf volatiles
GPCR	G-protein-coupled receptors
HA	Hydroxyapatite
HGT	Horizontal gene transfer
HHL	Hexanoyl homoserine lactone
HK sensor	Histidine kinase sensor
HSL	Homoserine lactone
Hwp1	Hyphal wall protein
ICD	Implantable cardioverter defibrillators
ISR	Induced systemic resistance
LEE	Locus of enterocyte effacement
LLO	Listeriolysin O
LOX	Lipoxygenase
MAP	Mitogen activated protein
MATE	Multidrug and toxic efflux family
MDR	Multidrug resistance
MF	Major facilitator family
MIC	Minimum inhibitory concentration
MTAN	5′-Methylthioadenosine/S-adenosylhomocysteine nucleosidase
N-AHL	N-Acyl-L-homoserine lactone
NIH	National Institutes of Health
OOHL	C3-Oxo-octanoyl homoserine lactone
PAC	Proanthocyanidins
PCN	Phenazine-1-carboxamide
PON	Paraoxonase
PPI	Proton pump inhibitors
PQS	*Pseudomonas* quinolone signal
Qnr	Quinolone resistance protein
QS	Quorum sensing
QSI	Quorum sensing interference
RAP	RNAIII activating peptide
RIF	Rifampin
RND	Resistance-nodulation-division family
ROS	Reactive oxygen species
ROS	Reactive oxygen species
R-THMF	(2R,4SL)-2-Methyl-2,3,3,4 tetrahydroxytetrahydrofuran
SAH	S-Adenosylhomocysteine
SAM	*S*-Adenosyl methionine

SAR	Structure-activity relationships
SAR	Systemic acquired resistance
SMS	Small multidrug resistance family
SOD	Superoxide dismutase
SSOs	Specific spoilage organisms
SSOs	Specific spoilage organisms
TCS	Two-component regulatory system
Tet(M), Tet(O)	Tetracycline resistance determinants
TLC	Thin-layer chromatography
TRAP	Target of RNAIII activating protein
TTO	Tea tree essential oil
TVB-N	Total volatile base nitrogen
VADs	Ventricular assist devices
X-Gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside

Part I

Role of Quorum Sensing in Prokaryotes and Eukaryotes

1 Novel Insights on the Functional Aspects of Quorum Sensing Systems and Its Applications in Medicine, Food Industry, and Agriculture

Pallaval Veera Bramhachari

Abstract

Microbes accustom to an array of behaviors and coordinate population signaled by AHL molecules to determine population density, trigger new patterns of gene expression, and mount virulence and pathogenesis. Diverse bacterial behaviors regulated by quorum sensing systems have a noteworthy impact on different fields of medicine, industry, and agriculture. Therefore, the ability of AHL molecules to modulate QS systems has remarkable real-world implications. This book exemplifies the magnitude and applications of QS and quorum sensing inhibitors (QSI) and its critical role in regulating diverse cellular functions. QS regulates interactions in both signal-producing organisms and between different species, viz., pathogenic, beneficial, and symbiotic microbes and higher organisms (symbiosis, growth promotion, pathogenicity). Its importance in agriculture (in the areas of plant–microbe interactions in connection to symbiosis and pathogenicity (plant growth promotion, biocontrol, virulence, and pathogenesis) ecology (microbial habitats), medicine (colonization of medical devices and disease causing human and animal hosts), food industry (food production, spoilage, and preservation), aquatic, industrial plant biofouling and activated sludge digestion. Nonetheless, complete management of bacterial quorum circuit in a biotechnological application is yet to be an incredulous goal. These strategies that are only in a preface and pose quite a lot of questions on exact nature of biofilms both in terms of implicated microbes and composition of extracellular matrix will remain a great task, but unquestionably this field stands high hope for future applications.

P. V. Bramhachari (✉)
Department of Biotechnology, Krishna University,
Machilipatnam, Andhra Pradesh, India
e-mail: veerabramha@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_1

Keywords
Acyl homoserine lactones (AHL) · Quorum sensing (QS) · Quorum sensing inhibitors (QSI) · Biofilm formation · Medicine · Agriculture · Food industry

1.1 Introduction

Microbes accustom to an array of behaviors and coordinate population behavior signaled by AHL molecules to determine population density, trigger new patterns of gene expression, and mount virulence and pathogenesis (Waters and Bassler 2005). However, biofilms are formed by one or more species engrossed in an extracellular matrix of diverse compositions varying in the nature of each ecosystem and colonizing species. Formation of biofilm is a critical outcome to persistent changes, for instance, transition from liquid environment to a surface. Toggling from one environment to another needs coordinated regulation and cross-talk among regulatory systems (Huang et al. 2019). Nevertheless, biofilm formation in medicine has various advantages to bacteria as they turn into resistant phenotype to disinfectants, antimicrobials, and unfavorable environmental conditions and they also swap over metabolic substances and genetic materials among the group members so that it becomes tricky to exterminate nonpathogenic organisms. Biofilm formation is mechanistically reliant on QS and acts as shield for microbes; thus, averting quorum sensing (QS)-based signaling is anticipated to prevent biofilms. The QS allows bacteria to coordinate with the quickly altering environmental conditions for their survival. These responses take account of adaptation with the existing nutrients, defense against other microorganisms which in turn compete for same nutrients, and protect them against the rapid changing environmental conditions.

Under these circumstances, microbes utilize certain chemicals to communicate among each other which is represented as QS. Notably, quorum sensing is one of the most incredible microbiological discoveries deemed to be global gene regulatory pathway for diverse traits in bacteria (van Bodman et al. von Bodman et al. 2008). QS process allows single-cell organisms, viz., bacteria, to communicate, cooperate, and act collectively. By QS process, microbes produce, release, and detect small chemical molecules called autoinducers to establish fiddly connections between friends and foes. Moreover, bacterial species that synthesize biofilm can possess wide genomic variants apropos involved in the formation of biofilms with different characteristics under diverse conditions (Waters and Bassler 2005). Furthermore, complexity of different biofilms together with diverse environments and wide range of colonizing species set hard tasks for biofilm eradication in medicine, food industry, and agriculture. However, as these biofilms are complex communities, their exceptional characteristics enhance the viewpoint of chemical and physical resistances, assembling their sticky structures incredibly intricate in some cases, and favoring their diligence.

Additionally, the association between QS and biofilm piloted a flurry of studies to appraise how microbial social behaviors affect the vital mode of growth;

nevertheless, it was revealed quickly that this connection solely differs with environmental conditions (Shrout et al. 2006). Therefore, based on the recent developments, new antibacterial approaches are currently directed onto biofilm formation inhibition, as substitute for its riddance is very significant in every sector (Gopal et al. 2015). This slimy matrix plays a structural role that is conscientious for strong perseverance of these biofilms in medicine and food industry in particular. This creates complex gradients with regard to oxygen diffusion and nutrients that hold extracellular enzymes utilized for nutritional purposes, allows for transfer of cell communication molecules, and protects embedded cells against toxic compounds. In short, biofilm formation facilitates physical resistance, mechanical resistance, and chemical protection to microbial cells (Flemming et al. 2016; Galiè et al. 2018).

1.2 Quorum Sensing and Its Importance in Biotechnology

In this book, we illustrate some significant QS-regulated behaviors that have noteworthy impact in medicine, food industry, and agriculture. The vital applications of QS inhibitors (QSI) comprise usage of QS components for upstream synthesis of natural or engineered bacterial products, furanone-like blockers, and therapeutic and industrial usage of acyl homoserine lactones (AHLs) and its analogues. There are three basic ways of reducing of biofouling and bacterial QS: (i) by blockage of AHL production, (ii) interference with signal receptor, and (iii) inactivation of AHL signal molecules. In addition, QS also plays a pivotal role in regulating the diversity of cellular activities (prokaryotic–prokaryotic–eukaryotic), for instance, virulence and biofilm formation considerably affect agriculture, human health, antibiotic synthesis, transport systems, activated sludges, biofouling on ships, commercial production, sporulation, virulence factor induction, cell differentiation, nutrient flux in pathogenic bacterial infections, bioluminescence, pigment production, plant–microbe interactions, starvation–genetic competence, and stress responses (Fig. 1.1).

QS mechanism regulates production of biosurfactants and was previously shown to reduce surface tension activities. Additionally, disruption of QS-based regulatory circuits proved to be efficient strategy for impediment of bacterial disease outbreaks (Mangwani et al. 2012). Furthermore, this is a promising alternative for antibiotics to combat bacterial infections which holds immense significance in aquaculture industry. A connection between QS and biofilm formation and virulence factor synthesis in numerous aquatic pathogens is undeniably a great threat to aquatic industry, which was an established fact (Defoirdt et al. 2004). It has been suggested that QS signals alone can be used as pathogenic diagnostics and therapeutic markers for the presence of pathogenic bacteria in environmental and clinical samples (Köhler et al. 2009). QS-based bioremediation of organic pollutants are identified to be employed in environmental restoration (Kothari et al. 2018). QS-regulated biofilm-based processes enhanced ability of biofuel production (Mangwani et al. 2012). Additionally, few advantages of this mechanism comprise concentration of cell-associated hydrolytic enzymes at the biofilm–substrate interface to augment reaction rates and the possibility of fungal–bacterial symbioses that consent to real-time

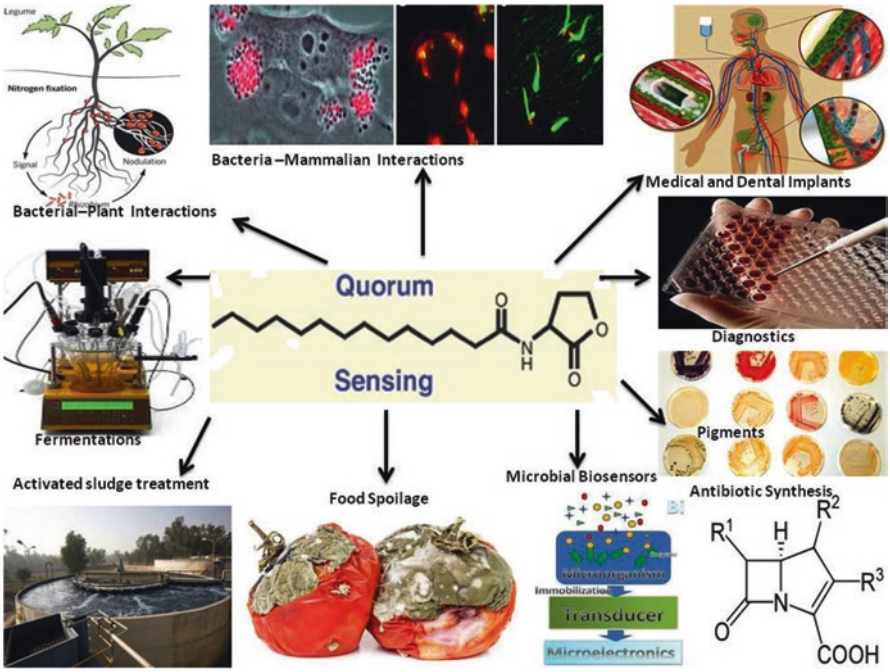


Fig. 1.1 Application of Quorum Sensing Systems in Agriculture, Medicine and Food Industry

delignification and saccharification (Wang and Chen 2009). Remarkably, a small number of AHL molecules were depicted to have metal-chelating properties and are employed for scavenging metals by many microbes. It is noteworthy that microbial biosensors were also designed to recognize cancer cell aggregation in vivo and can be subjugated to create biosensors that can target cancer cells (Mangwani et al. 2012).

1.3 Quorum Sensing and Biofilm Formation in Medicine

Bacterial biofilms have unique significance from a therapeutic perspective. Likewise, in the clinical perspective viewpoint, quite a few aspects of pathogenesis are unswervingly associated with progression of biofilms. There are abundant types of surfaces that contained types of clinical settings that can hold up biofilm development. Escalating antibiotic resistance among the pathogenic bacteria is anticipated as a nerve-racking dilemma as bacteria develop escape strategies against traditional and conventional antimicrobials. Cell-to-cell communication amid various bacterial pathogens is a population-dependent phenomenon used by several bacteria for regulating broad range of activities together with adaptation to numerous altering environmental conditions during growth for their better survival and characteristic

expression of phenotypes by synchronized gene regulation. Bacteria groups evolving as a biofilm have augmented resistance to antibiotic treatments, antiseptics, host immune defenses, and other cleansing agents proportionate to their planktonic counterparts (Welch et al. 2005). Nevertheless, disrupting the QS strategy against bacterial pathogens is devoid of giving rise to the development of antibiotic resistance. In general, technology innovations have propelled development and discovery of novel QSI molecules in medical use. However, QS signaling pathway impeding QS mechanism through QS inhibitors would be a novel approach undoubtedly. Formation of biofilm on medical appliances has resulted in the characterization of new infectious disease causing clones. It is not only the medical field which experiences the problem caused by biofilm formation, food processing industry and agriculture production also face similar problem where biofilms formed over the utensils act as a constant source of pathogen that contaminate the food products (Brooks and Flint 2008a; Bai and Vittal 2014a).

1.4 Quorum Sensing and Biofilm Formation in Agriculture

Bacterial QS shapes rhizosphere community in plants and important component for plant growth and development. Production of QS molecule has been established in bacterial isolates of numerous genera associated to plants, both beneficial and pathogenic types, respectively (Cao et al. 2009). However, stimulation of QS in plant microbes regulates and enhances indispensable actions in beneficial and pathogenic rhizosphere bacteria, including biofilm formation, antibiotic production, nitrogen fixation, integration of virulence genes, and disease establishment (Sanchez-Contreras et al. 2007). This strategy can also be extended to genetically engineer pathogen-specific AHL secretion into beneficial rhizosphere bacteria. Interestingly, these transgenic bacteria when supplemented to farm soil would anticipate augmenting crop defense against certain specific plant pathogens. Researchers also employed quorum quenching (QQ) enzymes to lessen bacterial virulence against plants. This study implies that engineering production and secretion of QQ enzymes into plants and plant-associated microbes may also serve as a unique crop protection strategy. However, despite the prospective agricultural benefits associated with AHL modulation of QS systems, this field, in general, has received plausibly modest attention. In principle, therefore, stimulation of these systems using small molecules could be desirable in an agricultural context (de Kievit et al. 2009).

1.5 Quorum Sensing and Biofilm Formation in Food Industry

QS signals are also implicated in food spoilage and affects food industry. Several studies reported the relationship between QS and biofilm formation in food contamination, persistent infections by food-related bacteria. Furthermore, it is quiet

complex to control the biofilm development for the reason that microorganisms in biofilm evolve diverse mechanisms in different nutritional and environmental conditions (Bramhachari 2018). Nonetheless, approaches to limit biofilm are yet inadequate. Yet, comprehensive understanding of the genetics in biofilm development can facilitate to improve preventive strategies. Thus, blocking QS signaling molecules in food-related bacteria possibly prevent QS-regulated phenotypes and is enormously conscientious for food spoilage. QS inhibitors may avert colonization of food surfaces, toxin formation, and proliferation of pathogenic microbes in food. For that reason, discovery of QSI/QQ compounds from plethora of natural sources and their evaluation for toxicological status may perhaps assist their use as food biopreservatives linked with reduction in food spoilage, economic losses, and pave way for the inventive methods in food preservation (Bai and Rai 2011).

1.6 Significance

The QS regulates communications in both signal producing organisms and amid different species, disease-causing and useful microbes, and higher organisms (growth promotion, pathogenicity, and symbiosis). It has importance in agriculture (in the areas of plant–microbe interactions in connection to symbiosis and pathogenicity (plant growth promotion, biocontrol and virulence) ecology (microbial habitats), medicine (colonization in medical devices and disease causing human and animal hosts, virulence, and pathogenesis), food industry (food spoilage, food production, and preservation), aquatic, industrial biofouling. Unsurprisingly, for that reason, QS field has gained remarkable concern in recent years. The range of potent activators and inhibitors of QS systems have been employed, endowed with an extensive set of chemical tools to investigate and maneuver this intricate signaling process. The book focuses on how bacteria orchestrate their responses to external signals and coordinate an activity by employing various applications. The concept of the book spins with the contemplation of QS systems controlling several physiological behaviors in bacteria and fungi. The chapter clearly illustrated the recent advancements on various functional aspects of QS systems and its applications, viz., survival behavior, antibiotic resistance, and application of QS inhibitors to counter antibiotic resistance. In summary, the book also explicates a complete yet an elaborative portrayal of basic and applied aspects of QS inhibitors/quorum quenchers, and large number of challenges associated with medicine, food industry, and agriculture. Nevertheless, these intricate quorum communications raise numerous fundamental questions which are progressively catching the attention of scientists in medicine, agriculture, and food industry (Bramhachari 2019).

Much research on QS is presented in the literature, but still result and application-oriented studies warrant the comprehensive mechanism of anti-QS and antibiofilm action of QSI/QQ against variety of bacterial pathogens. The identification and characterization of anti-QS compounds as new antipathogenic and antibacterial candidates might play imperative role in reducing the virulence and pathogenicity

of bacteria including drug-resistant bacteria, in reducing the biofilm hazard for safe infection-free health, and in combating the food-borne pathogens for safe food production. There are abundant opportunities for novel biotechnological applications to hinder/boost QS-controlled functions in bacteria. Natural anti-quorum sensing molecules and strategies already subsist, but more QSI/QQ molecules are yet to be uncovered. Numerous QS systems are discovered and established, along with several notable model systems that include regulation of genes essential for successful establishment of symbiotic and pathogenic microbial interactions. Thus, understanding the microbial QS machinery and outcome has indispensable implications to comprehend the diverse host–pathogen symbiotic interactions and may perhaps conceivably bestow with ground-breaking targets for antimicrobial therapies that block or interfere with their crisscross microbial communication networks (Bramhachari 2019).

Acknowledgments Dr.Bramhachari is grateful to Krishna University for extending the necessary support.

Conflict of Interest The author declare that there is no conflict of interest.

References

Bai AJ, Rai VR (2011) Bacterial quorum sensing and food industry. Compr Rev Food Sci Food Saf 10(3):183–193

Bai AJ, Vittal RR (2014a) Quorum sensing inhibitory and anti-biofilm activity of essential oils and their in vivo efficacy in food systems. Food Biotechnol 28:269–292

Bai AJ, Vittal RR (2014b) Quorum sensing inhibitory and anti-biofilm activity of essential oils and their in vivo efficacy in food systems. Food Biotechnol 28:269–292

Bramhachari PV (2018) Introduction to quorum sensing research in diverse microbial systems. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 3–6

Bramhachari PV (ed) (2019) Implication of quorum sensing system in biofilm formation and virulence. Springer, Cham

Brooks JD, Flint SH (2008a) Biofilms in the food industry: problems and potential solutions. Int J Food Sci Technol 43:2163–2176

Brooks JD, Flint SH (2008b) Biofilms in the food industry: problems and potential solutions. Int J Food Sci Technol 43:2163–2176

Cao HJ et al (2009) Complex quorum-sensing regulatory systems regulate bacterial growth and symbiotic nodulation in *Mesorhizobium tianshanense*. Arch Microbiol 191:283–289

De Kievit TR (2009) Quorum sensing in *Pseudomonas aeruginosa* biofilms. Environ Microbiol 11(2):279–288

Defoirdt T, Boon N, Bossier P, Verstraete W (2004) Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. Aquaculture 240:69–88

Flemming H-C, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14:563–575

Galiè S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F (2018) Biofilms in the food industry: health aspects and control methods. Front Microbiol 9:898

Gopal N, Hill C, Ross PR, Beresford TP, Fenelon MA, Cotter PD (2015) The prevalence and control of *Bacillus* and related spore-forming bacteria in the dairy industry. Front Microbiol 6:1418

Huang J, Yang Y, Zeng G, Gu Y, Shi Y, Yi K et al (2019) Membrane layers intensifying quorum quenching alginate cores and its potential for membrane biofouling control. Bioresour Technol 279:195–201

Köhler T, Buckling A, Van Delden C (2009) Cooperation and virulence of clinical *Pseudomonas aeruginosa* populations. Proc Natl Acad Sci 106(15):6339–6344

Kothari V, Patel P, Joshi C (2018) Modulation of microbial quorum sensing: Nanotechnological approaches. In: Design of nanostructures for versatile therapeutic applications. William Andrew Publishing, Oxford, pp 523–563

Mangwani N, Dash HR, Chauhan A, Das S (2012) Bacterial quorum sensing: functional features and potential applications in biotechnology. J Mol Microbiol Biotechnol 22(4):215–227

Sanchez-Contreras M et al (2007) Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos Trans R Soc B Biol Sci 362:1149–1163

Shrout JD, Chopp DL, Just CL, Hentzer M, Givskov M, Parsek MR (2006) The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. Mol Microbiol 62(5):1264–1277

von Bodman SB, Willey JM, Diggle SP (2008) Cell-cell communication in bacteria: united we stand. J Bacteriol 190(13):4377–4391

Wang ZW, Chen S (2009) Potential of biofilm-based biofuel production. Appl Microbiol Biotechnol 83(1):1–18

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Welch M et al (2005) Cell–cell communication in Gram-negative bacteria. Mol BioSyst 1:196–202

2 Quorum Sensing-Controlled Gene Expression Systems in Gram-Positive and Gram-Negative Bacteria

Meghanath Prabhu, Milind Naik, and Veda Manerikar

Abstract

In this chapter, quorum sensing (QS)-controlled gene expression systems in Gram-positive and Gram-negative bacteria is particularly emphasized. Acyl homoserine lactone (AHL) autoinducer (AI)-mediated signalling is a communication system in Gram-negative bacteria that control specific genes expression imparting physiological characteristics such as biofilm formation, bioluminescence, antibiotic synthesis, plasmid transfer, virulence factor, metal resistance and hydrocarbon degradation. AI concentrations reach a threshold level when adequate bacterial density is present that allows sensing a critical cell mass and in response they activate or repress target genes expression. Strikingly, AI binds the LuxR-type proteins, triggering them bind DNA and activate transcription of target genes. Synthesis of the AHL is dependent on a *luxI* homologue and a *luxR* homologue encoding a transcriptional activator protein, which is accountable for recognition of the cognate AHL and expression of correct gene. Gram-positive bacterial QS systems typically use secreted small oligopeptides via a dedicated ABC (ATP-binding cassette) exporter protein and two-component systems, which involve membrane-bound sensor kinase receptors and transcription factors present in cytoplasm which is responsible for alterations in gene expression. The process of signal transduction takes place as a phosphorelay cascade.

Keywords

Quorum sensing (QS) · Acyl homoserine lactone (AHL) · Autoinducer (AI) · ABC (ATP-binding cassette) · Controlled gene expression

M. Prabhu (✉) · M. Naik · V. Manerikar
Department of Microbiology, Goa University, Taleigao Plateau, Goa, India
e-mail: prabhumeghanath66@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_2

2.1 Introduction

Quorum sensing (QS) is a mechanism by which bacteria 'talk' with one another in response to population density through signalling molecules by controlling gene expression, thereby coordinating their behaviour. A process of bacterial QS depends on the production of extracellular chemical signalling molecules known as 'autoinducers' (AIs) (Nealson et al. 1970; Rutherford and Bassler 2012; LaSarre and Federle 2013; Naik et al. 2017; Mukherjee et al. 2017; Syal 2017). It is well documented that QS is used to coordinate global pattern of gene expression and regulation in bacteria (Turovskiy et al. 2007). It is used by both Gram-positive and Gram-negative bacteria to regulate expression of characters that permit bacteria to exploit particular niche. Such collective gene expression helps pathogenic bacteria to save energy during its host attack process which is otherwise energetically very expensive when taken up by individual cell (LaSarre and Federle 2013). QS is used to coordinate gene expression and regulation that promote invasion, defence and spread among local populations and is well documented particularly in human bacterial pathogens (LaSarre and Federle 2013; Papenfort and Bassler 2016).

In order to tolerate environmental changes, bacterial population have evolved QS mechanism to sense the adjacent environment, assimilate these signals and acclimatize their physiology to survive under changing conditions. The bacterial population in community perceive, respond and collectively modify behaviour through AIs, the concentration of which increases as bacterial population increases (Fuqua et al. 2001; Camilli and Bassler 2006; Papenfort and Bassler 2016). Through QS molecules, a species-specific cell-to-cell communication is achieved which plays important role in effective pathogenic or symbiotic interactions of diversity of bacteria with plant and animal hosts (Rutherford and Bassler 2012). Noteworthy that, AI relay mechanisms and target genes regulated by bacterial signalling systems vary in each case that allow bacteria to synchronize the gene expression and behaviour of the complete bacterial community. Bacterial community shows distinct characteristics due to QS such as biofilm formation, exopolysaccharide (EPS) production, bioluminescence, pigment and siderophores production, secretion of the virulence factor (toxin, hemolysin, enzymes, antibiotic resistance), metal resistance and aromatic hydrocarbon degradation (Fuqua et al. 2001; Rutherford and Bassler 2012; LaSarre and Federle 2013; Papenfort and Bassler 2016).

In a nutshell, all of the identified QS systems rely on three principles:

1. The members of the bacterial population synthesize AIs (signalling molecules).
2. AIs are sensed by the receptors that are present inside the bacterial cytoplasm or on the membrane.
3. Expression of genes essential for cooperative behaviours are triggered as well as detection of AIs results in activation of AI synthesis. Autoinduction loop apparently endorses synchrony in the population.

In this chapter, we describe the QS-controlled gene expression systems in Gram-negative and Gram-positive bacteria.

2.2 Gram-Negative and Gram-Positive Bacterial Quorum Sensing

2.2.1 AHL-Based Gene Expression Controlling System in Gram-Negative Bacteria

Different types of signalling systems are present in Gram-positive and Gram-negative bacteria. In the most described cases, *N*-acyl homoserine lactones (AHLs) (also referred as autoinducer-1, i.e. AI-1) signalling system is exclusively used by Gram-negative bacteria and is one of the best-investigated signalling mechanisms. The *V. fischeri* LuxR/LuxI bioluminescence system (R/I system) was first ever bacterial communication system investigated, after which many QS systems were studied in different Gram-negative bacteria (Lazdunski et al. 2004). The LuxI-like AHL synthases synthesize a specific AHL by joining the acyl side chain of a particular acyl–acyl carrier protein (acyl-ACP) from the fatty acid biosynthetic apparatus to the homocysteine moiety of *S*-adenosylmethionine (SAM) (Papenfort and Bassler 2016). These moieties are made up of homoserine lactone (HSL) ring attached to an acyl chain. The acyl side chain can vary in length from 4 to 16 (or up to18) carbons atoms, can be completely saturated and possess hydroxyls or carbonyls on the third carbon (Fuqua et al. 2001; LaSarre and Federle 2013). Some AHLs are also reported to be detected by membrane-bound sensor kinases, e.g. LuxN of *V. harveyi*, that start phosphorelay signalling cascades after ligand binding. After a threshold level of the AHL concentration is achieved in the surrounding, there is interaction between the AHL and a LuxR-type receptor in the cytosol of the bacteria. LuxR receptors are important transcriptional regulators whose DNA-binding properties initiate upon interaction with the AHL, resulting in target gene expression regulation in response to the AHL accumulation. A well known example of LuxI/R signalling is *Chromobacterium violaceum*, which utilizes CviI, CviR and AHL (C6HSL) to control violacein production (Stauff and Bassler 2011).

2.2.1.1 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative, opportunistic bacterial human pathogen associated with various kinds of serious illnesses such as respiratory infections, gastrointestinal infection, urinary tract infection, septic shock, etc. (Gupta et al. 2011; Prabhu et al. 2014). *P. aeruginosa* secrete various kinds of siderophores such as pyocyanin, pyoverdine, pyochelin and phenazine derivatives as well as pyorubin, pyomelanin, chloraphin or various combinations of these molecules (Prabhu et al. 2014). These siderophores are secreted as secondary metabolites when populations reach threshold level and also regulate growth of virulence factors in *P. aeruginosa* (Lamont et al. 2002). *P. aeruginosa* regulates several gene expressions via QS and employs three interconnected QS systems – LasRl, RhlRl and PQS – that each produces unique signalling molecules. *P. aeruginosa* possesses two recognized QS systems: LuxI/R-type QS systems produce and recognize N-3-oxododecanoyl-L-homoserine lactone (3OC12-HSL), whereas LasI/R and RhlI/R synthesize and detect C4-homoserine lactone. These dictate synchronous

synthesis of virulence factors and production of biofilms that are crucial in infections by *P. aeruginosa* (Mukherjee et al. 2017). The LasR:3OC12–HSL complex triggers transcription of numerous genes including RhlR. RhlR attaches to the autoinducer N-butanoyl-L-homoserine lactone (C4-HSL), the product of the RhlI synthase. RhlR:C4-HSL regulates a large genes cluster as well as those encoding virulence factors, viz. pyocyanin, elastases and rhamnolipids. RhlR is dependent on C4-HSL, but in the absence of C4-HSL, it is also able to work as a transcriptional regulator. QS-activated genes encoding products, viz. Pel and Psl exopolysaccharides, rhamnolipids and phenazines play important role in biofilms (Mukherjee et al. 2017). Biofilm enables horizontal gene transfer (HGT) between bacteria and thereby conferring resistance and metabolic benefit to the whole biofilm community (Syal 2017).

2.2.1.2 *Vibrio cholerae*

In *V. cholerae*, QS regulates genes accountable for the development of over 70 virulence factors such as cholera toxin. QS also regulates pilus formation and the expression of genes responsible for biofilm formation. QS activates type VI secretion, resulting in lysis of neighbouring non-kin cells which carry out the scavenging of DNA from lysed cells and HGT. Molecule (S)-3-hydroxytriecan-4-one was identified as an AI in *V. cholerae*, which is similar to (Z)-3-aminoundec-2-en-4-one as in *V. harveyi* (Papenfort and Bassler 2016). These molecules collectively are referred as cholera AI-1 (CAI-1). In *V. cholerae*, the CAI-1 AI synthase (CqsA) acts on SAM and decanoyl-CoA to produce amino-CAI-1 that may be spontaneously converted into CAI-1. Both amino-CAI-1 and CAI-1 are biologically active. Amino-CAI-1 is more stable than CAI-1 which increases the possibility that CAI-1 promotes a rapid response to fluctuations of AI (LaSarre and Federle 2013). The uncharacterized CAI-1 is made by CqsA (cholerae quorum sensing) and sensed by its similar sensor CqsS. AI-2 and CAI-1 are responsible for downregulation of several genes while upregulating the expression of Hap protease, the enzyme enabling the removal of *V. cholerae* from the intestine (Federle and Bassler 2003; Turovskiy et al. 2007).

2.2.2 Peptide-Based Gene Regulation System in Gram-Positive Bacteria

Peptide signals or oligopeptide-two-component-type QS system is used by Gram-positive bacteria which are comprised of membrane-bound sensor kinase receptors and cytoplasmic transcription factors that regulate alterations in gene expression (Papenfort and Bassler 2016). Many peptide AIs known till today are sensed by a membrane-bound sensor kinase, which changes its kinase/phosphatase activity in reply to communication with peptide, which changes the phosphorylation state of the response regulator. The final outcome is activation or repression of QS target genes. The *agr* QS system of *S. aureus* and the *fsr* system of *Enterococcus faecalis* both involve utilizing extracellular detection and both control virulence factor production like hemolysins (hly), Panton–Valentine leukocidin (*pvl*), enterotoxin A

(*entA*), coagulase (*coa*) and immune-modulatory factors encoding genes (Qazi et al. 2006; Oogai et al. 2011; Rutherford and Bassler 2012).

2.2.2.1 *S. aureus*

S. aureus is the normal flora of the human skin but is a foremost reason of nosocomial infections globally and leads to diseases from minor skin infections to fatal systemic disorders which include pneumonia, bacteraemia and sepsis (David and Daum 2010). Ability to cause disease by *S. aureus* based on expression of various adhesion molecules, toxins and compounds that hamper the immune system (Qazi et al. 2006). *Staphylococcus aureus* and various *Streptococci* have peptide-mediated QS systems (Qazi et al. 2006). Peptide signals in *S. aureus* are detected at the surface of the cell or intracellularly. Expression of genes encoding virulence factors are regulated by peptide-based QS (Garmyn et al. 2011; Oogai et al. 2011; Rutherford and Bassler 2012). Free-living *S. aureus* does not cause infection, for instance, endocarditis, osteomyelitis and foreign body-related infections, but are triggered by biofilms. Surface-associated adhesins, hemolysin, toxins and autolysins, the virulence factors important for staphylococcal infections, are regulated via the accessory gene regulator (agr) system (Oogai et al. 2011). The transcription of two different transcription units present on the agr locus in *S. aureus* is controlled by P2 and P3 promotors. *agr*BDCA is a P2 promotor which encodes four proteins that establish the *Agr*-sensing mechanism. In *S. aureus*, *agr* QS centres around cyclic autoinducing peptides (AIPs) belonging to four distinct groups that cooperate with cognate AgrC sensor kinases of the same group and control exotoxin production and biofilm production (Li and Tian 2016). Octapeptide is an AIP molecule in *S. aureus* with an exceptional thioester ring structure. As levels of AIP rises in extracellular atmosphere, it binds to the histidine kinase receptor (AgrC), which results in its autophosphorylation. The response regulator AgrA is further activated due to autophosphorylation, which along with SarA regulates the transcription at promotors (P2 and P3), resulting in increased intracellular concentration of RNAII (QS amplification) and RNAIII (exoproteins). Fascinatingly, Kaufmann et al. (2008) reported that AIP from *S. aureus* has the capacity for activating the *agr* regulon on its own and is also capable of inhibiting the *agr* activation of other strains. Such cross-strain inhibition of the *agr* response has been used to cure staphylococcal skin infections in animals. The peptide-based *agr* QS system is crucial for the progress of aggressive infections and disease, viz. subcutaneous abscesses, pneumonia, rabbit osteomyelitits and endocarditis (Kaufmann et al. 2008; Li and Tian 2016).

2.2.2.2 *Listeria monocytogenes (Lm)*

Gram-positive *Lm* is well-recognized bacterial pathogen causing listeriosis both in humans and animals (McGann et al. 2007). Sources of *Lm* infection are very diverse and range from contaminated foods, such as raw meat, raw milk, milking utensils, vegetables, dairy products, sausages, fruit juices, to ready-to-eat, packed foods and various environmental sources (Rieu et al. 2007). *Lm* is placed first in the list of emerging pathogens of concern for the public health and, therefore, for the food industry (Rantsiou et al. 2012). *Lm* is known to synthesize several virulence factors

such as invasion-associated proteins, internalin A, listeriolysin O (LLO), biofilm formation (Rieu et al. 2007; Naik et al. 2017) and various transcription factors (Rantsiou et al. 2012). Virulence factors encoding genes are under the control of the only QS mechanism involving *agr* peptide-sensing system as a complex controlling network for directing gene expression (Garmyn et al. 2009; Garmyn et al. 2011; Zetzmann et al. 2016). The virulence ability of an *agrD* deletion mutant was studied by Riedel et al. (2009), which approves the role played by *agr* QS system during the virulence of *Lm*. This report confirms that AgrD-dependent QS affects biofilm development, invasion, virulence and universal gene expression in *Lm* (Riedel et al. 2009). Production of AI-2 by Pfs and LuxS pathways in *Lm* has only been hypothesized, as genes *pfs (lmo1494)* and *luxS (lmo1288)* are existing in the genome of *Lm.* The *Lm*'s *agr* operon is similar to the *S. aureus* that comprises the four-gene operon *agr*BDCA system. Despite the resemblances at protein level, genetic organization and phenotypic characteristics regulation, *agr* systems vary about their mechanisms of target gene controlling in *Lm* (Zetzmann et al. 2016).

2.2.2.3 *Bacillus cereus*

B. cereus causes intestinal and non-intestinal infections in humans and is generally linked to food poisoning. QS regulates the ability of this bacteria to cause acute diarrheal disease through controlling the gene expression for the synthesis and secretion of a diversity of hemolysins, toxins, degradative enzymes and phospholipases (Rutherford and Bassler 2012). *B. cereus* members show the PlcR/PapR signalling system. PlcRa is a quorum sensing regulator with a signalling heptapeptide for PlcRa activity. 3D structural paralog of PlcR is PlcRa and bound explicitly to the abrB2 promoter. Inside the cells, PapR cooperates with PlcR, this results in complex formation which binds PlcR target spot on DNA leading to the stimulation of the PlcR regulon, which consists of 48 genes (Huillet et al. 2012).

2.3 Factors Affecting Quorum Sensing

The characteristics of surrounding physical environment of bacteria could influence QS (Decho et al. 2010). Similarly, diffusion of the signal through a specified environment will impact signal concentrations, with low flow rates encouragement and high flow rates controlling the AHL molecule build-up, respectively. In alkaline conditions generally, the AHL molecules are chemically unstable, thus accelerating the signal degradation under high pH environment (Fuqua et al. 2001). This effectively upsurge the concentration of quorum needed to trigger the associated quorum sensor. Interactions of the AHLs with extracellular reactants that weaken or sequester the signal molecules may also affect quorum build up. Expression of the AHLs synthase genes are directly affected and regulated by diverse environmental and physiological conditions, therefore directly affecting the production of signal.

2.4 Cross-Talk Between Different Bacterial Species

Gram-positive and Gram-negative microorganisms produce the AHL signal molecules and peptide signal molecules, respectively (LaSarre and Federle 2013; Papenfort and Bassler 2016; Naik et al. 2017). Signalling pathways from various bacterial communities collide resulting in interspecies communication (Qazi et al. 2006; Biswa and Doble 2013; Naik et al. 2017). *LuxS*-encoded autoinducer-2 (AI-2) system were reported to be used by both Gram-negative and Gram-positive bacteria that is major QS signalling systems involved in gene regulation. Also, there are QS signals which go beyond these classes, including *Pseudomonas* quinolone signal (PQS), diffusible signal factor (DSF) and autoinducer-3 (AI-3) (Lazdunski et al. 2004; Walters et al. 2006; Papenfort and Bassler 2016). Gram-negative bacterium *Salmonella enteritidis* was never reported to produce the AHL, but lately de Almeida et al. (2017) reported exogenous AHL stimulates biofilm formation in *Salmonella enteritidis*. Gram-negative bacteria producing peptide signal molecules has previously been reported (Han et al. 2011). Gram-negative bacteria such as *Proteus mirabilis*, *Pseudomonas aeruginosa, Citrobacter freundii* and *Enterobacter agglomerans* were reported to produce cyclic dipeptides which are capable of activating the AHL biosensor (Holden et al. 1999). Holden et al. (1999) confirmed that the Gram-negative bacteria were also capable of producing cyclic peptide molecules for quorum sensing cross-talk apart from the AHL. The AHL molecules produced by other bacteria are also believed to cross the bacterial cell envelope through diffusion, and existence of Gram-positive bacteria producing and responding to the AHL molecule has also been reported in marine *Exiguobacterium* sp. (Biswa and Doble 2013). *Chromobacterium violaceum* showed inhibition in the presence of C3-oxo-octanoyl homoserine lactone (OOHL) molecule produced by *Exiguobacterium* sp. (Biswa and Doble 2013); however, *Exiguobacterium* sp. was not inhibited by even at 100 μM of standard OOHL suggesting that the AHL molecules might be well recognized in the system of Gram-positive bacteria. This strain possesses a LuxR homolog designated as ExgR and also has LuxI homolog downstream to ExgR. Thus, suggesting a probability that bacteria might respond to the cross signal produced by another bacterium. The general observation was that the LuxR-type proteins can be activated by exogenous addition of the AHL molecules in heterologous hosts (Fuqua et al. 2001). *S. aureus* was reported to respond negatively to external AHL molecules. It has never been reported to harbour the AHL quorum sensing system. External *N*-acyl homoserine lactone was reported to antagonise virulence gene expression and QS in *S. aureus*. Also 3-oxo-C12-HSL was reported to inhibit *agr* QS expression in *S. aureus* (Qazi et al. 2006). In a very recent study by Naik et al. (2017), it was shown that Gram-positive *Lm* does not produce the AHL but responds to the AHL-forming biofilm. Naik et al. (2017) also observed the increase in biofilm formation by *Lm* with increasing concentration of the AHL molecule. The numbers of QS molecules will undoubtedly increase due to discovery of new QS molecules in novel bacteria. This will further reveal cross-talk between Gram-positive and Gram-negative bacteria.

2.5 Concluding Remarks

QS enables bacteria to coordinate communal behaviours. It is a very important gene expression and controlling mechanism used by various bacteria to regulate collective traits that permit bacteria to successfully exploit particular niches such as human gut and also in causing chronic infections in humans. Although through explosive research data in biochemistry, genetics, genomics and signal diversity of QS, we know the mechanism of QS systems and how it functions at the molecular level, but the correct biological function of many QS systems remains as a great scope. There are surely numerous QS systems to be discovered and there is a chance to separate out important differences between these QS systems. We are just starting to understand the cross-talk and bacterial sociality between various bacterial species via QS. The regulations of QS system open up a new area for treating infectious diseases and also use bacteria to realize the biology of sociality (Whiteley et al. 2017).

Acknowledgement Author MP and MN would like to thank for the financial support provided by Goa University. MN thanks the Science and Engineering Research Board (SERB), Department of Science and Technology, and Government of India for financial support by the Young Scientist Project (File Number: YSS/2014/000258).

References

Biswa P, Doble M (2013) Production of acylated homoserine lactone by gram-positive bacteria isolated from marine water. FEMS Microbiol Lett 343:34–41

Camilli A, Bassler BL (2006) Bacterial small-molecule signaling pathways. Science 311: 1113–1116

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23(3):616–687

de Almeida FA, Pimentel-Filho NJ, Pinto UM, Montavani HC, de Oliveira LL, Vanrtti MCD (2017) Acyl homoserine lactone-based quorum sensing stimulates biofilm formation by *Salmonella enteritidis* in aerobic conditions. Arch Microbiol 199:475–486

Decho AW, Norman RS, Visscher PT (2010) Quorum sensing in natural environments: emerging views from microbial mats. Trends Microbiol 18(2):73–80

Federle MJ, Bassler BL (2003) Interspecies communication in bacteria. J Clin Invest 112:1291–1299

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Garmyn D, Gal L, Lemaitre JP, Hartmann A, Piveteau P (2009) Communication and autoinduction in the species *Listeria monocytogenes*: a central role for the agr system. Commun Integr Biol 2(4):371–374

Garmyn D, Gal L, Briandet R, Guilbaud M, Lemaître JP, Hartmann A, Piveteau P (2011) Evidence of autoinduction heterogeneity via expression of the Agr system of *Listeria monocytogenes* at the single-cell level. Appl Environ Microbiol 77(17):6286–6289

Gupta RK, Chhibber S, Harjai K (2011) Acyl homoserine lactones from culture supernatants of *Pseudomonas aeruginosa* accelerate host immunomodulation. PLoS One. https://doi.org/10.1371/journal.pone.0020860

Han SW, Sriariyanun M, Lee SW, Sharma M, Bahar O, Bower Z, Ronald PC (2011) Small protein-mediated quorum sensing in a gram-negative bacterium. PLoS One 6:e29192

Holden MTG, Chhrabra SR, de Nys R, Stead P, Bainton NJ, Hill PJ, Manefield M, Kumar N, Labette M, England D, Rice S, Givskov M, Salmond GPC, Stewart GSAB, Bycroft BW, Kjelleberg S, Williams P (1999) Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. Mol Microbiol 33:1254–1266

Huillet E, Tempelaars MH, André-Leroux G, Wanapaisan P, Bridoux L, Makhzami S, Panbangred W, Martin-Verstraete I, Abee T, Lereclus D (2012) PlcRa, a new quorum-sensing regulator from Bacillus cereus, plays a role in oxidative stress responses and cysteine metabolism in stationary phase. PloS One. https://doi.org/10.1371/journal.pone.0051047

Kaufmann GF, Park J, Janda KD (2008) Bacterial quorum sensing: a new target for anti-infective immunotherapy. Expert Opin Biol Ther 8(6):719–724. https://doi.org/10.1517/14712598.8.6.719

Lamont IL, Beare PA, Ochsner U, Vasil AI, Vasil ML (2002) Siderophore-mediated signaling regulates virulence factor production in Pseudomonas aeruginosa. Proc Natl Acad Sci 99(10):7072–7077. https://doi.org/10.1073/pnas.092016999

LaSarre B, Federle MJ (2013) Exploiting quorum sensing to confuse bacterial pathogens. Microbiol Mol Biol Rev 77(1):73–111. https://doi.org/10.1128/MMBR.00046-12

Lazdunski AM, Ventre I, Sturgis JN (2004) Regulatory circuits and communication in Gram-negative bacteria. Nat Rev Microbiol 2(7):581–592. https://doi.org/10.1038/nrmicro924

Li Y-H, Tian X (2016) Quorum sensing and bacterial social interactions in biofilms. Sensors 12:2519–2538. https://doi.org/10.3390/s120302519

McGann P, Ivanek R, Wiedmann M, Boor KJ (2007) Temperature-dependent expression of Listeria monocytogenes internalin and internalin-like genes suggests functional diversity of these proteins among the listeriae. Appl Environ Microbiol 73(9):2806–2814. https://doi.org/10.1128/AEM.02923-06

Mukherjee S, Moustafa D, Smith CD, Goldberg JB, Bassler BL (2017) The RhlR quorum-sensing receptor controls *Pseudomonas aeruginosa* pathogenesis and biofilm development independently of its canonical homoserine lactone autoinducer. PLoS Pathog 13(7):e1006504. https://doi.org/10.1371/journal.ppat.1006504

Naik MM, Bhangui P, Bhat C (2017) The first report on Listeria monocytogenes producing siderophores and responds positively to N-acyl homoserine lactone (AHL) molecules by enhanced biofilm formation. Arch Microbiol. https://doi.org/10.1007/s00203-017-1416-8

Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 104:313–322

Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H (2011) Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl Environ Microbiol 77(22):8097–8105

Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol 14(9):576–588. https://doi.org/10.1038/nrmicro.2016.89

Prabhu MS, Walawalkar YD, Furtado I (2014) Purification and molecular and biological characterisation of the 1-hydroxyphenazine, produced by an environmental strain of *Pseudomonas aeruginosa*. World J Microbiol Biotechnol 30(12):3091–3099. https://doi.org/10.1007/s11274-014-1736-7

Qazi S, Middlenton B, Muharram SH, Cockayne A, Hill P, O'Shea P, Chhabra SR, Camera M, Williams P (2006) *N*-Acylhomoserine lactones antagonise virulence gene expression and quorum sensing in *Staphylococcus aureus*. Infect Immun:910–919

Rantsiou K, Mataragas M, Alessandria V, Cocolin L (2012) Expression of virulence genes of Listeria monocytogenes in food. J Food Safety 32(2):161–168. https://doi.org/10.1111/j.1745-4565.2011.00363.x

Riedel CU, Monk IR, Casey PG, Waidmann MS, Gahan CG, Hill C (2009) AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expres-

sion profiles in Listeria monocytogenes. Mol Microbiol 71(5):1177–89. https://doi.org/10.1111/j.1365-2958.2008.06589.x

Rieu A, Weidmann S, Garmyn D, Piveteau P, Guzzo J (2007) Agr system of Listeria monocytogenes EGD-e: role in adherence and differential expression pattern. Appl Environ Microbiol 73(19):6125–6133

Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a012427

Stauff DV, Bassler BL (2011) Quorum sensing in *Chromobacterium violaceum*: DNA recognition and gene regulation by the CviR receptor. J Bacteriol 193:3871–3878

Syal K (2017) Novel method for quantitative estimation of biofilms. Curr Microbiol 74(10):1194–1199. https://doi.org/10.1007/s00284-017-1304-0

Turovskiy Y, Kashtanov D, Paskhovar B, Chikindas ML (2007) Quorum sensing: fact, fiction, and everything in between. Adv Appl Microbiol 62:191–234

Walters M, Sircili MP, Sperandio V (2006) AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. J Bacteriol 188(16):5668–5681

Whiteley M, Diggle SP, Greenberg EP (2017) Progress in and promise of bacterial quorum sensing research. Nature 551:313–320. https://doi.org/10.1038/nature24624

Zetzmann M, Sanchez-Kopper A, Waidmann MS, Blombach B, Riedel CU (2016) Identification of *agr* peptide of *Listeria monocytogenes*. Front Microbiol 7:989. https://doi.org/10.3389/fmicb.2016.00989

Bacterial Quorum Sensing: Biofilm Formation, Survival Behaviour and Antibiotic Resistance

3

Ramesh Subramani and Mani Jayaprakashvel

Abstract

Biofilms are association of microorganisms that attach to each other to a surface enclosed in a self-generated extracellular matrix. Virtually (99.9%) all microorganisms have the competence to form biofilm. The formation of biofilm is a complex process, in which bacterial cells transform from planktonic cells to sessile mode of growth. The biofilm development results from the expression of specific genes. Biofilms have been developed as an adaptive strategy of bacterial species to survive in adverse environmental conditions as well as to establish antagonistic or beneficial interactions with their host. Molecular interaction and details of biofilm formation are not well-understood as bacteria in the biofilm have several orders of magnitude, more resistant to antibiotics compared to planktonic bacteria. Thus, the currently available drugs typically failed to target bacterial biofilms. Quorum sensing (QS) is a process of intercellular signalling or cell-cell communication and a vital regulatory mechanism for coordinating biofilm formation including common activities and physiological processes such as symbiosis, formation of spores or fruiting bodies, antibiotics synthesis, genetic competence, apoptosis and virulence in many bacterial species using extracellular QS signalling molecules, which is often referred to as autoinducers (AIs). Microorganisms produce a wide variety of QS signalling molecules that can be self-recognized in a concentration-dependent manner and subsequently induce

R. Subramani (✉)
School of Biological and Chemical Sciences, Faculty of Science, Technology & Environment, The University of the South Pacific, Suva, Republic of Fiji
e-mail: subramani_r@usp.ac.fj

M. Jayaprakashvel
Department of Marine Biotechnology, AMET deemed to be University, Kanathur, Chennai, Tamil Nadu, India
e-mail: jayaprakashvel@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_3

or suppress expression of QS-controlled genes. Bacterial QS regulation is established through a wide range of signals such as oligopeptides, N-acyl homoserine lactones (AHLs), furanosyl borate, hydroxy palmitic acid methyl ester and methyldodecanoic acid. In this chapter, we highlight the current understanding of the processes that lead to bacterial biofilm formation, survival behaviours and mechanisms of antimicrobial resistance in bacteria.

Keywords
Biofilm · Quorum sensing · Antibiotic resistance · Quorum quenchers · Acyl homoserine lactone · Microbial communication

3.1 Introduction

Biofilms are ubiquitous in nature and can occur on both animate and inanimate surfaces. Biofilms have been recognized as aggregates of many bacteria and simple eukaryotes growth on natural aquatic surfaces, clinical, industrial and domestic domains (Irie and Parsek 2008). The natural and clinical biofilms are formed by different types of microbial species with wide range of structural characteristics, however, majority of the biofilms are encased in self-produced extracellular matrix or extracellular polysaccharide (EPS) layer (Sutherland 2001a; Irie and Parsek 2008). The composition of extracellular matrix can differ between organisms, but are commonly abundant in proteins (<1–2%) including enzymes, polysaccharides (1–2%), nucleic acids (<1%) including DNA and RNA and water (up to 97%) (Lu and Collins 2007). The phenotype of matrix generally depends on environmental stress such as temperature, pH, osmolarity, UV radiation, desiccation, oxygen tension and nutrient availability (Staley et al. 2014). Bacterial communities in biofilm can switch from planktonic form to sessile form. The planktonic bacteria have relatively high cell growth and reproduction rate but low EPS production (Rabin et al. 2015). However, the sessile form exhibits slower growth of bacteria yet increased EPS production which is useful to form biofilms (Chadha 2014). It should be noted that biofilm forming bacterial mutants are unable to produce the EPS (Watnick and Kolter 1999). The synthesis of EPS matrix intends the bacterial cells to attach to a surface. Biofilm forms a thin layer eventually building to a thick layer (>100 layers) establishes a 'mushroom-' or 'tower'-shaped structure (Rabin et al. 2015). The bacterial arrangement in the biofilm depends on their metabolism and aero-tolerance; aerobic bacteria live in the upper layers, while anaerobic bacteria prefer to live in deeper layers of biofilm (Rabin et al. 2015).

Bacteria form biofilms for numerous advantages: enhance the tolerance of bacteria to harsh environmental conditions, avoid being washed away by water flow or blood stream and protection from antimicrobial agents and disinfectants (Jefferson 2004; Rabin et al. 2015). Further, the biofilm retards bacterial motility and increases cell density providing a suitable environment for plasmid exchange between bacterial communities by conjugation process. Some of these plasmids encode for antibiotic resistance (Hausner and Wuertz 1999; Rabin et al. 2015) and also enable them

to overcome different environmental stresses (Chadha 2014). The bacteria in a biofilm may communicate their presence to each other using chemical communication known as quorum sensing (QS). QS is a mechanism by which bacteria apparently regulate collective behaviours in response to cell density (Lyon and Muir 2003; West et al. 2012). The bacteria produce and release small diffusible signal molecules for cell-cell signalling. Diffusing of these small molecules into cells regulates (autoinduction) totally different behaviours including the production of a variety of small molecules that are released from bacterial cells to help growth, mobility, competence, sporulation, bioluminescence emission, symbiosis, antibiotic production and biofilm formation (West et al. 2012; Li and Tian 2012). In addition, the diffusion of these small molecules also contributes to an increase in the production of the signal molecule itself (autoregulation). Therefore, the production of these signalling or autoinducing molecules leads to high cell densities, which afford a considerable increase in the production of signal and QS-controlled factors (West et al. 2012; Darch et al. 2012). Generally, in infectious diseases, the invading bacteria need to reach a critical cell density before they show virulence and defeat the host defence mechanisms (Costerton et al. 2003; Li and Tian 2012). It is now apprehended that quorum sensing mechanisms occur in both unicellular prokaryotic and single-celled eukaryotic organisms such as fungi (Miller and Bassler 2001; Waters and Bassler 2005; van Bodman et al. 2008; Sordi and Muhlschlegal 2009; Li and Tian 2012). Furthermore, the cell-cell communication is apparent between microbial human pathogens through QS having important implications in the infections.

3.2 Structure, Biofilm Formation, Survivability and Quorum Sensing

Microbial biofilm formation is a dynamic process that floating (planktonic) cells transform to immobile (sessile) form of growth (Okada et al. 2005). Still controversy exists in QS involved in biofilm formation (Parsek and Greenberg 2005). However, it is demonstrated that QS are influencing the biofilm formation in several species (Parsek and Greenberg 2005). Further, it has been suggested that biofilm formation depends on the specific gene expression (Okada et al. 2005; Sauer et al. 2004). Biofilm formation is generally considered to occur through series of stages (Fig. 3.1): (1) adsorption or accumulation of organisms on an aggregator surface, i.e. substrate (deposition); (2) attachment to a surface or the desegregation of the interface between organisms and aggregator for formation of polymer bridges; (3) cell proliferation or growth of organisms on the aggregator's surface; (4) biofilm formation and maturation; and (5) detachment or dispersal (O'Toole et al. 2000; Garrett et al. 2008; Joo and Otto 2012).

In the accumulation step, microorganisms form initial conditioning layer composed of organic or inorganic molecules creating the foundation for biofilm growth. This layer provides a favourable environment for growth, nutrients and anchorage of the bacterial community (Characklis and Marshal 1990; Garrett et al. 2008). The attachment step is categorized as a two-stage process such as reversible attachment

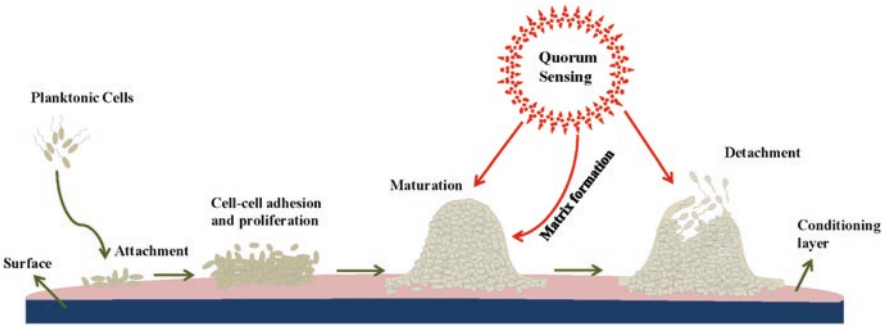


Fig. 3.1 Development process of biofilm

and irreversible attachment (Garrett et al. 2008; Renner and Weibel 2011). Bacteria should get closer enough to a surface for biofilm formation as bacterial cell meeting has both attractive and repulsive forces. When the bacterial cells have distance between 10 and 20 nm to a surface, the negative charges are repelled; however, van der Waals forces between the bacterial cells overcome the repulsion by attraction to a surface. Besides, fimbriae and flagella also provide the mechanical attachment to the surface (Palmer et al. 2007; Rabin et al. 2015). If the repulsive forces are higher than the attractive forces, the bacterial cells will disperse from the surface; this probably would occur before conditioning of a substrate (Garrett et al. 2008). In the early attachment, planktonic microbial cells are transferred from aqueous to the conditioned surface by either physical forces or bacterial flagella. Many environmental factors contribute to the reversible attachment of biofilm to a surface such as surface nature, temperature, pressure, available energy and bacterial orientation (Garrett et al. 2008). Besides, the reversibly attached cells persist immobilized and become irreversibly attached cells. The irreversibly attached biofilm can withstand greater physical or chemical forces (Sutherland 2001b; Liu et al. 2004; Rabin et al. 2015). The flagella and type IV pili play crucial role in irreversible attachment of cells to a surface and form microcolonies (Garrett et al. 2008; Rabin et al. 2015).

During the lag phase, the bacterial cells adapt to a surface or an environment for accumulation and attachment process. However, the rapid propagation in the population occurs in exponential phase or log phase. The bacterial rapid growth in the biofilm takes place with the sufficient nutrients accessible from the bulk fluid and the substrate surface depending on the nature of the environment (Garrett et al. 2008). During the cell division in the biofilm, daughter cells move outward and upward from the attachment point to form clusters, such interactions and growth provides mushroom-like structure (Hall-Stoodley and Stoodley 2002). It is believed that mushroom-like structure support the passage of nutrients to bacterial communities that lives in bottom of a biofilm. The secretions of extracellular matrix by bacteria in a biofilm aid to form bonding between cells due to interaction of polysaccharide, intercellular adhesion polymers and the presence of divalent cations (Dunne 2002; Garrett et al. 2008). Certain biofilm-related differential gene expression involved in the bacterial species for transforming planktonic to sessile form

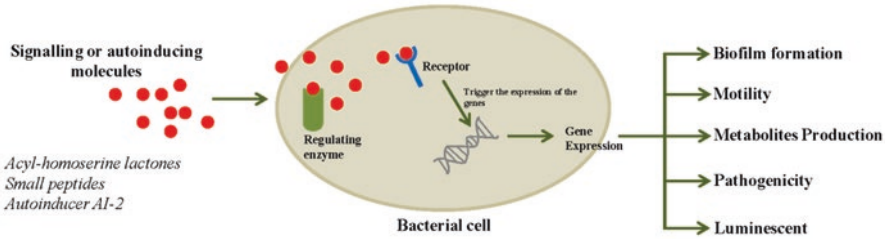


Fig. 3.2 General quorum sensing mechanism in biofilm formation

aids the cells for adhesion in the population. The motility of the sessile species is arrested and synthesis of external flagella is inhibited during this stage (Garrett et al. 2008). Meanwhile, the expression of number of genes in sessile species ameliorates the production of cell surface porin proteins and extracellular polysaccharides.

The cell surface porin proteins such as *OprC* and *OprE* provide the path of transportation of bacterial extracellular polysaccharides (homopolysaccharides or heteropolysaccharides) into the cell (Hancock et al. 1990; Sutherland 2001c). These polysaccharides play a key role in adhesion and cohesion of cells to form extracellular matrix. More than 50 cell surface proteins encoded for biofilm formation was found in the sessile cells which are absent in planktonic cells (Hall-Stoodley and Stoodley 2002). The fluid-filled matrix supports the distribution of nutrients consistently inside the biofilm (Parsek and Singh 2003). A cascade of cell signalling mechanisms are involved during high cell concentration in the biofilm. These signalling molecules or autoinducers (e.g. homoserine lactones and small peptides) are used to trigger gene expression by enzymatic process for developing and maturation of biofilm (Bassler 1999) (Fig. 3.2). The biofilm will break down during death phase by secreting of enzymes by the microbial community within the biofilm. The lytic enzymes produced by surface bacteria to break down the polysaccharides aggregate the biofilm for colonization of new substrates. Alginate lyase, N-acetyl-heparosan lyase and hyaluronidase are found to be generally used in the breakdown of the biofilm matrix in *Pseudomonas* spp., *Escherichia coli* and *Streptococcus equi*, respectively (Sutherland 1999). Concurrently, the gene coding proteins are upregulated for organisms' motility, pathogenicity, luminance and metabolites production (Garrett et al. 2008; Rabin et al. 2015) (Fig. 3.2).

3.3 Quorum Sensing in Bacteria

All microorganisms have the capability to form biofilm on any surface (Sekhar et al. 2009). During biofilm formation microbial communities including intraspecies and interspecies are able to communicate between them through a mechanism known as quorum sensing. There are well-known QS systems described in bacteria: acyl homoserine lactones (AHLs) are a major class of autoinducer signalling molecules used by Gram-negative species for quorum sensing. AHLs are composed of

homoserine lactone (HSL) rings containing acyl chains of C_4 to C_{18} in length (Ng and Bassler 2009). These side chains entertain occasional alteration, particularly at the C_3 position, or unsaturated double bonds (Fig. 3.3a) (Ng and Bassler 2009). Gram-positive bacterial species predominantly use modified oligopeptides as autoinducers in quorum sensing-regulated gene expression systems (Fig. 3.3b). Furthermore, the autoinducer molecules called AI-2 and HAI-1 system were found in both Gram-positive and Gram-negative bacterial species (Fig. 3.3c) (Fuqua et al. 2001; Bassler 2002; Sturme et al. 2002).

These systems play the central role for the formation of biofilm. QS relies upon the intercommunication of a small diffusible signal molecule with a sensor or transcriptional activator to trigger gene expression for QS-coordinated activities (Li and Tian 2012) (Fig. 3.2). It is constituted that during QS bacteria concurrently regulate gene expression in response to changes in high cell population densities and complexity of microbial species (Ng and Bassler 2009). There are two types of gene expression systems on QS, i.e. low-cell density dependent for individual and non-social behaviours and high-cell density dependent for group and social behaviours (Parsek and Greenberg 2005; Waters and Bassler 2005; Williams et al. 2007; Novick and Geisinger 2008). The detecting and responding to variations in cell density is the essential phases of QS. The low molecular weight molecules of autoinducers are produced intracellularly and secreted outside the cells either passively or actively. The increase of concentration of autoinducer is proportional to increase in the number of cells of a population. When autoinducers accumulate to meet minimal threshold, QS-related receptors bind to the autoinducers and activate signal transduction cascades leading to gene expression in population-wide changes (Ng and Bassler 2009) (Fig. 3.2).

3.4 Quorum Sensing in Gram-Negative Bacteria

An archetypal Gram-negative bacterial quorum sensing circuit is shown in Fig. 3.4a. Acyl homoserine lactone (AHL)-mediated Lux-type QS is common in many Gram-negative bacterial species. The LuxR and LuxI homologs in Gram-negative bacterial species are responsible for production of autoinducers (Fuqua et al. 2001; Parsek and Greenberg 2005). The first AHL autoinducer identified in the marine bacterium *Vibrio fischeri* inhabits as an endosymbiont in the light organ of Hawaiian squid *Euprymna scolopes* (Ruby 1996). The luminescence produced by *V. fischeri* is utilized by host *E. scolopes* for its anti-predation mechanism (Ruby 1996). The LuxI and LuxR are important proteins for QS regulation of bioluminescence in *V. fischeri*. LuxI is an autoinducer of *N*-3-(oxo-hexanoyl) homoserine lactone of the QS synthases (Fig. 3.3a) (Engebrecht and Silverman 1984; Schaefer et al. 1996). The autoinducer diffuses passively through the bacterial membrane, and its concentration increases both intra- and extracellularly as the cell density of the population increases (Bassler 2002). The LuxR is the cytoplasmic receptor for autoinducer and also the transcriptional activator of the luciferase luxICDABE operon (Engebrecht and Silverman 1984). The autoinducer ligand is necessary for the stability of LuxR

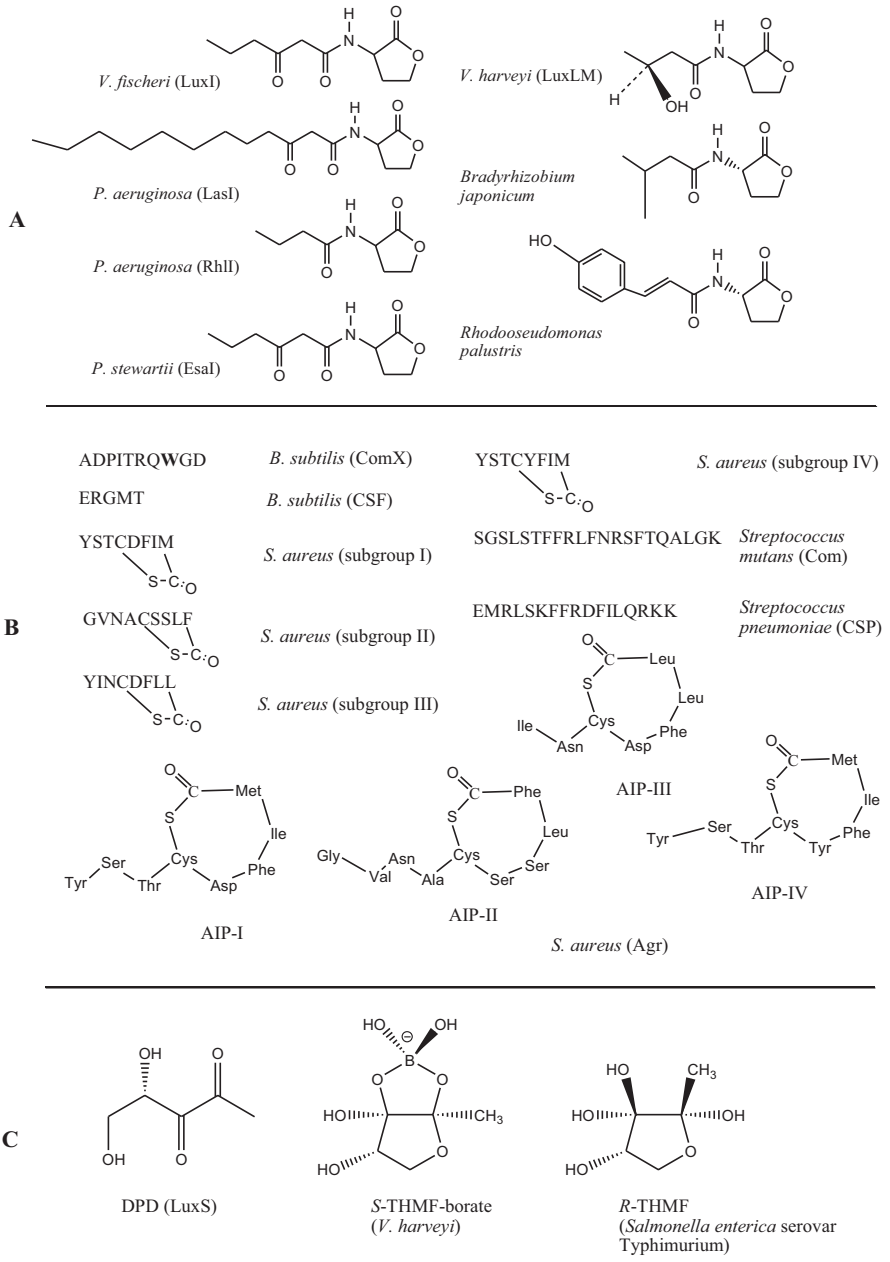


Fig. 3.3 Representative chemical structures of bacterial autoinducers and the responsible enzymes for their production. (**a**) Gram-negative *N*-acyl-homoserine lactone autoinducers. (**b**) Oligopeptide autoinducers and amino acid sequences of the peptide autoinducers produced by Gram-positive bacteria. The bolded tryptophan in *Bacillus subtilis* (ComX) is isoprenylated. (**c**) Autoinducer-2 family quorum sensing molecules. DPD (4,5-dihydroxy-2,3-pentanedione), the precursor to AI-2. In the presence of boron, AI-2 exists as *S*-THMF-borate ((2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate). In the absence of boron, AI-2 exists as *R*-THMF ((2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran)

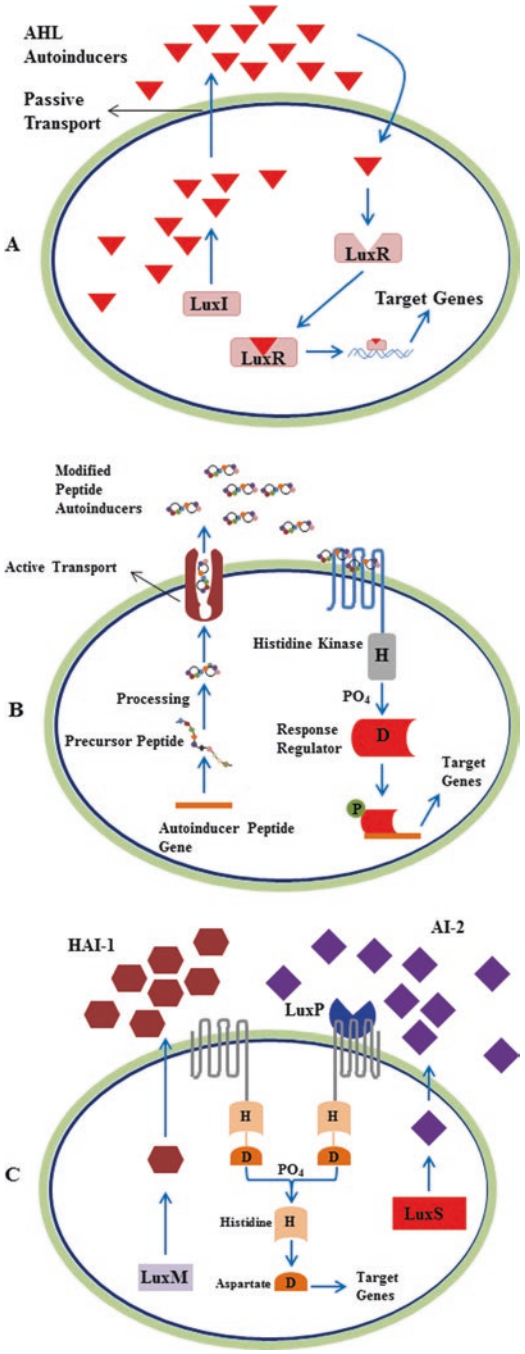


Fig. 3.4 Types of bacterial quorum sensing systems. (**a**) Gram-negative bacteria, (**b**) Gram-positive bacteria, (**c**) the *V. harveyi* quorum sensing circuit

protein. When the QS autoinducer accumulates, it is bound by LuxR and the LuxRAHL complex identifying a unanimous binding system upstream of the luxICDABE operon and activates its expression (Stevens et al. 1994).

Pseudomonas aeruginosa is an opportunistic pathogen that primarily infects the immunocompromised individuals. It is a well-studied pathogen in terms of the regulation of virulence factors and the role the QS acts in pathogenicity. *P. aeruginosa* virulence characters are regulated by two different Lux-type QS systems such as *las* and *rhl* (Sifri 2008). This *las* and *rhl* system regulates the cascade of virulence regulators in *P. aeruginosa* including various virulence traits such as exoprotease secretion, toxin production, motility and biofilm formation (Van Delden and Iglewski 1998). The AHL quorum sensing in *P. aeruginosa* has been described by detection of *las* and *rhl* AHLs *N*-3-oxododecanoyl homoserine lactone and *N*-butanoyl-homoserine lactone in sputum samples collected from cystic fibrosis-infected patients (Singh et al. 2000; Erickson et al. 2002). The AHL autoinducer molecules are normally unique among which a specific AHL molecule is recognized only by the bacterial species that produces it. Therefore, the AHL-quorum sensing systems largely nurture intra-species cell-cell communication (Ng and Bassler 2009). In addition, the AHL especially *N*-3-oxododecanoyl homoserine lactone exhibits antibacterial activity exclusively against Gram-positive bacteria. Therefore, the production of AHLs enhance *P. aeruginosa* is more determined in a mixed bacterial population or in a biofilm formation (Sifri 2008). The third non-AHL-QS system that cell signalling occurs through quinolone compounds has been discovered in *P. aeruginosa* in addition to *las* and *rhl* systems (Sifri 2008). Production of quinolones (4-hydroxy-2-alkylquinolines) is regulated by the transcriptional regulator MvfR (Pesci et al. 1999). The MvfR regulates the expression of many genes through the trigger of PqsH in the production of anthranilic acid and its conversion to 4-hydroxy-2-heptylquinoline, and its further conversion to 3,4-dihydroxy-2-heptylquinoline is known as *Pseudomonas* quinolone signal (Gallagher et al. 2002; Deziel et al. 2004). Synthesis of MvfR and PqsH is regulated by LasR, thereby intertwining the mvfR signalling pathway with AHL quorum sensing (Deziel et al. 2004). Expression of QS-regulated genes is controlled by MvfR but is different from those regulated by AHL autoinducers (Deziel et al. 2005). The large network of QS-regulators such as *las*, *rhl* and *mvfR* are controlled by a wide range of cellular functions in *P. aeruginosa*.

3.5 Quorum Sensing in Gram-Positive Bacteria

Figure 3.4b displays the typical quorum sensing circuit of Gram-positive bacteria. Quorum sensing is a cell-cell communication and regulation of gene expression in Gram-positive bacterial species. In Gram-positive bacteria, the signalling molecules or autoinducers are mostly small post-translationally processed peptides called autoinducing peptides (AIPs) (Fig. 3.3b) (Monnet and Gardan 2015). The AIPs are impermeable to cell membranes; thereby secretion of QS small peptides is usually actively mediated by specialized transport proteins and secreted into the

extracellular environment (Ng and Bassler 2009). Furthermore, in many cases, the initially produced small peptides are modified by processing and cyclization during secretion (Havarstein et al. 1995; Solomon et al. 1996; Ji et al. 1997; Ng and Bassler 2009). One of the major differences between Gram-positive and Gram-negative QS systems is the site of the cognate receptors. The Gram-negative species of LuxR-type receptors are cytoplasmic-bound, while the sensors for small oligopeptides in Gram-positive species are cell membrane-bound. Therefore, the signal transduction in Gram-positive species occurs through a series of phosphorylation cascade using membrane-bound two signalling proteins (Simon et al. 2007; Ng and Bassler 2009). The two-component signalling proteins such as membrane-bound histidine kinase receptor and a cognate cytoplasmic response regulator function as a transcriptional regulator (Simon et al. 2007; Ng and Bassler 2009). Similarly in AHL-QS systems, the concentration of secreted small oligopeptide autoinducers increases the cell density (Ng and Bassler 2009). A membrane-bound histidine kinase receptor activates its intrinsic autophosphorylation process by quorum sensing via detection of oligopeptide autoinducer accumulation and reaching a threshold concentration in the extracellular environment (Ji et al. 1995). This ATP-driven phosphorylation activity ensuring a conserved histidine residue (H) in the cytoplasm subsequently transfers the phosphate group to the conserved aspartate residue (D) of a cognate response regulator (Ng and Bassler 2009). Phosphorylation action triggers the regulators to employ as DNA-binding transcription factors to control expression of target genes (Ng and Bassler 2009).

Gram-positive peptide autoinducers are different from Gram-negative bacterial AHLs as they are genetically encoded, not showing similarity on a single core molecule. Therefore, Gram-positive bacterial species can produce a signal with a unique sequence (Fig. 3.3b) (Ng and Bassler 2009). The quorum sensing system of *Staphylococcus aureus* is a well-studied system, which is encoded by the accessory gene regulator (*agr*) locus (Sifri 2008). The *agr* system plays a crucial role in regulating the syntheses of a wide range of *S. aureus* virulence factors (Novick 2003) and complex association with biofilm formation (Sifri 2008). The *agr* locus consists of two different transcripts such as RNAII and RNAIII. The RNAII encoding *agrB*, *agrD*, *agrC* and *agrA* and RNAIII are instrumental in suppression of cell wall-related protein production and increase the production of exoprotein secretion in response to high cell concentration (Sifri 2008). The colonization facilitated by cell wall-associated adhesins however secreted products of *S. aureus* inevitably for invasion and dissemination. The four genes encoded by RNAII are involved in the production and sensation of the AIPs (Sifri 2008). The *agrD* encodes the precursor of the AIP; however, integral membrane protein *AgrB* controls its processing and excretion as a thiolactone-modified cyclic oligopeptide. The extracellular accumulation of the AIP is regulated by a two-component histidine kinase that constituted *AgrA* and *AgrC*, whereas the transcription of RNAII and RNAIII is induced by activation of *AgrA-AgrC*. Interestingly, RNAIII undergo self-transcription and acts as the regulatory effector molecule for the *agr* system, mainly by translational inhibition of the virulence gene repressor and possibly other gene regulators (Geisinger et al. 2006; Boisset et al. 2007; Sifri 2008). The autoinduction and signal

transduction of *agr* system regulates the staphylococcal virulence (Novick 2003; Sifri 2008).

3.6 Quorum Sensing in Intraspecies or Cross-Species

The third type of quorum sensing known as autoinducer 2 (AI-2) has been reported in both Gram-negative and Gram-positive species (Miller and Bassler 2001; Federle and Bassler 2003; Waters and Bassler 2005). The AI-2 quorum sensing system is different from the other two quorum sensing systems that are particularly implemental in signalling in intraspecies or cross-species communication (Schauder and Bassler 2001; Federle and Bassler 2003). However, two autoinducers, namely HAI-1 and AI-2, are produced by a marine bacterium *V. harveyi* (Fig. 3.3c). Notably, HA-1 is archetypal to the Gram-negative QS metabolite AHL. However, HA-1 synthesis is not dependent on a LuxI-like enzyme. AI-2 is a furanosyl borate diester that regulates cell density-dependent bioluminescence in *V. harveyi* (Chen et al. 2002; Vendeville et al. 2005). Both the HAI-1 and AI-2 signal transductions occur through similar phosphorylation cascade of Gram-positive species. Later, Miller et al. (2004) also reported a chemically distinct form of the quorum sensing signalling molecule AI-2 from *Salmonella typhimurium* (Fig. 3.3c).

The model system of quorum sensing circuit controlling bioluminescence shown in Fig. 3.4c is that of the Gram-negative bacterium *V. harveyi*. A highly conserved catalytic enzyme *LuxS* plays a vital role in AI-2 synthesis. The AI-2 quorum sensing is predominant among prokaryotic organisms as *luxS* gene has been determined in several bacterial species (Li and Tian 2012). In AI-2 synthesis, *LuxS* primarily converts *S*-ribosylhomocysteine to homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) (Fig. 3.3c) where the DPD is a precursor molecule for synthesizing AI-2 (Irie and Parsek 2008). The LuxP protein acts as a cytoplasmic receptor and a transcriptional activator in the *V. harveyi* system (Vendeville et al. 2005). However, in *Salmonella enterica* sv. Typhimurium system, AI-2 is first transported inside the cell prior to initiating a signalling cascade (Taga et al. 2001; Irie and Parsek 2008). A number of diverse interspecies cell-cell communication have been reported by AI-2 that regulates specific target gene expression in *V. harveyi, Vibrio cholerae, Escherichia coli, Salmonella typhimurium, Shigella flexneri, Streptococcus pyogenes, Clostridium perfringens, Porphyromonas gingivalis, Neisseria meningitidis, Borrelia burgdorferi* and *Actinobacillus actinomycetemcomitans* (Bassler 2002).

3.7 Biofilm as a Mechanism of Resistance to Antibiotics

It is now confirmed that intra- and intercellular communications in microorganisms govern its microbial ecology (Penesyan et al. 2015). The complex cell-cell signalling in natural bacterial communities promotes the evolution and leads the bacterial species as super bugs that are extremely resistant antibiotics. More than 75% of bacterial infections involved biofilm formation and encompasses surface-attached

bacterial colonies that are protected by an extracellular matrix (Musk and Hergenrother 2006). It is reported that the immobile or sessile bacterial species in a biofilm are 1000 times more resistant to antibiotic treatment than the same organism that were grown as free-floating planktonic cells, which rigorously complicates treatment choices (Rasmussen and Givskov 2006). The knowledge about the molecular mechanisms of antibiotic resistance in biofilms is meagre, despite the existence of a decade of research. However, some intrinsic and extrinsic resistance factors are documented to biofilm resistance to antibiotics (Anderson and O'Toole 2008).

The intrinsic factors also known as innate resistance are associated with biofilm development and life cycle (Paraje 2011). There are many different types of intrinsic biofilm factors that are influential of the antibiotic resistance in bacterial communities in biofilm. The factors being: (1) Biofilms can act as diffusion barriers to preclude antibiotics to reach their targets. Biofilm constitutes a rich exopolysaccharide, enzymes, DNA, protein, water channel and bacterial cells (Rabin et al. 2015). These physical and chemical properties of the matrix make the antibiotics liable to limited diffusion or render it ineffective against bacterial species in biofilm and make them more resistant against antibiotics. (2) The deficiency of nutrients and oxygen inside biofilms facilitates the bacterial communities to establish the microenvironment within the biofilm. This microenvironment might induce alternative metabolic activity resulting in slow growth of the bacteria (Paraje 2011). Furthermore, many studies are evidential to the oxygen limitation, hypoxic zones, restricted nutrient diffusion and slow or no growth of bacteria within biofilms (Patel 2005; Paraje 2011). The slow growth certainly leads resistance to killing of bacteria which are occupied within the biofilm (Costerton et al. 1999). However, the stationary planktonic cells are being killed due to slow growth in the microenvironments that undermine the activity of antibiotics by pH variations (Patel 2005; Høiby et al. 2010). (3) A small subpopulation of bacteria within biofilm possibly differentiates into persister cells (Paraje 2011). Generally, the non-growing or slow-growing bacteria in the biofilms that differentiate into dormant cells are considered as persister cells, which are highly resistant to antibiotic treatment (Lewis 2005). The persister cells undergo phenotypic variations by stable genetic changes for withstanding in the extreme antibiotic treatment environment (Keren et al. 2004). However, the association among planktonic persisters and biofilm resistance and the mechanisms of antibiotic tolerance are unclear (Paraje 2011). (4) Microbial communities in biofilms producing imbalanced or increased oxidants such as the free radicals, peroxide and nitric oxide lead to overproduction of reactive oxygen species (ROS) (Paraje 2011). Resulting in the detoxification of ROS by antioxidant defence enzymes particularly, superoxide dismutase (SOD) and catalase (CAT) are inadequate to eliminate the free radicals in the biofilms (Sardesai 1995). Cumulatively, ROS known as oxidative stress may result in significant damage to cell structures including the matrix, DNA, proteins and lipids (Becerra et al. 2006; Baronetti et al. 2011; Arce Miranda et al. 2011).

Furthermore, the amplified synthesis of oxidative stress stimulates specific variations in the physiology of bacteria (Paraje 2011). It is also important to note that

biofilm development is determined by the balanced production of oxidants (ROS and NO) and antioxidant defences (SOD) which can be much affected by diverse environmental stress factors that would lead to cellular stress causing a reduction in the extracellular matrix of the biofilms (Arce Miranda et al. 2011). In addition, bacteria in biofilms can trigger oxidative stress mode through inducing the SOS response and activating DNA repair systems, such as methyl mismatch repair (MMR) or the DNA oxidative repair system (GO) (Jolivet-Gougeon and Bonnaure-Mallet 2014). Unexpectedly, the immense number of bacteria with mutations in DNA repair genes has been detected inside biofilms, contributing in a hypermutator phenotype with a mutation rate up to 1000-fold (Jolivet-Gougeon and Bonnaure-Mallet 2014). This phenotype grants a critical advantage for strong mutator species relating to adhesion capability (Le Bars et al. 2012), growth in biofilms (Lujan et al. 2011) and persistence (Mena et al. 2007). (5) Bacteria within biofilms produce a prophylactic shield against phagocytes through QS-regulated synthesis of virulence factors such as enzymes and cellular lysins (Paraje 2011). In addition, QS influence the biofilm development and regulate the tolerance of biofilms to antibiotic action (Bjarnsholt et al. 2005).

The extrinsic or induced resistance factors are induced transcriptionally in biofilm-growing bacteria against antibiotic treatment. The highest occurrence of mutation has been observed in sessile bacteria compared to free-floating planktonic bacteria residing in biofilm through horizontal gene transmission (Paraje 2011). Studying horizontal gene transfer in natural environments contributes emergence of multidrug-resistant bacteria and genetic diversity of microbial communities (Martínez 2009, 2012). Bacteria can accumulate high levels of enzymes in response to antibiotics. For example, *Pseudomonas aeruginosa* biofilm secrete high amount of beta-lactamase regulated by beta-lactamase gene (*ampC*) by expression of the green fluorescent protein (GFP) after exposure to high dose of ceftazidime (Bagge et al. 2004; Jolivet-Gougeon and Bonnaure-Mallet 2014). Therefore, the bacterial cells within biofilms might concurrently produce antibiotic degrading enzymes that affect the affinity of antibiotic target and over express efflux pumps that have a broad range of substrates (Paraje 2011; Jolivet-Gougeon and Bonnaure-Mallet 2014). The multidrug efflux pumps in biofilm-growing bacteria importantly contribute to biofilm formation, and this mechanism is arsenal in defeating various classes of antibiotics.

3.8 Conclusion

The bacterial physiological functions such as motility, development of antibiotic resistance and virulence factors and biofilm formation are regulated by quorum sensing mechanism. It is undeniable that increased antibiotic resistance in biofilm forming bacteria and further comprehensive studies of molecular mechanisms are needed to understand the regulatory system of QS.

References

Anderson GG, O'Toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. In: Romeo T (ed) Bacterial biofilms, Current topics in microbiology and immunology, vol 322. Springer-Verlag, Berlin/Heidelberg, pp 85–105

Arce Miranda JE, Sotomayor CE, Albesa I, Paraje MG (2011) Oxidative and nitrosative stress in *Staphylococcus aureus* biofilm. FEMS Microbiol Lett 315:23–29

Bagge N, Hentzer M, Andersen JB, Ciofu O, Givskov M, Hoiby N (2004) Dynamics and spatial distribution of beta-lactamase expression in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 48:1168–1174

Baronetti JL, Angel Villegas N, Paraje MG, Albesa I (2011) Nitric oxide-mediated apoptosis in rat macrophages subjected to Shiga toxin 2 from *Escherichia coli*. Microbiol Immunol 55:231–238

Bassler BL (1999) How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 2:582–587

Bassler BL (2002) Small talk: cell-to-cell communication in bacteria. Cell 109:421–424

Becerra MC, Paez PL, Larovere LE, Albesa I (2006) Lipids and DNA oxidation in *Staphylococcus aureus* as a con sequence of oxidative stress generated by ciprofloxacin. Mol Cell Biochem 285:29–34

Bjarnsholt T, Jensen PØ, Burmølle M, Hentzer M, Haagensen JAJ, Hougen HP, Calum H, Madsen KG, Moser C, Molin S, Høiby N, Givskov M (2005) *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. Microbiology 151:373–383

Boisset S, Geissmann T, Huntzinger E, Fechter P, Bendridi N, Possedko M, Chevalier C, Helfer AC, Benito Y, Jacquier A, Gaspin C, Vandenesch F, Romby P (2007) *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator rot by an antisense mechanism. Genes Dev 21:1353–1366

Chadha T (2014) Bacterial biofilms: survival mechanisms and antibiotic resistance. J Bacteriol Parasitol 5:190

Characklis WG, Marshal KC (1990) Biofilms. Wiley, New York

Chen X, Schauder S, Potier N, Van Dorssealaer A, Pelczer I, Bassler BL, Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415:545–549

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322

Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G (2003) The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 112:1466–1477

Darch SE, West SA, Winzer K, Diggle SP (2012) Density-dependent fitness benefits in quorum sensing bacterial populations. Proc Natl Acad Sci U S A 109:8259–8263

Deziel E, Lepine F, Milot S, He J, Mindrinos MN, Tompkins RG, Rahme LG (2004) Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. Proc Natl Acad Sci U S A 101:1339–1144

Deziel E, Gopalan S, Tampakaki AP, Lépine F, Padfield KE, Saucier M, Xiao G, Rahme LG (2005) The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting lasRI, rhlRI or the production of N-acyl-L-homoserine lactones. Mol Microbiol 55:998–1014

Dunne WM (2002) Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 15:155–166

Engebrecht J, Silverman M (1984) Identification of genes and gene products necessary for bacterial bioluminescence. Proc Natl Acad Sci U S A 81:4154–4158

Erickson DL, Endersby R, Kirkham A, Stuber K, Vollman DD, Rabin HR, Mitchell I, Storey DG (2002) *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. Infect Immun 70:1783–1790

Federle MJ, Bassler BL (2003) Interspecies communication in bacteria. J Clin Invest 112:1291–1299

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468
Gallagher LA, McKnight SL, Kuznetsova MS, Pesci EC, Manoil C (2002) Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. J Bacteriol 184:6472–6480
Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18:1049–1056
Geisinger E, Adhikari RP, Jin R, Ross HF, Novick RP (2006) Inhibition of rot translation by RNAIII, a key feature of agr function. Mol Microbiol 61:1038–1048
Hall-Stoodley L, Stoodley P (2002) Developmental regulation of microbial biofilms. Curr Opin Biotechnol 13:228–233
Hancock REW, Siehnel R, Martin N (1990) Outer membrane proteins of *Pseudomonas*. Mol Microbiol 4:1069–1075
Hausner M, Wuertz S (1999) High rates of conjugation in bacterial biofilms as determined by quantitative *in situ* analysis. Appl Environ Microbiol 65:3710–3713
Havarstein LS, Coomaraswamy G, Morrison DA (1995) An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*. Proc Natl Acad Sci U S A 92:11140–11144
Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35:322–332
Irie Y, Parsek MR (2008) Bacterial biofilms. In: Romeo T (ed) Quorum sensing and microbial biofilms. Springer, Berlin/Heidelberg, pp 67–84
Jefferson KK (2004) What drives bacteria to produce a biofilm? FEMS Microbiol Lett 236:163–173
Ji G, Beavis RC, Novick RP (1995) Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci U S A 92:12055–12059
Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276:2027–2030
Jolivet-Gougeon A, Bonnaure-Mallet M (2014) Biofilms as a mechanism of bacterial resistance. Drug Discov Today Technol 11:49–56
Joo HS, Otto M (2012) Molecular basis of *in vivo* biofilm formation by bacterial pathogens. Chem Biol 19:1503–1513
Keren I, Kaldalu N, Spoering A, Wang YP, Lewis K (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230:13–18
Le Bars H, Le Gall-David S, Renoux VM, Bonnaure-Mallet M, Jolivet-Gougeon A, Bousarghin L (2012) Impact of a mutator phenotype on motility and cell adherence in *Salmonella* Heidelberg. Vet Microbiol 159:99–106
Lewis K (2005) Persister cells and the riddle of biofilm survival. Biochemistry 70:267–274
Li YH, Tian X (2012) Quorum sensing and bacterial social interactions in biofilms. Sensors 12:2519–2538
Liu Y, Yang SF, Li Y, Xu H, Qin L, Tay JH (2004) The influence of cell and substratum surface hydrophobicities on microbial attachment. J Biotechnol 110:251–256
Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. PNAS 104:11197–11202
Lujan AM, Macia MD, Yang L, Molin S, Oliver A, Smania AM (2011) Evolution and adaptation in *Pseudomonas aeruginosa* biofilms driven by mismatch repair system-deficient mutators. PLoS One 6:e27842
Lyon GJ, Muir TW (2003) Chemical signaling among bacteria and its inhibition. Chem Biol 10:1007–1021
Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci 276:2521–2530
Martínez JL (2012) Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. Front Microbiol 3:1
Mena A, Macia MD, Borrell N, Moya B, de Francisco T, Perez JL, Oliver A (2007) Inactivation of the mismatch repair system in Pseudomonas aeruginosa attenuates virulence but favors persistence of oropharyngeal colonization in cystic fibrosis mice. J Bacteriol 189:3665–3668

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199
Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM (2004) *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol Cell 15:677–687
Monnet V, Gardan R (2015) Quorum-sensing regulators in Gram-positive bacteria: 'cherchez le peptide'. Mol Microbiol 97:181–184
Musk DJ Jr, Hergenrother PJ (2006) Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets. Curr Med Chem 13:2163–2177
Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. Annu Rev Genet 43:197–222
Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449
Novick RP, Geisinger E (2008) Quorum sensing in staphylococci. Annu Rev Genet 42:541–564
O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79
Okada M, Sato I, Cho SJ, Iwata H, Nishio T, Dubnau D, Sakagami Y (2005) Structure of the Bacillus subtilis quorum-sensing peptide pheromone ComX. Nat Chem Biol 1:23–24
Palmer J, Flint S, Brooks J (2007) Bacterial cell attachment, the beginning of a biofilm. J Ind Microbiol Biotechnol 34:577–588
Paraje M (2011) Antimicrobial resistance in biofilms. In: Méndez-Vilas A (ed) Science against microbial pathogens: communicating current research and technological advances. Formatex Research Center, Badajoz, pp 736–744
Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol 13:27–33
Parsek MR, Singh PK (2003) Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol 57:677–701
Patel R (2005) Biofilms and antimicrobial resistance. Clin Orthop Relat Res 437:41–47
Penesyan A, Gillings M, Paulsen IT (2015) Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules 20:5286–5298
Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 96:11229–11234
Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E, Sintim HO (2015) Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem 7:493–512
Rasmussen TB, Givskov M (2006) Quorum-sensing inhibitors as anti-pathogenic drugs. Int J Med Microbiol 296:149–161
Renner LD, Weibel DB (2011) Physicochemical regulation of biofilm formation. MRS Bull 36:347–355
Ruby EG (1996) Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri-Euprymna scolopes* light organ symbiosis. Annu Rev Microbiol 50:591–624
Sardesai VM (1995) Role of antioxidants in health maintenance. Nutr Clin Pract 10:19–25
Sauer FG, Remaut H, Hultgren SJ, Waksman G (2004) Fiber assembly by the chaperone-usher pathway. Biochim Biophys Acta 1694:259–267
Schaefer AL, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP (1996) Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. Proc Natl Acad Sci U S A 93:9505–9509
Schauder S, Bassler BL (2001) The languages of bacteria. Genes Dev 15:1468–1480
Sekhar S, Kumar R, Chakraborti A (2009) Role of biofilm formation in the persistent colonization of *Haemophilus influenzae* in children from northern India. J Med Microbiol 58:1428–1432
Sifri CD (2008) Quorum sensing: bacteria talk sense. Healthcare Epidemiol 47:1070–1076
Simon MI, Crane BR, Crane A (2007) Two-component signaling systems. Academic Press, San Diego

Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 407:762–764

Solomon JM, Lazazzera BA, Grossman AD (1996) Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. Genes Dev 10:2014–2024

Sordi LD, Muhlschlegal FA (2009) Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communication network regulating microbial coexistence and virulence. FEMS Yeast Res 9:990–999

Staley C, Dunny GM, Sadowsky MJ (2014) Environmental and animal associated enterococci. Adv Appl Microbiol 87:147–186

Stevens AM, Dolan KM, Greenberg EP (1994) Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. Proc Natl Acad Sci U S A 91:12619–12623

Sturme MH, Kleerebezem M, Nakayama J, Akkermans AD, Vaugha EE, de Vos WM (2002) Cell to cell communication by autoinducing peptides in gram-positive bacteria. Antonie Van Leeuwenhoek 81:233–243

Sutherland IW (1999) Polysaccharases for microbial exopolysaccharides. Carbohydr Polym 38:319–328

Sutherland IW (2001a) The biofilm matrix – an immobilized but dynamic microbial environment. Trends Microbiol 9:222–227

Sutherland I (2001b) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9

Sutherland IW (2001c) Microbial polysaccharides from Gram-negative bacteria. Int Dairy J 11:663–674

Taga ME, Semmelhack JL, Bassler BL (2001) The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol Microbiol 42:777–793

van Bodman SB, Willey JM, Diggle SP (2008) Cell-cell communication in bacteria: united we stand. J Bacteriol 190:4377–4391

Van Delden C, Iglewski BH (1998) Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg Infect Dis 4:551–560

Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR (2005) Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. Nat Rev Microbiol 3:383–396

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Watnick PI, Kolter R (1999) Steps in the development of a *Vibrio cholerae* El Tor biofilm. Mol Microbiol 34:586–595

West SA, Winzer K, Gardner A, Diggle SP (2012) Quorum sensing and the confusion about diffusion. Trends Microbiol 20:586–594

Williams P, Winzer K, Chan WC, Camara M (2007) Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond Ser B Biol Sci 362:1119–1134

Modulation of Bacterial Quorum Sensing by Eukaryotes

4

Sathish Rajamani, Lukuo Lee, Emma Smith, Max Majireck, and Rajinikanth Mohan

Abstract

Bacterial quorum sensing (QS) mediated by small chemical signaling molecules is an important phenomenon that helps bacterial populations to synchronize and regulate their key cellular functions. Important to this behavior is employment of often unique signaling molecules that are recognized and bound by dedicated cognate receptors that result in modulation of gene expression. Well-documented examples are those of gram-negative bacterial *N*-acyl homoserine lactone (AHL) QS signaling pathways. The ability to disrupt or alter bacterial communication using signal analogues (agonist and antagonist) and other signal disruption mechanisms offers selective advantage to competitors and coinhabitants that share the environment with the bacterium. Such mechanisms are often found pervasive in both bacterial genre and higher eukaryotic hosts. Examples include employment of small molecular signals that target QS signaling and/or enzymes that inactivate QS signals. This report aims to highlight well-documented key mechanisms adapted by eukaryotes such as algae, fungi, plants, and animals both to sense bacterial QS signals and to employ bacterial QS modulators to regulate interkingdom interactions such as symbiosis, altered biofilm production, pathogenesis, etc. This knowledge could pave ways for developing nature-inspired non-antibiotic drugs, antifouling agents, and other bacterial fitness disruptor

S. Rajamani (✉)
Division of Molecular Translational Sciences, US Army Medical Research Institute of Infectious Diseases, General Dynamics Information Technology, Fort Detrick, MD, USA
e-mail: sathish.rajamani.ctr@mail.mil

L. Lee · E. Smith · R. Mohan (✉)
Department of Biology, Colgate University, Hamilton, NY, USA
e-mail: veerabramha@gmail.com

M. Majireck
Department of Chemistry, Hamilton College, Clinton, NY, USA

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_4

drugs that potentially bypasses bacterial drug resistance mechanisms, providing longer-term solutions for bacterial disease control.

Keywords
Quorum sensing (QS) · QS signaling pathways · Modulation · Gene expression · Eukaryotes

4.1 Introduction

The capacity to distinguish between colonizable commensal or beneficial bacteria and pathogens is a challenge inherent to all eukaryotic organisms. The ability to recruit useful bacteria and conversely to deter harmful pathogens offers critical advantages to the eukaryotic host. Among bacteria, interbacterial communications are largely accomplished with a language of chemicals through small molecule production, exchange, and detection. Found ubiquitous among prokaryotes are bacterial quorum sensing (QS) signaling mechanisms that are highly evolved and well adapted for intracellular and intercellular communications (Waters and Bassler 2005). Gram-positive bacteria primarily utilize peptide derivatives for QS behavior, while well-studied examples of gram-negative signaling molecules are *N*-acyl homoserine lactone (AHL) and autoinducer-2 class signaling molecules (Waters and Bassler 2005). Bacterial sensing of their self-produced QS molecules to determine their cell densities offer them an opportunity to draw consensus on their population number and coordinate their gene expression.

Alternatively, interkingdom sensing of bacterial QS signals by higher eukaryotes such as plants and animals can aid in the establishment of a census of its bacterial neighbors. This process could help inform eukaryotes to make informed decisions on altering its physiological behavior for successful adaptation while coexisting in their shared habitat. Alternatively, QS molecules could serve the dual role of bacterial communication molecules and act as chemical agents that alter eukaryotic fitness behavior as noted in fungi and mammals to 3-oxo-C_{12}-HSL produced by *Pseudomonas aeruginosa*. As detailed in this chapter, documented examples establish that higher eukaryotes have also developed sophisticated mechanisms to sense and respond to bacterial small molecules. As detailed further, this accentuates the belief that interkingdom signal transactions between bacteria and eukaryotes are broadly attributed to achieve (i) favorable interactions between the two (e.g., symbiosis) and (ii) alter bacterial communications via employing mimics or signal abrogation mechanisms to curtail harmful bacterial interactions with the host. Our primary objective of this discussion is to highlight well-documented mechanisms adapted by eukaryotes, mainly plants, algae, and mammals, to sense and respond to bacterial signal sensing and to underscore strategies administered by eukaryotes to manipulate bacterial AHL signaling.

4.2 Sensing of Bacterial AHL Signals by Eukaryotes

Plants host a great diversity of bacteria in mutualistic and pathogenic interactions, many of them being gram-negative species utilizing AHL QS system(s). Using a proteomics-based approach, plant legume *Medicago truncatula* was shown to sense the presence of nanomolar to micromolar concentrations of 3-oxo-C_{12}-HSL and 3-oxo-$C_{16:1}$-HSL signals and respond by altering their global gene expression (Mathesius et al. 2003). Using reporter gene fusions, tissue-specific activation of target genes in response to AHL was further demonstrated. Similarly, proteomic analysis with *Arabidopsis thaliana* seedlings demonstrated that the plant can alter its proteome in response to 3-oxo-C_8-HSL sensing (Miao et al. 2012). AHL sensing by plants can prime them for enhanced resistance to pathogens. In investigating the protective role of AHL-producing bacteria against the fungal pathogen *Alternaria alternata*, it was demonstrated that AHL-deficient bacterial mutants triggered less resistance to tomato plants against the fungal pathogen (Schuhegger et al. 2006).

Subsequently, AHL was shown to increase the production of salicylic acid, salicylic acid biosynthetic pathways, and ethylene-dependent defense genes, signal molecules, and signaling pathways which impact plant defense (Schuhegger et al. 2006). Other notable studies showed that overall plant responses to AHL signal could be varied depending on the chain length and modification of acyl chain (Bauer and Mathesius 2004; Miao et al. 2012; Schenk et al. 2012; Schuhegger et al. 2006; von Rad et al. 2008). AHL sensing in *Arabidopsis* induced growth promotion in the form of root elongation and changes in root development (Hartmann et al. 2014; Ortiz-Castro et al. 2008; von Rad et al. 2008). In an effort to understand the mechanism of AHL sensing in *Arabidopsis*, it was shown that a simple heterotrimeric Gα subunit GPA1 and G-protein G-protein-coupled receptors (GPCRs) such as GCR1, Cand2, and Cand7 are involved in short-chain 3-oxo-C6HSL and 3-oxo-C8HSL sensing and root elongation (Jin et al. 2012; Liu et al. 2012). On the other hand, AHL-stimulated growth increase was not observed in barley and yam (Gotz-Rosch et al. 2015), indicating varied plant responses to AHLs.

Like plants, animals can also sense bacterial signaling molecules and the host responses could be varied to AHL sensing. For instance, opportunistic pathogen *P. aeruginosa* QS signal 3-oxo-C_{12}-HSL effect on mammalian cells has been extensively studied and found to have both anti-inflammatory and proinflammatory properties and proapoptotic functions (Cooley et al. 2008; Hooi et al. 2004; Kravchenko et al. 2008; Rumbaugh and Kaufmann 2012; Tateda et al. 2001). Using engineered mammalian cells that sensed AHLs (Shiner et al. 2004) and utilizing radiolabeled 3-oxo-C_{12}-HSL (Ritchie et al. 2007), it was shown that the AHL molecule can enter the eukaryotic cell and alter cellular response, underscoring the possible existence of an AHL-specific intracellular target. Similarly, transcriptional profiling using microarray analysis of human lung epithelial cells exposed to 3-oxo-C_{12}-HSL revealed that xenobiotic sensing and drug transport transcripts increased upon AHL exposure. This study also showed that the AHL accumulated rapidly in A549 cell lines and that inhibition of ABC transporter ABCA1 resulted in decreased expulsion

of 3-oxo-C_{12}-HSL, suggesting a mechanism employed by eukaryotic cells to remove these signals from cells (Bryan et al. 2010).

In a landmark finding, it was shown that 3-oxo-C_{12}-HSL can affect innate immune responses in cell lines and in vivo. More specifically, the AHL altered NF-κB-mediated immune function, evidently facilitating *P. aeruginosa* infection progression via suppression of innate immunity (Kravchenko et al. 2008). Alternatively, mice exposed to short- and long-chain AHLs increased the survivability of mice to *Aeromonas hydrophila* infection challenge, perhaps by priming the host response to defend against the pathogen (Khajanchi et al. 2011). Significantly, AHL pretreatment enhanced bacterial clearance from infected animals possibly by phagocytosis of bacteria by activated innate immune cells. Further, there are also a number of other documented examples of gram-positive and gram-negative non-AHL QS signal sensing by mammalian cells, which is beyond the scope of this discussion (Rumbaugh and Kaufmann 2012). Collectively these observations demonstrate that plants and animals can sense and respond to bacterial QS signals. In some cases, AHLs can target the cellular metabolism and immune system; in others, cells can sense AHLs and activate cellular defenses and prime immune response.

4.3 Plants Secrete Compounds That Alter Bacterial QS

Secretion of compounds to alter bacterial QS is a widely emerging theme in the prokaryotic and eukaryotic world (Rasmussen et al. 2005; Saurav et al. 2017). Plants comprise a large untapped source of antimicrobial compounds, with only a small portion of the known species studied (Savoia 2012). Additionally, most studies previously conducted on the antimicrobial modes of plants have only focused on the ability of the plant to inhibit growth or kill bacteria (Adonizio et al. 2006). However, since the discovery of the QS disrupting halogenated furanones from the marine red algae *Delisea pulchra*, there has been an emergence of studies investigating the anti-QS properties of plants (Manefield et al. 2002; Venturi and Fuqua 2013). Pivotal work in the field of anti-QS agents produced by plants was reported by Dr. Bauer's laboratory (Teplitski et al. 2000). Interestingly, in addition to production and secretion of anti-QS activity, the exudate from pea (*Pisum sativum*) plants was found to contain a number of high-performance liquid chromatography (HPLC) separable QS-active (QS stimulatory) as well as QS inhibitory compounds that altered bacterial QS reporter responses (Teplitski et al. 2000).

Rightfully designated as "QS mimics" for their QS modulatory (agonistic and antagonistic) activities, the presence of such compounds was also found in other plants, including crown vetch, *Medicago truncatula*, rice, soybean, and tomato. It was also shown that *Medicago truncatula* QS mimic profiles changed substantially with the age of the plant seedling (Gao et al. 2003), indicating a possible role for these molecules in aiding the plant in choosing its bacterial partners. Importantly, in nearly two decades since the discovery of these activities, the chemical identities of these compounds remain undetermined. Using *Medicago truncatula* as a model, it was also shown that plants can sense externally administered bacterial AHLs and

can alter the secretion profile of their AHL mimics (Bauer and Mathesius 2004; Mathesius et al. 2003). Rice plant extracts containing QS mimics, like that of AHLs, were demonstrated to be AHL lactonase (AiiA) enzyme-sensitive group (Degrassi et al. 2007), offering some clues to its shared chemical feature to AHLs. More recently, a plant-derived compound rosmarinic acid, predicted to bind to QS regulators, was shown to act as QS agonist for RhlRI QS system in *P. aeruginosa* (Corral-Lugo et al. 2016). Similarly, flavonoids produced by plants were shown to inhibit *P. aeruginosa* LasRI QS system and inhibit its QS and QS-regulated biofilm production (Paczkowski et al. 2017). More recently, plant phenolics such as carvacrol and eugenol were shown to target ExpRI QS system of plant pathogen *Pectobacterium* sp., resulting in QS inhibition (Joshi et al. 2016). Significantly, structural analysis of rosmarinic acid, flavonoids, and plant phenolics carvacrol and eugenol indicates no characteristic functional group that can be hydrolyzed by AiiA enzyme and possibly represents a chemically different class of plant-derived QS mimic(s). Combining this knowledge, it is reasonable to infer that plants secrete diverse QS mimics which aid with their phyllosphere and rhizosphere bacterial interactions.

Medicinal plants in particular, due to their long history of usage in traditional practices for purposes such as infection control, serve as a promising area in the search of QS interfering compounds (Koh and Tham 2011; Savoia 2012). Studies have investigated the effects of medicinal plant extracts on QS activity by screening them on the bacterial reporter strains *Chromobacterium violaceum* and *P. aeruginosa* (Adonizio et al. 2006; Al-Hussaini and Mahasneh 2009; Khan et al. 2009; Koh and Tham 2011; Krishnan et al. 2012; Tolmacheva et al. 2014; Yeo and Tham 2012). *C. violaceum* produces the purple pigment violacein through QS, where violacein is produced after threshold levels of AHL are reached (Adonizio et al. 2006). Inhibition or disruption of *C. violaceum*'s violacein production by medicinal plant extracts would indicate QS disruption through interruption of AHL signaling (Adonizio et al. 2006). *P. aeruginosa* demonstrate QS-dependent swarming, and thus inhibition of swarming by medicinal plant extracts would also indicate QS inhibition (Koh and Tham 2011). Table 4.1 summarizes notable medicinal plant extracts with QS inhibitory activity through violacein or swarming inhibition. Though the structural information on these activities are limited or unknown, these results demonstrate the potential of medicinal plants as sources of QS inhibitory compounds.

4.4 Algae Secrete Compounds That Alter Bacterial QS

Marine macroalga *D. pulchra* endemic to the Australian seacoast was found to have limited surface bacterial colonization. Subsequently, it was established that they produced and stored a wide range of halogenated furanones in their secretory glands that aided in preventing bacterial colonization (de Nys et al. 1993). Importantly, halogenated furanones that have structural resemblance to bacterial QS AHL signals were determined to possess AHL antagonistic properties. This was established using bacterial bioassays that were used to monitor AHL-mediated QS functions such as swarming and bioluminescence production (Givskov et al. 1996). Furanone's

Table 4.1 Medicinal plants with QS inhibitory activity

Medicinal plant species and common name	Medicinal use	Violacein inhibition	Swarming inhibition	Reference
Astragalus membranaceus (milkvetch)	Treatment of cold, arthritis, loss of appetite, weakness, night sweats, numbness of muscles, boils, diarrhea, asthma, nervousness	+	–	Yeo and Tham (2012)
Lilium brownii (lily bulb)	As sedative and tonic for treating cough, lung disorders, urinary disorders, deafness, earache, nervousness, carminative	+	+	Yeo and Tham (2012)
Panax notoginseng (ginseng)	Treatment of dizziness, vertigo, nosebleed, injuries, fractures, heart problems; to stop bleeding, for reducing swelling and pain	+	+	Koh and Tham (2011), Yeo and Tham (2012)
Conocarpus erectus (buttonwood)	Febrifuge, respiratory ailments, venereal disease	+	Unknown	Adonizio et al. (2006)
Hemidesmus indicus (anantamul)	Febrifuge, venereal disease	+	+	Zahin et al. (2010)
Laurus nobilis (bay laurel)	Carminative, digestive problems, cold, bronchitis	+	Unknown	Al-Hussaini and Mahasneh (2009)
Syzygium aromaticum (spice clove)	Treatment against oral bacteria, asthma, allergic disorder	+	Unknown	Khan et al. (2009), Krishnan et al. (2012)
Arctostaphylos uva-ursi (bearberry)	Antimicrobial and a mild diuretic used for urinary tract complaints that includes cystitis and urolithiasis	+	Unknown	Tolmacheva et al. (2014)

+: significant inhibition, −: insignificant inhibition, Unknown: inhibition untested

inhibitory function was shown to be partly relieved with increasing AHL concentrations in these assays, indicative of competition for AHL receptor binding site (Manefield et al. 1999). Furthermore, in an in vivo ligand-binding assay employing labeled AHL and halogenated furanones showed that furanones displaced bound AHLs from the LuxR protein. In validating the direct interaction between halogenated furanone and LuxR AHL receptor protein, it was determined that the furanones inhibit AHL-mediated gene expression via accelerated degradation of the AHL receptor (Manefield et al. 2002) and the site for furanone-AHL receptor interaction was also determined (Koch et al. 2005). Since its discovery, halogenated furanones both natural and synthetic derivatives of these molecules have been

shown to alter QS-regulated properties, including virulence, antibiotic production, biofilm formation, etc. (Hentzer et al. 2002; Martinelli et al. 2004). These results underscored the natural competitive evolutionary mechanisms employed by eukaryotes to inhibit AHL signaling via competing for a common binding site in LuxR and/or its homologues.

To understand whether unicellular green algae found in soil and water bodies have evolved mechanisms to regulate bacteria QS, algae-bacterial reporter overlay studies and solvent extraction of spent cell-free media coupled to HPLC fractionation and bacterial QS reporter studies were undertaken. Bacterial reporter overlays on *Chlamydomonas* and *Chlorella* sp. indicated that these algae produced both inhibitory and stimulatory QS mimic activities. Like plants (Teplitski et al. 2000), HPLC fractionation of spent algal media had an array of chromatographically separable fractions tested against bacterial reporters expressing *Burkholderia cepacia* CepR and *P. aeruginosa* LasR AHL receptors that modulate QS-regulated promoter gene fusions (Fig. 4.1) (Teplitski et al. 2004). As shown in Fig. 4.1, these QS stimulatory activities were secreted and accumulate in the growth media over time. Interestingly, the production of these mimics were growth medium dependent, wherein the secretion of these mimics were negligible when the algae were grown in carbon-rich acetate (TAP or tris-acetate-phosphate) medium than when cultured in minimal (HS or high salt) medium (Fig. 4.1). Proteomic analysis with bacterium *Sinorhizobium meliloti* and partially purified LasR algae QS mimic indicated that the mimic affected the accumulation of 16 out of 25 proteins that were regulated by the bacterial AHL molecule (Teplitski et al. 2004). Treatment of CepR

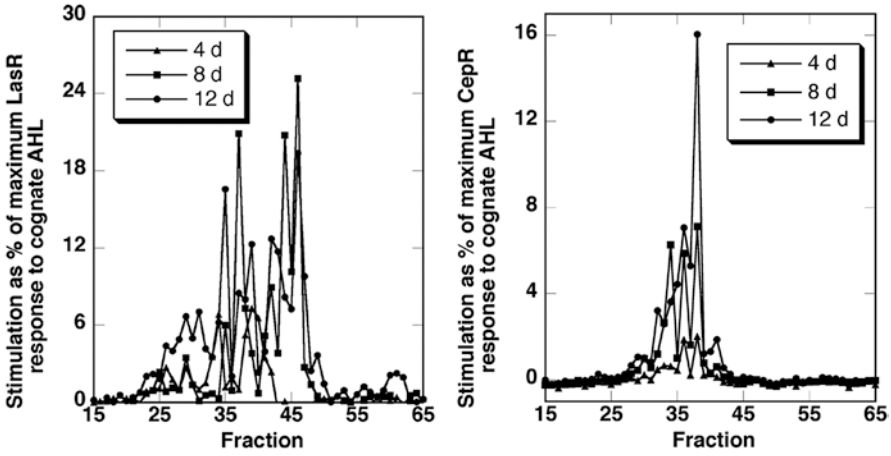


Fig. 4.1 *Chlamydomonas reinhardtii* secretes compounds that are mimics of bacterial AHL signaling. Samples of HPLC fractions of *C. reinhardtii* culture filtrates from 4, 8, and 12 d were bioassayed with the *E. coli* LasR AHL reporter (left panel) and *Pseudomonas putida* CepR (right panel). Responses are shown as percentage of the maximum response recorded with the reporter to saturating levels of the cognate AHL (3-oxo-C_{12}-HSL for LasR and C_8-HSL for CepR). (Adapted with permission from Teplitski et al. (2004). Copyright 2004 American Society of Plant Biologists)

receptor-active AHL mimic HPLC fractions with AHL lactonase (AiiA) inactivated most of these mimic activities, demonstrating the potential existence of conserved homoserine lactone ring (Rajamani et al. 2011). Transgenic algae expressing AiiA with substantially reduced accumulation of AHL mimics showed limited ability to prevent biofilm formation by competing bacteria found in pond water. These observations validate that AHL mimics play a critical role in modulating bacterial QS response behavior in nature. In pursuit of identifying the chemical identity of a QS mimic from algae, a LasR-active QS mimic that accumulated in TAP media culture of *Chlamydomonas reinhardtii* was purified and determined to be a vitamin riboflavin derivative compound lumichrome (Rajamani et al. 2008). Lumichrome and riboflavin, despite being structurally unrelated to AHL molecules and insensitive to AiiA, docked at a similar location as 3-oxo-C_{12}-HSL in LasR receptor binding site. Mutational studies with LasR receptor coupled with reporter studies confirmed that the binding locations were indeed common for AHL and lumichrome/riboflavin binding (Rajamani et al. 2008).

4.5 Mechanisms Employed by Eukaryotes for QS Disruption

Apart from the production of signaling molecules to disrupt QS, eukaryotes also employ other mechanisms for bacterial QS alteration. One of the key attributes of eukaryotic protective mechanisms against pathogenic bacteria includes inactivation of QS signals. Though there are limited studies to the fate of AHLs in plants, it was demonstrated that plants can employ enzymatic mechanisms to degrade AHLs (Palmer et al. 2014). Using in vitro assays and *A. thaliana* wild-type and mutant plants, the fatty acid amide hydrolase (FAAH) was shown to play a role in degradation of AHL by AHL amidolysis. Further, the product of this enzymatic activity L-homoserine is proposed to play a key role in plant physiology (Palmer et al. 2014). While plants do not have specialized immune cells, lower animals rely on the innate immune system, whereas higher animals have sophisticated acquired immunity and cell types that navigate bacterial encounters. In animals, the ability to negotiate various bacterial species and selectively permit or ward off certain bacteria rests upon the immune system. The simple freshwater metazoan, *Hydra*, in a recent study, has been show to produce oxidoreductase enzymes that convert the inhibitory 3-oxo-modifed AHL into a 3-hydroxy substituted AHL that signals a phenotype change in the colonizing *Curvibacter*, favoring host colonization (Pietschke et al. 2017). In essence, the host modulates bacterial gene expression by modifying a QS signal to alter bacterial behavior and promote interaction and colonization.

Cells in higher animals have been shown to exhibit the ability to neutralize pathogenic bacterial AHL signals and this ability appears to vary between cell types. AHL lactonases are frequently used to degrade bacterial AHL signals to dampen QS and associated processes. The best-known lactonases produced by animal hosts belong to the paraoxonase (PON) family, including PON1–3, which degrade the 3-oxo-substituted AHLs (Chun et al. 2004; Draganov et al. 2005). Cells such as mammalian airway epithelia display the ability to specifically degrade some, but not

other, AHLs. Sera of mammals contains activity reminiscent of PONs and is conserved between mammalian species (Yang et al. 2005) and could be related to bacterial AHL lactonases (Bar-Rogovsky et al. 2013). Heterologous expression of human PON1 in *Drosophila* suppressed *P. aeruginosa* infection, in a PON1 activity-dependent manner, illustrating the importance of PON enzymes in degrading AHL signals and containing pathogen infections (Stoltz et al. 2008). Suppression of QS processes by animal hosts can potentially suppress virulence-associated processes including biofilm formation. Animal immune cells also exhibit other mechanisms to suppress biofilm formation. For instance, lactoferrins produced by neutrophils sequester iron, depriving colonizing bacteria of this nutrient, which is essential for many microbial processes including formation of the biofilm structure (Ammons and Copie 2013). An immunomodulatory role has also been attributed to AHL signals, with host perceiving AHLs as a cue for defense. In response to AHL signals in *P. aeruginosa*, elevated local defense through phagocytosis has been reported, although other reports suggest that AHLs may assist in evasion of host defense (reviewed in (Hansch 2012)).

4.6 Nature-Inspired Synthetic QS Analogues

Since the discovery of naturally occurring small-molecule autoinducer signals of QS (Adonizio et al. 2006; Bassler and Losick 2006; Camilli and Bassler 2006; Ng and Bassler 2009a, b), researchers have developed a preponderance of synthetic analogues based on their natural molecular structures. This section highlights some representative examples of synthetic analogues targeting AHL-based QS. Several excellent reviews on the discovery and use of small-molecule modulators of QS have been published (Delago et al. 2016; Galloway et al. 2011; Kalia 2013; Mattmann and Blackwell 2010; Pan and Ren 2009), and the reader is directed to these manuscripts for additional examples and analyses.

As noted in a comprehensive 2011 review describing small-molecule perturbation of QS in gram-negative bacteria (Galloway et al. 2011), difficulties arise when attempting to classify active compounds based on either their structure or activity. For example, lack of standardization between the various studies in terms of measuring potency prevents one from making absolute comparisons between IC_{50} values. Another complication arises from the fact that some small-molecule modulators of QS can switch from agonist to antagonist, depending on concentration and even the target QS pathway under investigation (e.g., a specific target QS receptor small-molecule interaction and downstream reporter activation). Furthermore, differences in bacterial strain, media, assays, and impurities provide further variables that can affect the ultimate measurement of biological activity. Nevertheless, comparison between different studies provide researchers valuable qualitative information that may be used to study relative activity, or extract basic trends in the structure-activity relationships (SAR) that guide medicinal chemists to develop analogues with improved and/or new properties.

Synthetic small molecules designed to modulate QS in bacteria generally target one of the three components within the overall QS biochemical pathway: the gene(s) responsible for production of autoinducers, the receptor(s), or the autoinducer itself. Although simplified, these broad categories fit well with the general targets for which most synthetic and natural autoinducers interact. For example, in the gram-negative bacteria *V. fischeri*, LuxI-type synthases utilize *S*-adenosyl methionine (SAM) and an acyl carrier protein (ACP) to manufacture the AHL product via an enzyme-mediated acylation-lactonization cascade (Watson et al. 2002). Accordingly, many synthetic small-molecule modulators of QS are structural mimics of AHL, SAM, and corresponding cofactors/biosynthetic intermediates designed to disrupt this key biochemical pathway (Alfaro et al. 2004; Musk and Hergenrother 2006; Parsek et al. 1999; Shen et al. 2006; Zhao et al. 2003).

The majority of synthetic analogues derived from natural QS modulators are based on AHLs. A typical, naturally occurring AHL may be represented by C4-HSL or BHL **1** and 3-oxoC_{12}-HSL or OdDHL **2**, both found in *P. aeruginosa*, in which the amino group on the L-homoserine lactone "head" is decorated by an acyl group "tail" (Fig. 4.2).

For the most part, bacteria generate AHL autoinducers that have a high specificity for modulation of their specific QS system (Pomianek and Semmelhack 2007). Remarkably, however, most of the currently known natural AHLs maintain the unsubstituted L-homoserine lactone head group. Consequently, the length and placement of functional groups on the acyl tail vary greatly between species, since this molecular feature strongly influences selective binding to their corresponding AHL receptors (e.g., LuxR) and modification of synthetic AHL analogues at the acyl tail have been explored in great detail. For example, in the earliest study of AHL analogues, Eberhard et al. (1986) investigated the effect of varying the chain length of the acyl group and/or C3-oxo substituent on the agonistic/antagonistic activity in *V. fischeri* B-61. This strain is particularly reliant on exogenous *N*-(3-oxo-C_6)-HSL (OHHL) for bioluminescence, and so production of light serves as a useful measurement for inhibitory versus activating phenotypes.

Eberhard's seminal study, and subsequently many others, has demonstrated that AHL receptors can accommodate a variety of chain lengths, degrees of steric bulk, and an array of functional groups within natural and synthetic AHL analogues, which may demonstrate either antagonistic or agonistic behavior. Representative examples of synthetic AHL analogues that exploit this structural flexibility include AHL **3**, a QS inducer bearing a C4 cycloalkyl group, and AHL **4**, a QS inhibitor bearing C4 phenyl group (Fig. 4.3) (Reverchon et al. 2002). Moreover, many synthetic analogues designed using the principles of bioisosterism have yielded a range of synthetic AHLs with enhanced properties, such as hydrolytically stable

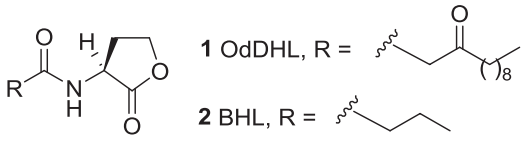


Fig. 4.2 Representative naturally occurring AHLs

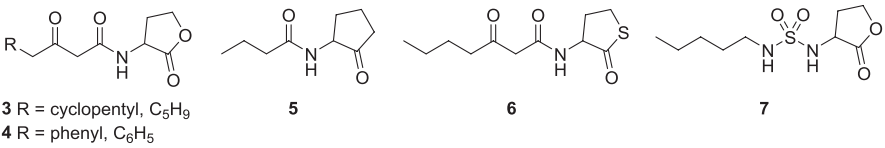


Fig. 4.3 Representative synthetic analogues of AHLs

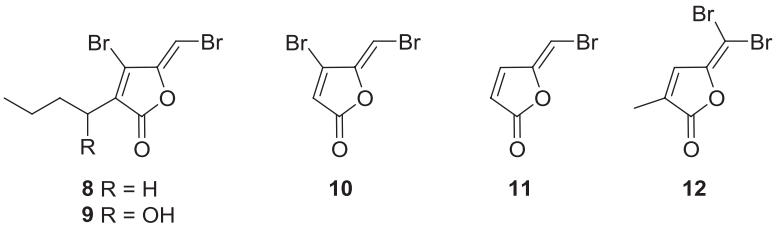


Fig. 4.4 Representative natural and synthetic bromofuranones

cyclopentanone **5** (which circumvents the ready hydrolysis of AHL lactones in vivo), super-agonist thiolactam **6** (J. C. A. Janssens et al. 2007), and sulfonyl urea antagonist **7** (Frezza et al. 2008). Thus, there are myriad possibilities for development of novel small-molecule modulators of QS by modification of the acyl tail and lactone head of native AHLs.

While AHL-based synthetic analogues predominate, there are a variety of other synthetic QS modulators based on non-AHL molecular scaffolds. Numerous halogenated furanones inspired by those naturally occurring in *D. pulchra* (Denys et al. 1993) among other algae have provided researchers with a broad array of antagonists toward QS-regulated behaviors in bacteria, including virulence expression, antibiotic production, transport function, motility, biofilm formation, and siderophore synthesis. For example, bromofuranones such as **8** and **9** have been found to inhibit AHL-mediated gene expression in a number of bacterial species (Fig. 4.4). Accordingly, numerous synthetic analogues have been designed, such as simplified QS antagonists **10** and **11** (Hentzer et al. 2003), which lack a bromo- and/or butyl side chain in comparison to natural product **8** and bromofuranone **12**, a potent inhibitor of biofilm formation in *E. coli*.

From these notable examples, it is clear that both the continued isolation of QS-mediating small molecules from natural sources and design of new synthetic analogues with novel properties are imperative for advancing our fundamental understanding of QS mechanisms and applying these insights toward new therapeutic strategies for infectious disease.

4.7 Significance and Conclusions

The ability to modulate bacterial QS is witnessed in wide-ranging eukaryotes from lower plantlike marine algae like *Delisea* to higher plants like *Medicago* and lower metazoans like *Hydra* to humans. Employment of chemical signals that alter bacterial communication and functions are potentially beneficial to the employing host. Importantly these signal molecules are not toxic or lethal to bacterial cells, but certainly alter key bacterial functions that provide fitness in their environment (e.g., biofilm production, siderophore production, virulence, etc.). Production of a diverse array of QS mimics as observed in chromatographically separable fractions from plant and algae exudate (Gao et al. 2003; Teplitski et al. 2004; Teplitski et al. 2000) indicates clearly the demonstrable investment made by eukaryotes to alter QS in bacterial counterparts associated in their niche. With the increasing occurrence of drug-resistant bacterial infections and the limited success in finding newer antibiotics (Center for Disease Control (CDC) – Antibiotic Resistance Threats in the United States 2013; World Health Organization (WHO) Publishes List of Bacteria for which New Antibiotics are Urgently Needed 2017), it is imperative to continue the study of QS interference mechanisms to develop novel therapeutics that alter bacterial fitness. Further this need is exacerbated in biothreat pathogens such as *Burkholderia pseudomallei* that are inherently resistant to number of antibiotics (Schweizer 2012). It is well documented that bacteria encapsulated in biofilms are notoriously difficult to treat with antibiotics (Stewart 2002) and might lead to antimicrobial resistance (AMR) due to delivery of sublethal doses of antibiotic to the target pathogens. Identification of QS analogues that limits the bacterial biofilm phenotype and render them susceptible to antibiotics will be most beneficial for combination therapies. Alternatively, QS analogues that limit virulence and provide extended therapeutic window for infection treatment would also be an important addition to limiting disease progression. Since furanones can, surprisingly, also inhibit enteric pathogens such as *Salmonella* and *E. coli*, which utilize a different mechanism of QS signaling other than AHL signals, they have a broad spectrum of applications, including to target pathogenic biofilm formation in therapeutic settings (Janssens et al. 2008). QS inhibitory furanones from *Delisea* and similar agents have great potential for application in the control of biofouling, primary caused by buildup of marine bacterial biofilm and followed by algae and other organisms on marine vessels and other surfaces (Dworjanyn et al. 2006).

Understanding modulation of bacterial QS by plants should have palpable implications toward agricultural applications. Bacteria possess enzymes such as AHL lactonases, acylases, and oxidoreductases that can modify or degrade their own AHL signals or those of competing bacteria, thus limiting their QS, growth, and virulence processes (Grandclement et al. 2016). Expression of such a lactonase in plants degraded the AHL signals of the soft rot pathogen *Erwinia carotovora* in rice and leads to a reduction of disease (Zhang et al. 2007). Such a capability could be transferred to plants by expressing lactonases that interfere with pathogen QS and virulence. Indeed, potato plants expressing such an enzyme exhibited enhanced

resistance to soft rot disease. However, such a targeting needs to be specific to minimize the effects on the general homeostasis of the plant microbiome.

In nature, while the AHL mimics produced by *Delisea* inhibits bacterial QS, plant AHL mimics such as rosmarinic acid and lumichrome from microalgae unexpectedly appear to stimulate bacterial QS signaling (Corral-Lugo et al. 2016). Pathogenic bacteria tend to delay virulence processes by rendering them QS dependent, such that virulence is launched only when a sufficient threshold of pathogen is reached (Bassler 1999). Plant AHL mimics are believed to defeat this strategy by stimulating premature activation of QS signaling that could alert plant defenses (Corral-Lugo et al. 2016). Alternatively, stimulation of QS may be beneficial in promoting plant interaction with bacterial symbionts. Importantly, further work needs to be undertaken to understand the nature and role of these QS signal mimics. With the existing bacterial reporters, novel protein-based biosensors, and advanced analytical techniques with improved sensitivities (Lepine et al. 2018; Rajamani et al. 2007, 2008; Rajamani and Sayre 2018; Rothballer et al. 2018; Teplitski et al. 2004; Teplitski et al. 2000), determining their structure-activity relationship should be possible. Structural identification of these natural products might find applications for treating antibiotic-resistant pathogens by altering bacterial fitness (e.g., limited virulence production, poor biofilm growth, etc.) to aid in the clearance by host immune system or as combination with other drugs that eliminate the pathogen. As these QS modulators do not have deleterious effects on bacterial growth and survival (not bactericidal or bacteriostatic), the probability that the bacteria will develop resistance against these chemical agents is highly unlikely.

Acknowledgments Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

References

Adonizio AL, Downum K, Bennett BC, Mathee K (2006) Anti-quorum sensing activity of medicinal plants in southern Florida. J Ethnopharmacol 105(3):427–435. https://doi.org/10.1016/j.jep.2005.11.025

Alfaro JF, Zhang T, Wynn DP, Karschner EL, Zhou ZS (2004) Synthesis of LuxS inhibitors targeting bacterial cell-cell communication. Org Lett 6(18):3043–3046. https://doi.org/10.1021/ol049182i

Al-Hussaini R, Mahasneh AM (2009) Microbial growth and quorum sensing antagonist activities of herbal plants extracts. Molecules 14(9):3425–3435. https://doi.org/10.3390/molecules14093425

Ammons MC, Copie V (2013) Mini-review: Lactoferrin: a bioinspired, anti-biofilm therapeutic. Biofouling 29(4):443–455. https://doi.org/10.1080/08927014.2013.773317

Bar-Rogovsky H, Hugenmatter A, Tawfik DS (2013) The evolutionary origins of detoxifying enzymes: the mammalian serum paraoxonases (PONs) relate to bacterial homoserine lactonases. J Biol Chem 288(33):23914–23927. https://doi.org/10.1074/jbc.M112.427922

Bassler BL (1999) How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 2(6):582–587

Bassler BL, Losick R (2006) Bacterially speaking. Cell 125(2):237–246. https://doi.org/10.1016/j.cell.2006.04.001

Bauer WD, Mathesius U (2004) Plant responses to bacterial quorum sensing signals. Curr Opin Plant Biol 7(4):429–433. https://doi.org/10.1016/j.pbi.2004.05.008

Bryan A, Watters C, Koenig L, Youn E, Olmos A, Li G et al (2010) Human transcriptome analysis reveals a potential role for active transport in the metabolism of *Pseudomonas aeruginosa* autoinducers. Microbes Infect 12(12–13):1042–1050. https://doi.org/10.1016/j.micinf.2010.07.006

Camilli A, Bassler BL (2006) Bacterial small-molecule signaling pathways. Science 311(5764):1113–1116. https://doi.org/10.1126/science.1121357

Center for Disease Control (CDC) (2013) Antibiotic resistance threats in the United States. http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf

Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP (2004) Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. Proc Natl Acad Sci U S A 101(10):3587–3590. https://doi.org/10.1073/pnas.0308750101

Cooley M, Chhabra SR, Williams P (2008) N-Acylhomoserine lactone-mediated quorum sensing: a twist in the tail and a blow for host immunity. Chem Biol 15(11):1141–1147. https://doi.org/10.1016/j.chembiol.2008.10.010

Corral-Lugo A, Daddaoua A, Ortega A, Espinosa-Urgel M, Krell T (2016) Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. Sci Signal 9(409):ra1. https://doi.org/10.1126/scisignal.aaa8271

de Nys R, Wright AD, König GM, Sticher O (1993) New halogenated furanones from the marine alga *Delisea pulchra* (cf. fimbriata). Tetrahedron 49(48):11213–11220. https://doi.org/10.1016/S0040-4020(01)81808-1

Degrassi G, Devescovi G, Solis R, Steindler L, Venturi V (2007) *Oryza sativa* rice plants contain molecules that activate different quorum-sensing N-acyl homoserine lactone biosensors and are sensitive to the specific AiiA lactonase. FEMS Microbiol Lett 269(2):213–220. https://doi.org/10.1111/j.1574-6968.2006.00624.x

Delago A, Mandabi A, Meijler MM (2016) Natural quorum sensing inhibitors – small molecules, big messages. Israel J Chem 56(5):310–320. https://doi.org/10.1002/ijch.201500052

Denys R, Wright AD, Konig GM, Sticher O (1993) New halogenated furanones from the marine alga *Delisea pulchra* (cf fimbrata). Tetrahedron 49(48):11213–11220

Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN (2005) Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res 46(6):1239–1247. https://doi.org/10.1194/jlr.M400511-JLR200

Dworjanyn SA, Nys R d, Steinberg PD (2006) Chemically mediated antifouling in the red alga *Delisea pulchra*. Mar Ecol Prog Ser 318:153–163

Eberhard A, Widrig CA, McBath P, Schineller JB (1986) Analogs of the autoinducer of bioluminescence in *Vibrio fischeri*. Arch Microbiol 146(1):35–40. https://doi.org/10.1007/bf00690155

Frezza M, Soulere L, Reverchon S, Guiliani N, Jerez C, Queneau Y, Doutheau A (2008) Synthetic homoserine lactone-derived sulfonylureas as inhibitors of *Vibrio fischeri* quorum sensing regulator. Bioorg Med Chem 16(7):3550–3556. https://doi.org/10.1016/j.bmc.2008.02.023

Galloway, W. R., Hodgkinson, J. T., Bowden, S. D., Welch, M., & Spring, D. R. (2011). Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. Chem Rev, 111(1), 28–67. doi:https://doi.org/10.1021/cr100109t

Gao M, Teplitski M, Robinson JB, Bauer WD (2003) Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Mol Plant-Microbe Interact 16(9):827–834. https://doi.org/10.1094/MPMI.2003.16.9.827

Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, Eberl L et al (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178(22):6618–6622

Gotz-Rosch C, Sieper T, Fekete A, Schmitt-Kopplin P, Hartmann A, Schroder P (2015) Influence of bacterial N-acyl-homoserine lactones on growth parameters, pigments, antioxidative capacities and the xenobiotic phase II detoxification enzymes in barley and yam bean. Front Plant Sci 6:205. https://doi.org/10.3389/fpls.2015.00205

Grandclement C, Tannieres M, Morera S, Dessaux Y, Faure D (2016) Quorum quenching: role in nature and applied developments. FEMS Microbiol Rev 40(1):86–116. https://doi.org/10.1093/femsre/fuv038

Hansch GM (2012) Host defence against bacterial biofilms: "Mission Impossible"? ISRN Immunol 2012:17. https://doi.org/10.5402/2012/853123

Hartmann A, Rothballer M, Hense BA, Schroder P (2014) Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. Front Plant Sci 5:131. https://doi.org/10.3389/fpls.2014.00131

Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR et al (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology 148(Pt 1):87–102. https://doi.org/10.1099/00221287-148-1-87

Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N et al (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22(15):3803–3815. https://doi.org/10.1093/emboj/cdg366

Hooi DSW, Bycroft BW, Chhabra SR, Williams P, Pritchard DI (2004) Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules. Infect Immun 72(11):6463–6470. https://doi.org/10.1128/iai.72.11.6463-6470.2004

Janssens JCA, Metzger K, Daniels R, Ptacek D, Verhoeven T, Habel LW et al (2007) Synthesis of *N*-acyl homoserine lactone analogues reveals strong activators of SdiA, the *Salmonella enterica* serovar Typhimurium LuxR homologue. Appl Environ Microbiol 73(2):535–544. https://doi.org/10.1128/aem.01451-06

Janssens JC, Steenackers H, Robijns S, Gellens E, Levin J, Zhao H et al (2008) Brominated furanones inhibit biofilm formation by *Salmonella enterica* serovar Typhimurium. Appl Environ Microbiol 74(21):6639–6648. https://doi.org/10.1128/AEM.01262-08

Jin G, Liu F, Ma H, Hao S, Zhao Q, Bian Z et al (2012) Two G-protein-coupled-receptor candidates, Cand2 and Cand7, are involved in Arabidopsis root growth mediated by the bacterial quorum-sensing signals N-acyl-homoserine lactones. Biochem Biophys Res Commun 417(3):991–995. https://doi.org/10.1016/j.bbrc.2011.12.066

Joshi JR, Khazanov N, Senderowitz H, Burdman S, Lipsky A, Yedidia I (2016) Plant phenolic volatiles inhibit quorum sensing in pectobacteria and reduce their virulence by potential binding to ExpI and ExpR proteins. Sci Rep 6:38126. https://doi.org/10.1038/srep38126

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31(2):224–245. https://doi.org/10.1016/j.biotechadv.2012.10.004

Khajanchi BK, Kirtley ML, Brackman SM, Chopra AK (2011) Immunomodulatory and protective roles of quorum-sensing signaling molecules N-acyl Homoserine lactones during infection of mice with Aeromonas hydrophila. Infect Immun 79(7):2646–2657. https://doi.org/10.1128/iai.00096-11

Khan MS, Zahin M, Hasan S, Husain FM, Ahmad I (2009) Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol 49(3):354–360. https://doi.org/10.1111/j.1472-765X.2009.02666.x

Koch B, Liljefors T, Persson T, Nielsen J, Kjelleberg S, Givskov M (2005) The LuxR receptor: the sites of interaction with quorum-sensing signals and inhibitors. Microbiology 151(Pt 11):3589–3602. https://doi.org/10.1099/mic.0.27954-0

Koh KH, Tham FY (2011) Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. J Microbiol Immunol Infect 44(2):144–148. https://doi.org/10.1016/j.jmii.2009.10.001

Kravchenko VV, Kaufmann GF, Mathison JC, Scott DA, Katz AZ, Grauer DC et al (2008) Modulation of gene expression via disruption of NF-kappaB signaling by a bacterial small molecule. Science 321(5886):259–263. https://doi.org/10.1126/science.1156499

Krishnan T, Yin WF, Chan KG (2012) Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* PAO1 by ayurveda spice clove (*Syzygium aromaticum*) bud extract. Sensors (Basel) 12(4):4016–4030. https://doi.org/10.3390/s120404016

Lepine F, Milot S, Groleau MC, Deziel E (2018) Liquid chromatography/mass spectrometry (LC/MS) for the detection and quantification of N-acyl-L-Homoserine lactones (AHLs) and 4-Hydroxy-2-Alkylquinolines (HAQs). Methods Mol Biol 1673:49–59. https://doi.org/10.1007/978-1-4939-7309-5_4

Liu F, Bian Z, Jia Z, Zhao Q, Song S (2012) The GCR1 and GPA1 participate in promotion of Arabidopsis primary root elongation induced by N-acyl-homoserine lactones, the bacterial quorum-sensing signals. Mol Plant-Microbe Interact 25(5):677–683. https://doi.org/10.1094/MPMI-10-11-0274

Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg P, Kjelleberg S (1999) Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology 145(Pt 2):283–291. https://doi.org/10.1099/13500872-145-2-283

Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, Kjelleberg S, Givskov M (2002) Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology 148(Pt 4):1119–1127. https://doi.org/10.1099/00221287-148-4-1119

Martinelli D, Grossmann G, Sequin U, Brandl H, Bachofen R (2004) Effects of natural and chemically synthesized furanones on quorum sensing in Chromobacterium violaceum. BMC Microbiol 4:25. https://doi.org/10.1186/1471-2180-4-25

Mathesius U, Mulders S, Gao M, Teplitski M, Caetano-Anolles G, Rolfe BG, Bauer WD (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. Proc Natl Acad Sci U S A 100(3):1444–1449. https://doi.org/10.1073/pnas.262672599

Mattmann ME, Blackwell HE (2010) Small molecules that modulate quorum sensing and control virulence in *Pseudomonas aeruginosa*. J Org Chem 75(20):6737–6746. https://doi.org/10.1021/jo101237e

Miao C, Liu F, Zhao Q, Jia Z, Song S (2012) A proteomic analysis of *Arabidopsis thaliana* seedling responses to 3-oxo-octanoyl-homoserine lactone, a bacterial quorum-sensing signal. Biochem Biophys Res Commun 427(2):293–298. https://doi.org/10.1016/j.bbrc.2012.09.044

Musk DJ Jr, Hergenrother PJ (2006) Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets. Curr Med Chem 13(18):2163–2177

Ng W-L, Bassler BL (2009a) Bacterial quorum-sensing network architectures. Annu Rev Genet 43(1):197–222. https://doi.org/10.1146/annurev-genet-102108-134304

Ng WL, Bassler BL (2009b) Bacterial quorum-sensing network architectures. Annu Rev Genet 43:197–222. https://doi.org/10.1146/annurev-genet-102108-134304

Ortiz-Castro R, Martinez-Trujillo M, Lopez-Bucio J (2008) N-acyl-L-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ 31(10):1497–1509. https://doi.org/10.1111/j.1365-3040.2008.01863.x

Paczkowski JE, Mukherjee S, McCready AR, Cong JP, Aquino CJ, Kim H et al (2017) Flavonoids suppress *Pseudomonas aeruginosa* virulence through allosteric inhibition of quorum-sensing receptors. J Biol Chem 292(10):4064–4076. https://doi.org/10.1074/jbc.M116.770552

Palmer AG, Senechal AC, Mukherjee A, Ané J-M, Blackwell HE (2014) Plant responses to bacterial N-acyl l-homoserine lactones are dependent on enzymatic degradation to l-homoserine. ACS Chem Biol 9(8):1834–1845. https://doi.org/10.1021/cb500191a

Pan J, Ren D (2009) Quorum sensing inhibitors: a patent overview. Expert Opin Ther Pat 19(11):1581–1601. https://doi.org/10.1517/13543770903222293

Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP (1999) Acyl homoserine-lactone quorum-sensing signal generation. Proc Natl Acad Sci U S A 96(8):4360–4365

Pietschke C, Treitz C, Foret S, Schultze A, Kunzel S, Tholey A et al (2017) Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts. Proc Natl Acad Sci U S A 114(40):E8488–e8497. https://doi.org/10.1073/pnas.1706879114

Pomianek ME, Semmelhack MF (2007) Making Bacteria behave: new agonists and antagonists of quorum sensing. ACS Chem Biol 2(5):293–295. https://doi.org/10.1021/cb700098c

Rajamani S, Sayre R (2018) Biosensors for the detection and quantification of AI-2 class quorum-sensing compounds. Methods Mol Biol 1673:73–88. https://doi.org/10.1007/978-1-4939-7309-5_6

Rajamani S, Zhu J, Pei D, Sayre R (2007) A LuxP-FRET-based reporter for the detection and quantification of AI-2 bacterial quorum-sensing signal compounds. Biochemistry 46(13):3990–3997. https://doi.org/10.1021/bi602479e

Rajamani S, Bauer WD, Robinson JB, Farrow JM 3rd, Pesci EC, Teplitski M et al (2008) The vitamin riboflavin and its derivative lumichrome activate the LasR bacterial quorum-sensing receptor. Mol Plant-Microbe Interact 21(9):1184–1192. https://doi.org/10.1094/MPMI-21-9-1184

Rajamani S, Teplitski M, Kumar A, Krediet CJ, Sayre RT, Bauer WD (2011) N-acyl homoserine lactone lactonase, AiiA, inactivation of quorum-sensing agonists produced by *Chlamydomonas reinhardtii* (chlorophyta) and characterization of AiiA transgenic algae(1). J Phycol 47(5):1219–1227. https://doi.org/10.1111/j.1529-8817.2011.01049.x

Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO et al (2005) Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. Microbiology 151(Pt 5):1325–1340. https://doi.org/10.1099/mic.0.27715-0

Reverchon S, Chantegrel B, Deshayes C, Doutheau A, Cotte-Pattat N (2002) New synthetic analogues of N-acyl homoserine lactones as agonists or antagonists of transcriptional regulators involved in bacterial quorum sensing. Bioorg Med Chem Lett 12(8):1153–1157

Ritchie AJ, Whittall C, Lazenby JJ, Chhabra SR, Pritchard DI, Cooley MA (2007) The immunomodulatory *Pseudomonas aeruginosa* signalling molecule N-(3-oxododecanoyl)-l-homoserine lactone enters mammalian cells in an unregulated fashion. Immunol Cell Biol 85(8):596–602. https://doi.org/10.1038/sj.icb.7100090

Rothballer M, Uhl J, Kunze J, Schmitt-Kopplin P, Hartmann A (2018) Detection of the bacterial quorum-sensing signaling molecules N-acyl-homoserine lactones (HSL) and N-acyl-homoserine (HS) with an enzyme-linked immunosorbent assay (ELISA) and via ultrahigh-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS). Methods Mol Biol 1673:61–72. https://doi.org/10.1007/978-1-4939-7309-5_5

Rumbaugh KP, Kaufmann GF (2012) Exploitation of host signaling pathways by microbial quorum sensing signals. Curr Opin Microbiol 15(2):162–168. https://doi.org/10.1016/j.mib.2011.12.003

Saurav K, Costantino V, Venturi V, Steindler L (2017) Quorum sensing inhibitors from the sea discovered using bacterial N-acyl-homoserine lactone-based biosensors. Mar Drugs 15(3). https://doi.org/10.3390/md15030053

Savoia D (2012) Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol 7(8):979–990. https://doi.org/10.2217/fmb.12.68

Schenk ST, Stein E, Kogel KH, Schikora A (2012) Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal Behav 7(2):178–181. https://doi.org/10.4161/psb.18789

Schuhegger R, Ihring A, Gantner S, Bahnweg G, Knappe C, Vogg G et al (2006) Induction of systemic resistance in tomato by N-acyl-L-homoserine lactone-producing rhizosphere bacteria. Plant Cell Environ 29(5):909–918

Schweizer HP (2012) Mechanisms of antibiotic resistance in Burkholderia pseudomallei: implications for treatment of melioidosis. Future Microbiol 7(12):1389–1399. https://doi.org/10.2217/fmb.12.116

Shen G, Rajan R, Zhu J, Bell CE, Pei D (2006) Design and synthesis of substrate and intermediate analogue inhibitors of S-ribosylhomocysteinase. J Med Chem 49(10):3003–3011. https://doi.org/10.1021/jm060047g

Shiner EK, Reddy S, Timmons C, Li G, Williams SC, Rumbaugh KP (2004) Construction of a bacterial autoinducer detection system in mammalian cells. Biol Proced Online 6:268–276. https://doi.org/10.1251/bpo98

Stewart PS (2002) Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol 292(2):107–113. https://doi.org/10.1078/1438-4221-00196

Stoltz DA, Ozer EA, Taft PJ, Barry M, Liu L, Kiss PJ et al (2008) Drosophila are protected from *Pseudomonas aeruginosa* lethality by transgenic expression of paraoxonase-1. J Clin Invest 118(9):3123–3131. https://doi.org/10.1172/jci35147

Tateda K, Moore TA, Newstead MW, Tsai WC, Zeng X, Deng JC et al (2001) Chemokine-dependent neutrophil recruitment in a murine model of legionella pneumonia: potential role of neutrophils as immunoregulatory cells. Infect Immun 69(4):2017–2024. https://doi.org/10.1128/iai.69.4.2017-2024.2001

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant-Microbe Interact 13(6):637–648. https://doi.org/10.1094/MPMI.2000.13.6.637

Teplitski M, Chen H, Rajamani S, Gao M, Merighi M, Sayre RT et al (2004) *Chlamydomonas reinhardtii* secretes compounds that mimic bacterial signals and interfere with quorum sensing regulation in bacteria. Plant Physiol 134(1):137–146. https://doi.org/10.1104/pp.103.029918

Tolmacheva AA, Rogozhin EA, Deryabin DG (2014) Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. Acta Pharma 64(2):173–186. https://doi.org/10.2478/acph-2014-0019

Venturi V, Fuqua C (2013) Chemical signaling between plants and plant-pathogenic Bacteria. Annu Rev Phytopathol 51(1):17–37. https://doi.org/10.1146/annurev-phyto-082712-102239

von Rad U, Klein I, Dobrev PI, Kottova J, Zazimalova E, Fekete A et al (2008) Response of *Arabidopsis thaliana* to N-hexanoyl-DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229(1):73–85. https://doi.org/10.1007/s00425-008-0811-4

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346. https://doi.org/10.1146/annurev.cellbio.21.012704.131001

Watson WT, Minogue TD, Val DL, von Bodman SB, Churchill ME (2002) Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. Mol Cell 9(3):685–694

World Health Organization (WHO) (2017) Publishes list of bacteria for which new antibiotics are urgently needed. WHO, Geneva

Yang F, Wang LH, Wang J, Dong YH, Hu JY, Zhang LH (2005) Quorum quenching enzyme activity is widely conserved in the sera of mammalian species. FEBS Lett 579(17):3713–3717. https://doi.org/10.1016/j.febslet.2005.05.060

Yeo SSM, Tham FY (2012) Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia. Malays J Microbiol 8(1):11–20

Zhang L, Ruan L, Hu C, Wu H, Chen S, Yu Z, Sun M (2007) Fusion of the genes for AHL-lactonase and S-layer protein in *Bacillus thuringiensis* increases its ability to inhibit soft rot caused by Erwinia carotovora. Appl Microbiol Biotechnol 74(3):667–675. https://doi.org/10.1007/s00253-006-0696-8

Zhao G, Wan W, Mansouri S, Alfaro JF, Bassler BL, Cornell KA, Zhou ZS (2003) Chemical synthesis of S-ribosyl-L-homocysteine and activity assay as a LuxS substrate. Bioorg Med Chem Lett 13(22):3897–3900. https://doi.org/10.1016/j.bmcl.2003.09.015

5 Cell to Communication Between Mammalian Host and Microbial Quorum Sensing Orchestrates the Complex Relationships

Prudhvi Lal Bhukya, Ganugula Mohana Sheela, A. M. V. N. Prathyusha, and Pallaval Veera Bramhachari

Abstract

Bacteria have to adapt to dynamic environmental condition in order to survive. Quorum sensing is a communication between bacterial cells for modification in behavior by sensing change in cell density. This communication includes the production and release of signaling molecules termed as autoinducer. This is to check the cell number and to modify gene expression accordingly to perform certain cooperative actions. Quorum-sensing phenomenon is executed by both gram-positive and gram-negative bacteria, but it differs in their mechanism. It is not restricted to bacteria only. As prokaryotes and eukaryotes coexist for their effective survival, there is a crosstalk between bacterial signaling molecule and host-derived hormones. The current chapter highlights inter-kingdom relationship between bacteria and host, especially focus is on mammals and how they crosstalk with each other. It emphasizes on how autoinducers produced by bacteria affect mammalian host cell behavior and its signaling mechanism.

Keywords

Bacterial gene expression · Mammalian gene expression · Autoinducers

Prudhvi Lal Bhukya, Ganugula Mohana Sheela, A. M. V. N. Prathyusha and Pallaval Veera Bramhachari contributed equally with all other contributors.

P. L. Bhukya (✉)
Hepatitis Division, National Institute of Virology, Pune, India

Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
e-mail: bhukyaprudvi@gmail.com

G. M. Sheela · A. M. V. N. Prathyusha · P. V. Bramhachari
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
e-mail: veerabramha@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_5

5.1 Introduction

Quorum sensing is an intracellular communication between bacterial species in order to survive and modulate host immune response. This phenomenon works through small and diffusible signaling molecule termed as autoinducers (Waters and Bassler 2005). Gram-positive and gram-negative bacteria differ in the quorum-sensing mechanism (Papenfort and Bassler 2016). The most common signal molecules used by gram-negative bacteria are N-Acyl homoserine lactone (AHL) synthesized by LuxI homologue proteins (Papenfort and Bassler 2016). Gram-positive bacteria use peptides for quorum sensing. These peptides cannot freely diffuse across cell membrane as AHL. It requires transporter in order to cross cell membrane, generally ATP-binding cassette (ABC) transporter (Federle and Bassler 2003).

Recent evidence shows, however, that quorum-sensing signaling is not restricted to bacterial cell-to-cell communication but also allows communication between microorganisms and their hosts. The mammalian immune system has an excellent defense mechanism in identifying and destroying evaded pathogens; conversely, at times, the pathogen evades these mechanisms and establishes disease in its host. Bacteria encounter scores of different types of cells within the host. This may result from bacteria moving into new tissue sites, or the nature of the environment could change eventually as the host inflammatory response alters the composition of cells at interface of infection. This interplay has the potential to trigger gene expression patterns in the bacterium specific to the composition of attacking cells (Davis and Isberg 2018). Bacteria communicate with one another through hormone-like signals to alter their gene expression through QS (Fuqua et al. 1996). Furthermore, these signals also modify mammalian cell-signal transduction (Telford et al. 1998), and host hormones can cross signal to regulate bacterial gene expression (Sperandio et al. 2003) in an inter-kingdom signaling.

This signaling is not confined to bacteria but also affects host in which bacteria are residing; in turn, host can also modify quorum sensing executed by bacteria (Hughes and Sperandio 2008). Research over decades has exemplified that *P. aeruginosa* QS signals, through different mechanisms, interfered with RNA and DNA processes, protein synthesis, homeostasis, calcium signaling, mitochondrial, and cytoskeletal dynamics in host cells. However, *P. aeruginosa* communication via AHLs can disturb few important cellular processes in the host, viz. cell morphology, proliferation, differentiation, and apoptosis, and thereby many biological activities and functions (Turkina and Vikström 2019). In this chapter, specifically we shed a light on: (1) How bacterial QS phenomenon affects host, taking example of gram-negative and gram-positive bacteria? (2) How host affects/interferes with bacterial QS mechanism? (3) What are the inhibitors known/designed so far to protect from pathogenic bacteria QS?

5.2 How Bacterial QS Phenomenon Affects Host?

5.2.1 How Gram-Positive Bacteria Quorum Sensing Affects Host?

Gram-positive bacteria use two-component signaling mechanism for quorum sensing. It uses bioactive peptides as autoinducers. These peptides need transporters in order to penetrate into cell membrane (Ji et al. 2016). When the accumulation of secreted peptides reaches threshold concentration, it interacts with sensor kinase at cell membrane and thereby initiates series of phosphorylation of regulatory proteins and serves as transcription factor with alterations in gene expression (Miller and Bassler 2001). *Bacillus* group including *B. thuringiensis, B. anthracis*, and *B. cereus* produces and secretes a variety of hemolysins, phospholipases, and toxins which cause acute diarrheal disease (Rutherford and Bassler 2012; Bottone 2010). As a notable example, *Bacillus subtilis* outlines a typical mechanism for inter-kingdom signaling between gram-negative bacteria and the host. Nonetheless, *B. subtilis* is a well-characterized gram-positive bacterium which is a part of avian, mammalian, and human enteric flora (Gonzalez et al. 2011). The QS pentapeptide competence and sporulation factor (CSF) of *B. subtilis* activates the key eukaryotic survival pathways such as p38 MAP kinase and AKT in intestinal epithelial cells (Fig. 5.1). It is noteworthy that CSF particularly prevents the oxidant-induced intestinal epithelial cell injury and loss of barrier function by induction of cytoprotective heat shock proteins (HSPs). Interestingly, this cytoprotective effect depends on its uptake by organic cation transporter-2 (OCTN2). In another example, *Staphylococcus aureus* is a part of human gut microflora. This bacterium can cause minor skin infections if any damage occurs at the epithelial barrier which may perhaps lead to pneumonia, bacteremia, and sepsis (Fujiya et al. 2007). This pathogenic microbe has

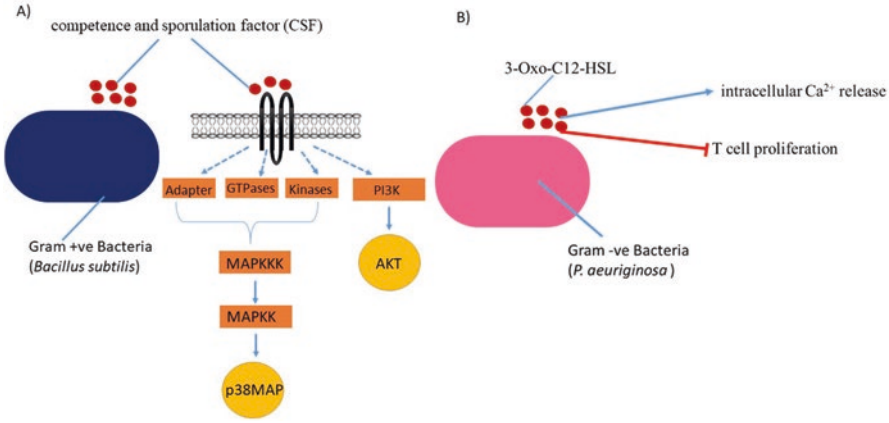


Fig. 5.1 Interaction of B.Subtilis with host cellular pathways

been a leading cause of hospital-related infections in the United States. Remarkably, the disease-causing ability of this bacterium solely depends on adhesion molecule expression, toxins, and compounds that affect basic functioning of the immune system (Rutherford and Bassler 2012).

5.2.2 How Gram-Negative Bacteria Quorum Sensing Influences the Host?

In gram-negative bacteria, signaling molecules include N-acyl-homoserine lactone (HSL) compounds, autoinducer-2 (AI-2) and its precursor 4,5-dihydroxy-2,3-pentanedione (DPD), diffusible signal factor (DSF), and 2-heptyl-3-hydroxy-4-quinolone (PQS), in addition to dialkylresorcinol (DAR) and a-hydroxyketone (AHK) molecules. While DAR, PQS, and HSL AIs directly bind cytosolic transcription factors, DSF, AI-2, and AHKs are detected through specific membrane-bound sensor kinases of two-component systems (TCS) (Papenfort and Bassler 2016) (4). *Pseudomonas aeruginosa* is a gram-negative bacterium, which causes cystic fibrosis and nosocomial infections in immunocompromised individuals Chastre and Fagon 2002; Subramanian et al. 2013). QS-regulated factors impact *P. aeruginosa* communities and pathogenicity by following modes: motility and adhesion, lipopolysaccharides, enzymes and exotoxins, protein secretion systems, iron uptake, biofilm formation. Remarkably, *P. aeruginosa* decoy molecules regulate an ensemble of host functions by different modes: inflammatory response, host transcription, mitochondrial physiology, pro-apoptosis, calcium signaling, directional cell migration, chemotaxis, single cell and multicellular migration (Turkina and Vikström Turkina and Vikström 2019). It is noteworthy that *P. aeruginosa* makes use of host during a weakened state for its own benefit. *P. aeruginosa* releases pyocyanin extracellularly which in turn induces neutrophil apoptosis and epithelial cell damage (Lau et al. 2004) and permits to subvert immune surveillance and gain placement in lungs. PQS also controls the expression of the lasB (elastase) gene (Pesci et al. 1999), and a synergistic effect is attained in existence of both PQS and C4-HSL (McKnight et al. 2000).

P. aeruginosa autoinducer molecule 3-oxo-C12-HSL inhibits T-cell proliferation and inhibits release of IL-2, while PQS (pseudomonas quinolone signal) inhibits cell proliferation without affecting IL-2 release in human monocytes. $3OC_{12}$-HSL triggers intracellular Ca^{2+} release, endoplasmic reticulum (ER) structure, and eukaryotic translation initiation factor, resulting in endoplasmic reticulum stress, thereby affecting host protein synthesis (Fig. 5.1). Both flagella and pili are potent virulence factors enabling to move and bind to host cells mucus layers, sense mechanical features of their environment, and facilitate early biofilm development at surface (Fig. 5.2) (Haiko and Westerlund-Wikström 2013). In another example, lipopolysaccharides (LPS) stimulated bone marrow dendritic cells, and the release of TNF-α is inhibited by PQS. PQS also inhibits dendritic cell-induced T-cell proliferation and innate immune response in mouse monocyte/macrophage cell line J774A.1 by NF-κβ-signaling pathway (Skindersoe et al. 2009). Flagellin and LPS

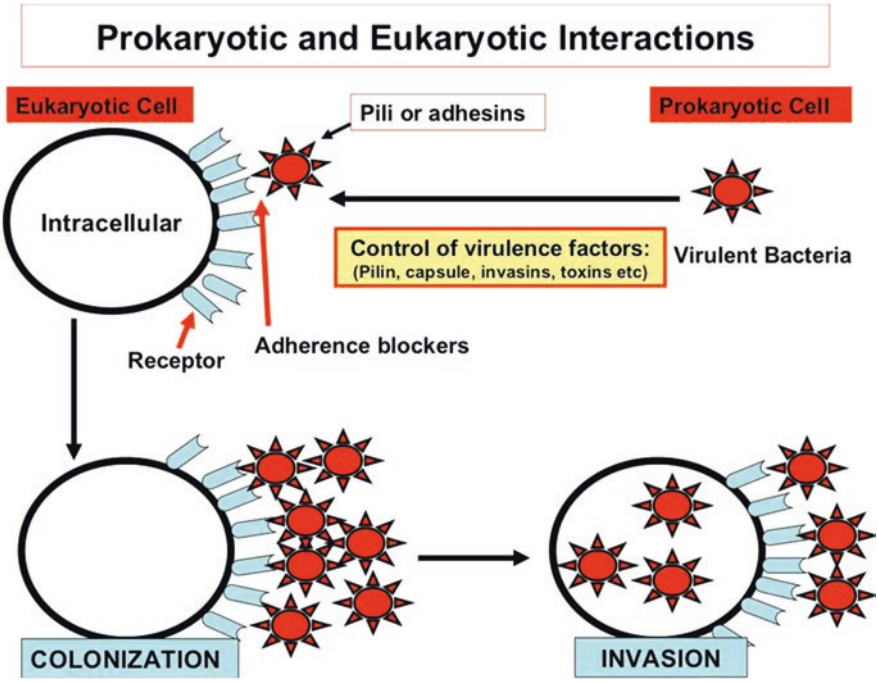


Fig. 5.2 Bacterial adhesins in host-microbe interactions

associate with host pattern recognition receptors, e.g., toll-like receptors (TLR5 and TLR4) expressed on different host cells, and thereby initiate an inflammatory response via NF-κB-signaling pathway (Gellatly and Hancock 2013). *V. cholera* produces and responds to two AI molecules: (S)-3-hyroxytridecan-4-one (CAI-1) and AI-2 (Sheela et al. 2018). These QS molecules produce enterotoxin that causes chronic watery diarrhea which can lead to dehydration and death if not treated appropriately (Fig. 5.2) (Rutherford and Bassler 2012). Yet, in another study conducted in *L. pneumophila*, *FlaA* gene represented a pathogen-associated molecular pattern (PAMP), produced by wild-type activates in mammalian host cell cytoplasm, viz., NAIP5/NLRC4 inflammasome and the associated caspase-1 and caspase-7. Inflammasome activation necessitates the bacterial Icm/Dot T4SS, further impairs LCV formation and supports the release of pro-inflammatory cytokines as well as pyroptosis (Schell et al. 2016).

5.3 Host Responses to Bacterial Quorum Sensing

It has long been known that there is symbiotic relationship between bacteria and mammalian host for maintaining homeostasis (Chow et al. 2010). Mammalian host and residential microflora prevent invasion, colonization, and survival of pathogenic microorganism by physical, chemical, and cell-mediated antimicrobial strategies

(Curtis and Sperandio 2011) (Fig. 5.2). Specifically talking about humans, about 500–1000 species of bacteria reside in gut. Residential microflora helps in digestion of food and nutrient absorption (Walsh et al. 2014). However, pathogens can affect host and residential bacteria quorum sensing for virulence (Hughes and Sperandio 2008). Mucosal surface which is constantly in contact with bacteria secretes antimicrobial peptides such as lysozyme and defensin to protect from bacterial infection by rapidly killing bacteria and prevents acute invasive infection (Diamond et al. 2009). In chronic infections, free-living bacteria form biofilms which are untreatable as it is resistant to antimicrobial peptides (Bjarnsholt 2013). *P. aeruginosa* which is highly pathogenic bacteria forms biofilm in airway and causes lung destruction which leads to death eventually (Gellatly and Hancock 2013). *Lactoferrin* can block biofilm formation of *P. aeruginosa*. It is a highly abundant and ubiquitous molecule present in human external secretion. This biofilm formation is required for long-duration invasion of colonization of surfaces (Fig. 5.2). In order to form biofilm, it requires higher amount of iron than needed for its growth. If an iron level drops or not sufficient to maintain biofilms, bacteria move to survival. Lactoferrin chelates iron, thereby, cause bacteria to wander across the surface instead of biofilm formation (Ammons and Copié 2013).

It is not only by secreting antimicrobial peptide/protein or antibiofilm molecule but also host can act on specific autoinducer secreted by bacteria. Human colon cell line CaCo-2 inactivates 3OC12-HSL when it is exposed to environmental pathogens (Ammons and Copié 2013). This effect was also seen with the human lung cell line A549. The aforementioned two human cell lines evidenced maximum amount of *3OC12-HSL* inactivation. The colon and lung cell lines are constantly exposed to bacteria. This phenomenon does not hold true with all the mammalian cell lines. Notably, 293 T, COS-7, and MDCK kidney cell lines from human, monkey, and canine showed very less or no inactivation of 3O2-HSL. There is an immense variation in the inactivation of QS-signaling molecule OC12-HSL between different mammalian cell lines. It will be very tempting to learn a) what signaling components does the cell line have that can inactivate bacterial QS? b) What are the lacking factors in other cell lines that are unable to inactive bacterial QS? However, the reports are very few showing the modulation of quorum sensing by host (Chun et al. 2004).

5.4 What Are the Inhibitors Known/Designed So Far to Protect from Pathogenic Bacteria QS?

Antibiotics are very well accepted and standardized in treating health ailments caused by bacteria. As bacteria are developing resistance to antibiotic, there is a need to develop other strategies for targeting pathogenic microorganisms. So, disease caused by antibiotic-resistant bacteria causes major deaths worldwide (Clatworthy et al. 2007, Ventola 2015). Pathogenic bacteria reside in host and synthesize different compound in order to colonize and damage the host, known as

virulence factors (Fig. 5.2) (Deep et al. 2011). Rather than aiming at bacterial eradication which is a general theme in the development of antibiotic for disease treatment, we can target this virulence factor synthesized by bacteria to prevent damage to host (Ellis and Kuehn 2010). As the virulence factor production is costly for bacteria at metabolic level, production of virulence factor expression goes through complex array regulatory network. One of the major regulatory network which controls virulence factor production is quorum sensing. It is not only the quorum sensing which regulates, but there are also other factors which regulate virulence factor production such as regulatory RNAs, secondary messengers, and alternative sigma factors (Peyraud et al. 2016).

Quorum sensing is an upcoming and intensively studied field, which is showing promiscuous alternative to antibiotic (Tay and Yew 2013). Quorum-sensing process can be disrupted by several mechanisms, such as downregulating activity of acyl-homoserine-lactone cognate receptor protein or AHL synthase, AHL degradation or designing of molecules which can mimic the analogous signaling, and enzymatic degradation of QS molecule. The proposed criteria for selection of proper QS inhibitors are as follows: (1) small molecule and efficiently reduce QS-related gene expression, (2) specificity to the given QS molecule without side effects, (3) longer than AHL, and (4) should not be antigenic. Decoy receptors and antibodies are known to inhibit QS signal. These are the novel approaches for anti-infective therapy (Tay and Yew 2013; Defoirdt et al. 2013). Ap4–24 H11 is a hapten, which inhibits the production of auto-inducing peptide (AIP)-4 by *S. aureus* (Park et al. 2007). There are array of QS inhibitors available such as plant based, marine system based, animal based, and fungal based which have been extensively studied (Galloway et al. 2012; Koh et al. 2013; Saurav et al. 2017). Targeting bacterial QS and designing inhibitors to disrupt quorum sensing are an expanding and evolving area to target pathogenic bacterial diseases.

5.5 Summary

QS, an very old signaling mechanism firstly used by unicellular organisms to synchronize the function and augment the health of cell populations limited in a niche, is also employed by cells of the immune systems in mammalian organisms, representing the immune system a robust and well-controlled apparatus that is able to fight intruders from both outside and inside cells. Bacteria QS system is a very complex intracellular communication devised for their effective survival. In case of pathogenic organisms, novel strategies are to be developed beyond inhibition of bacterial QS by educating and enhancing host defense against the invasion of pathogenic bacterium. This will be more helpful and reliable future strategy in disease treatment rather than developing drugs against bacteria and solving multidrug resistance. While we need to gain a better understanding of the complex relationship among the host and invading pathogens, rationally designed therapies are warranted that specifically target virulence traits incurred by pathogens.

References

Ammons MC, Copié V (2013) Lactoferrin: a bioinspired, anti-biofilm therapeutic. Biofouling 29:443–455

Bjarnsholt T (2013) The role of bacterial biofilms in chronic infections. APMIS Suppl 136:1–51

Bottone EJ (2010) Bacillus cereus, a volatile human pathogen. Clin Microbiol Rev 23:382–398

Chastre J, Fagon JY (2002) Ventilator-associated pneumonia. Am J Respir Crit Care Med 165:867–903

Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK (2010) Host–bacterial symbiosis in health and disease. Adv Immunol 107:243–274

Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP (2004) Inactivation of a Pseudomonas aeruginosa quorum-sensing signal by human airway epithelia. Proc Natl Acad Sci U S A 101:3587–3590

Clatworthy AE, Pierson E, Hung DT (2007) Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3:541–548

Curtis MM, Sperandio V (2011) A complex relationship: the interaction among symbiotic microbes, invading pathogens, and their mammalian host. Mucosal Immunol 4:133–138

Davis KM, Isberg RR (2018) One for all, but not all for one: social behavior during bacterial diseases. Trends Microbiol 27:64–74

Deep A, Chaudhary U, Gupta V (2011) Quorum sensing and bacterial pathogenicity: from molecules to disease. J Lab Phys 3:4–11

Defoirdt T, Brackman G, Coenye T (2013) Quorum sensing inhibitors: how strong is the evidence? Trends Microbiol 21:619–624

Diamond G, Beckloff N, Weinberg A, Kisich KO (2009) The roles of antimicrobial peptides in innate host defense. Curr Pharm Des 15:2377–2392

Ellis TN, Kuehn MJ (2010) Virulence and Immunomodulatory roles of bacterial outer membrane vesicles. Microbiol Mol Biol Rev 74:81–94

Federle, Bassler (2003a) Chemical communication among bacteria. Proc Natl Acad Sci 100:14549–14554

Federle MJ, Bassler BL (2003b) Interspecies communication in bacteria. J Clin Investig 112:1291–1299

Fujiya M, Musch MW, Nakagawa Y, Hu S, Alverdy J, Kohgo Y, Schneewind O, Jabri B, Chang EB (2007) The Bacillus subtilis quorum-sensing molecule CSF contributes to intestinal homeostasis via OCTN2, a host cell membrane transporter. Cell Host Microbe 1:299–308

Fuqua C, Winans SC, Greenberg EP (1996) Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu Rev Microbiol 50:727–751

Galloway WR, Hodgkinson JT, Bowden S, Welch M, Spring DR (2012) Applications of small molecule activators and inhibitors of quorum sensing in gram-negative bacteria. Trends Microbiol 20:449–458

Gellatly SL, Hancock RE (2013) Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis 67(3):159–173

Gonzalez DJ, Haste NM, Hollands A, Fleming TC, Hamby M, Pogliano K, Nizet V, Dorrestein PC (2011) Microbial competition between Bacillus subtilis and Staphylococcus aureus monitored by imaging mass spectrometry. Microbiology 157:2485–2492

Haiko J, Westerlund-Wikström B (2013) The role of the bacterial flagellum in adhesion and virulence. Biology (Basel) 2(4):1242–1267

Hughes DT, Sperandio V (2008) Inter-kingdom signalling: communication between bacteria and their hosts. Nat Rev Microbiol 6:111–120

Ji Q, Chen PJ, Qin G, Deng X, Hao Z, Wawrzak Z, Yeo W-S, Quang JW, Cho H, Luo G-Z, Weng X, You Q, Luan C-H, Yang X, Bae T, Yu K, Jiang H, He C (2016) Structure and mechanism of the essential two-component signal-transduction system WalKR in Staphylococcus aureus. Nat Commun 7:11000

Koh C-L, Sam C-K, Yin W-F, Tan LY, Krishnan T, Chong YM, Chan K-G (2013) Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors (Basel, Switzerland) 13:6217–6228

Lau GW, Hassett DJ, Ran H, Kong F (2004) The role of pyocyanin in Pseudomonas aeruginosa infection. Trends Mol Med 10:599–606

McKnight SL, Iglewski BH, Pesci EC (2000) The Pseudomonas quinolone signal regulates rhl quorum sensing in Pseudomonas aeruginosa. J Bacteriol 182:2702–2708

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Papenfort K, Bassler B (2016) Quorum-sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol 14:576–588

Park J, Jagasia R, Kaufmann GF, Mathison JC, Ruiz DI, Moss JA, Meijler MM, Ulevitch RJ, Janda KD (2007) Infection control by antibody disruption of bacterial quorum sensing signaling. Chem Biol 14:1119–1127

Pesci EC et al (1999) Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 96:11229–11234

Peyraud R, Cottret L, Marmiesse L, Gouzy J, Genin S (2016) A resource allocation trade-off between virulence and proliferation drives metabolic versatility in the plant pathogen Ralstonia solanacearum. PLoS Pathog 12:e1005939

Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2:a012427

Saurav K, Costantino V, Venturi V, Steindler L (2017) Quorum sensing inhibitors from the sea discovered using bacterial N-acyl-homoserine lactone-based biosensors. Mar Drugs 15:53

Schell U, Simon S, Hilbi H (2016) Inflammasome recognition and regulation of the Legionella flagellum. Curr Top Microbiol Immunol 397:161–181

Sheela GM, Prathyusha AMVN, Neelapu NRR, Bramhachari PV (2018) Intra and inter-species communication in microbes: living with complex and sociable neighbors. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 7–16

Skindersoe ME, Zeuthen LH, Brix S, Fink LN, Lazenby J, Whittall C, Williams P, Diggle SP, Froekiaer H, Cooley M, Givskov M (2009) Pseudomonas aeruginosa quorum-sensing signal molecules interfere with dendritic cell-induced T-cell proliferation. FEMS Immunol Med Microbiol 55:335–345

Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB (2003) Bacteria-host communication: the language of hormones. Proc Natl Acad Sci U S A 100:8951–8956

Subramanian P, Choy KL, Gobal SV, Mansor M, Ng KH (2013) Impact of education on ventilator-associated pneumonia in the intensive care unit. Singap Med J 54:281–284

Tay SB, Yew WS (2013) Development of quorum-based anti-virulence therapeutics targeting gram-negative bacterial pathogens. Int J Mol Sci 14:16570–16599

Telford G et al (1998) The Pseudomonas aeruginosa quorum-sensing signal molecule N-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. Infect Immun 66:36–42

Turkina MV, Vikström E (2019) Bacteria-host crosstalk: sensing of the quorum in the context of Pseudomonas aeruginosa infections. J Innate Immun 11(3):263–279

Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharm Therap 40:277–283

Walsh CJ, Guinane CM, O'Toole PW, Cotter PD (2014) Beneficial modulation of the gut microbiota. FEBS Lett 588:4120–4130

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

6 In Silico Approaches for Unearthing Bacterial Quorum-Sensing Inhibitors Against Pathogenic Bacteria

Shrikant Pawar, Pallaval Veera Bramhachari, and Chandrajit Lahiri

Abstract

The bacterial phenotypic traits of biofilm formation, bioluminescence, swarming motility, and even virulence are being highly influenced by the phenomenon of cell density-dependent gene regulation a.k.a. quorum sensing (QS) through which the bacteria communicate within themselves. Essentially, QS is an intracellular signaling system which are different for the different gram characters of bacteria. While gram-negative bacteria use chemical autoinducer molecules like acyl-homoserine lactones (AHLs) for such signaling, the gram-positive bacteria use peptide-based signaling systems. These quorum-sensing peptides (QSPs) can initiate a signaling cascade of events via two-component system or even by direct binding to transcription factors. After the detection of QSPs by bacteria, response regulators or transcriptional factors are activated, which further stimulates change in the target gene expression. Owing to the therapeutic potential of the AHLs and QSPs as drug targets, different in silico approaches were utilized for the identification of inhibitors and their modeling which can help in combatingthe respective bacterial pathogenicity. Thus, certain group of researchers also

S. Pawar
Yale Center for Genome Analysis, Yale University, New Haven, CT, USA
e-mail: spawar2@student.gsu.edu; shrikant.pawar@yale.edu

P. V. Bramhachari
Department of Biotechnology, Krishna University,
Machilipatnam, Andhra Pradesh, India
e-mail: veerabramha@gmail.com

C. Lahiri (✉)
Department of Biological Sciences, Sunway University, Petaling Jaya, Selangor, Malaysia
e-mail: chandrajitl@sunway.edu.my

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_6

developed machine learning tools based on support vector machine (SVM) and hidden Markov models (HMM) for the identification of novel and effective biofilm inhibitory peptides (BIPs), while others used in silico approaches for predicting and designing of antibiofilm peptides usingbidirectional recursive neural network (BRNN) and Random Forest (RF) algorithms. Moreover, biological network visualization techniques and analysis enabled the identification of QSPs in different bacteria using related information from the curated databases. To this end, identification of the binding pocket(s), motif search, and other physicochemical properties will help in predicting the three-dimensional structure of such target. Furthermore, ultra-high-throughput screening is another approach which unveils QS inhibitors (QSI) based on the characterization of natural products and screening for naturally occurring enzymes. This review specifically focuses on all such in silico approaches in predicting QSI in different bacterial species. Such in silico QSI predictions and their docking onto QS targets can help to shape up a promising future for making newer therapeutic options available against different pathogenic bacteria.

Keywords
Quorum sensing (QS) · Inhibitors · Bioinformatics

6.1 Targeting the Bacterial Communication System (QS)

6.1.1 Quorum Sensing

Over the last decade, the phenomenon of bacterial QS has emerged to be the favorite alternative for the community of drug discoverers. This is because such QS systems offered a novel controlling point of growth in the treatment of bacterial infectious diseases. This enables a group of researchers to aim for new drug discovery targets to combat the increasing multidrug resistance in these pathogens. Once discovered as a unique phenotype for gram-positive bacteria, *S. pneumoniae* (Tomasz 1965), and for the marine gram-negative microbes *V. fischeri* and *V. harveyi* (Nealson et al. 1970), QS has eventually been discovered to be related with almost all pathogenic bacteria. In these bacteria, the phenotypes encompass bioluminescence, swarming motility, biofilm formation, and virulence (Wynendaele et al. 2013). Essentially, all these phenotypes involve communication and coordination among the bacteria themselves. This particular biological phenomenon, through which bacteria can communicate with each other, is carried out through sensing the auto-secreted signaling molecules in a cell density-dependent manner. Rightly, thus, such sensing threshold concentration of the essential biomolecules has been coined the term QS (Miller and Bassler 2001; Garsin 2004).

QS biomolecules can be the autoinducers (AI), like acylated homoserine lactone (AHL) in gram negatives, and quorum-sensing peptides (QSPs), like diketopiperazines (DKPs) in gram positives (Miller and Bassler 2001; Garsin 2004; Michiels et al. 2001). Other signaling molecules like pseudomonas quinolone signal (PQS)

were also reported to function as QSPs in some bacteria (Pesci et al. 1999). Of these different types of QS molecules, the AI can be of three different types, namely, AI-1, AI-2, and AI-3, as reported in clinically important gram-negative pathogens, viz., *E. coli* and *S. enterica*, which may or may not be mediated by the canonical LuxS or LuxR homologues (Lahiri 2018). Moreover, the QSPs can initiate a signaling cascade of events after reaching a threshold concentration. This is done via the prokaryotic two-component signal transduction system or by direct binding to transcription factor, which further induces change in target gene expression (Jimenez and Federle 2014; Schauder and Bassler 2001).

Ideally, most pathogenic bacteria utilize the QS mechanisms for biofilm formation and virulence factor production. For instance, in *Pseudomonas aeruginosa*, the QS controlling phenotypes contribute toward colonization in patients of cystic fibrosis (Pawar and Lahiri 2018). This is performed through two hierarchically organized systems, each consisting of an autoinducer synthetase (LasI/RhlI) and a corresponding regulator protein (LasR/RhlR). Other clinically relevant bacteria like *S. epidermidis* and *E. faecalis* utilize QS through expression of pathogenicity-related extracellular proteases (Krämer and Jung 2010; Nishiguchi et al. 2009). In some other gram positives like *S. pneumoniae* and *S. gordonii*, QS is controlled by competence-stimulating peptides (CSPs) (Havarstein et al. 1997). In pathogenic *Escherichia coli*, QSPs are reported as linear pentapeptides responsible for programmed cell death (Kolodkin-Gal et al. 2007).

It is worthwhile to mention here that despite its high importance in mediating virulence and biofilm formation, QS is also seen in nonclinically relevant organisms like halophiles, acidophiles, psychrophiles, thermophiles, piezophiles, and archaea (DasSarma and DasSarma 2006; Montgomery et al. 2013). Some halophilic eukaryotic micro-algae, *Dunaliella salina*, were shown to engage in the QS process in saline environments (Baker-Austin et al. 2010). An acidophile, *Ferroplasma acidarmanus*, was found to exhibit distinct morphological changes in biofilm formations and contain many related genes to biofilm formation and motility without the canonical LuxR or LuxS homologues being identified (Nichols et al. 2009). Hyperthermophilic archaeons, *Pyrococcus furiosus* and *Thermotoga* maritima, together could produce an autoinducer-2 (AI-2) type signal through a series of biotic and abiotic steps (Medigue et al. 2005). The psychrophile *P. haloplanktis* was observed to contain the mtnN gene involved in the production of putative AI-2 signals (Bodor et al. 2008). *S. benthica* and *S. violacea* are piezophilic microorganisms documented to contain the luxS gene (Zhang et al. 2012). Certain methanogenic archaeon like *M. harundinacea* produces carboxylated AHLs, N-carboxyl-decanoyl-homoserine lactone, N-carboxyl-dodecanoyl-homoserine lactone, and N-carboxyl-tetradecanoyl-homoserine lactone, all of which contribute to QS in archaea (Kumar et al. 2013).

6.1.2 Quorum-Sensing Inhibitors

With the aforesaid background in QS, it is imperative that the inhibitors of the phenomenon are likely to be compounds having the ability to impair the communication signals between the bacteria. Such QS-inhibitory molecules were found in

marine environment. For instance, a range of halogenated furanone compounds with anti-AHL properties were found in the red seaweed *Delisea pulchra* (Manefield et al. 1999). Generally, such compounds alter the composition and abundance of the bacterial community, thereby hampering the subsequent development of the biofilms. Moreover, synthetic derivatives of natural furanone compounds can also act as a potent antagonist of bacterial QS as reported for *P. aeruginosa* PAO1. A microarray analysis of the transcriptome revealed QS system genes to be specifically targeted by the furanone drug which further inhibited the virulence factor expression (Manefield et al. 1999). One such synthetic drug, 4-nitro-pyridine-N-oxide (4-NPO), mainly affected the genes regulated by either RhlR alone or RhlR and LasR in concert (Hentzer et al. 2003). Thus, several molecular libraries of both natural and synthetic chemicals were screened to identify numerous QS-inhibitory compounds (Rasmussen and Givskov 2006). Experimentally, these were validated through bacterial clearance and mortality reduction for animal pulmonary infection models (Rasmussen and Givskov 2006). Besides these, several enzymes were identified having the capacity to block the QS-governed virulence of plant pathogens (Rasmussen and Givskov 2006).

Catering to the need of dealing with such QS inhibition, genes of QS and QSPs became important targets for combating bacterial pathogenicity (Thoendel and Horswill 2010). Essentially, the QS mechanisms are disrupted by several small molecules, monoclonal antibodies, and antagonists against the receptors (Dong et al. 2007; Nakayama et al. 2009). Thus, the fungal metabolite ambuic acid was reported to act like drug in gram-positive bacteria to inhibit cyclic peptide quormones (Nakayama et al. 2009). Likewise, in *E. faecalis*, QS was found to be inhibited by Siamycin I (Nakayama et al. 2007). Furthermore, in *Staphylococcus aureus*, the small molecule, RNA III-inhibiting peptide (RIP), has been shown to impede QS mechanism (Giacometti et al. 2003), besides the anti-autoinducer monoclonal antibody which effectively hinders the autoinducing peptide (AIP)-4 of the same bacteria (Park et al. 2007). Again, for bacteria exhibiting QS mediated by AI like AHL, several research groups investigated the effects of sublethal concentrations (SLC) of bioactive dietary phytochemical extracts from common dietary fruit, herb, and spice extracts (Vattem et al. 2007).

While other researchers were experimenting with existing targets and inhibitors, the predictive power of bioinformatics tools and techniques was deployed to identify targets of QS and its mechanism. For instance, a computational biological analysis was also efficient in suggesting the proteins PqsR and PqsE acting as receptors in *P. aeruginosa* (Schaadt 2013). Both are required together with an AI to form pyocyanin. This was experimentally demonstrated through the inhibition of the AI formation with enzymes, while the pyocyanin biosynthesis was blocked by PqsR antagonists (Schaadt 2013). Moreover, certain other groups developed machine learning tools for identification of novel and effective biofilm inhibitory peptides (BIPs), while others employed in silico approaches for predicting and designing antibiofilm peptides (Sharma et al. 2016a; Mohammed Zaghlool Saeed Al-Khayyat and Ammar Ghanem Ameen Al-Dabbagh 2016a). Furthermore, the bioinformatics approaches were also been applied in form of prediction and docking techniques for

studying QS structures (Ute et al. 2006a). For example, in *P. aeruginosa*, ultrahigh-throughput screening was shown to be an efficient technique for identifying QS inhibitors (Rajput et al. 2015). Thus, this review article primarily focuses on all these computational and predictive techniques of studying and targeting QS in different organisms.

6.1.3 Predictive Approaches

6.1.3.1 Machine Learning and Novel Algorithms

QSPs drive QS phenomenon in gram-positive bacteria (Miller and Bassler 2001). Targeting QSPs can be an alternative strategy to combat bacterial pathogenicity (Medigue et al. 2005). Therefore, analysis and prediction of QSPs are of immense importance in gram-positive bacteria. A machine learning tool for identification of novel and effective biofilm inhibitory peptides (BIPs) was recently been proved an efficient method of classification (Guo et al. 2011). This study utilizes peptide sequence properties like amino acid composition, position, and motifs for characterizing QSPs and non-QSPs. The QSPs identified with this technique were rich in aromatic amino acids, namely, Phe, Trp, and Tyr, while the larger amino acids like Trp, Phe, Lys, and Gln were preferred at the C terminus along with Cys. A dipeptide composition analysis discovered that Leu-Phe, Asn-Asn, Ile-Phe, Ser-Thr, Ser-Leu, Cys-Val, Pro-Cys, Val-Gly, and Phe-Phe were seen as preferred consecutive residues. The data validation performed with nonredundant positive dataset identified QS motifs reported to comprise QS functions in gram-positive and gram-negative bacteria and archaea (Paggi et al. 2003; Tian et al. 2009).

Some of the known signaling mechanisms and potential antipathogenic drugs can specifically target QS systems (Hentzer and Givskov 2003). Thus, with an aim to competitively inhibit the transcriptional regulators LuxR and LasR, designing and biological screening of parallel solution-phase synthesis of sulfide AHL analogues were reported (Persson et al. 2005). Essentially, these comprised sulfoxides, sulfones, and dithianes having similarity both to sulfides and to bioactive structures from garlic (Persson et al. 2005). Furthermore, physicochemical properties like aromaticity, molecular weight, and secondary structure were also observed to differentiate QSPs from non-QSPs (Persson et al. 2005). Another study utilizes support vector machine (SVM) to extract physicochemical indices, where QSPs are seen to prefer secondary structure conformations (α-helix, coil, and β-sheet) similar to QSPs of *S. mutans* with random coil α-helix conformations (Syvitski et al. 2007; Samanta and Chakrabarti 2001). The abundant Cys and Trp residues identified by this technique can be imperative in forming binding sites with anchoring disulfide bonds to increase activity and stability (Rasmussen et al. 2007; De Jesus and Allen 2013; Haag et al. 2012; Hall et al. 2004). SVM was also used to develop QSP prediction algorithm (QSPpred) which achieved an accuracy of 93% during tenfold cross-validation (Persson et al. 2005). QSPpred is a web server hosting several SVM-based predictive models, viz., QSPepPred, QSPepDesign, and QSPepMap. Of these, QSPepPred predicts the extent of an input peptide as QSP or non-QSP,

QSPepDesign designs all possible single position mutants of a given peptide sequence to predict their QS status, while QSPepMap helps to identify potential regions of QSPs in proteins (Persson et al. 2005). Predictor QSPpred, thus, helps in accelerating QS research with QSP prediction.

Biofilms are sessile communities constituted by cells adhering to a substratum and embedded in polysaccharides, proteins, and extracellular DNA (Sauer et al. 2002). Biofilms are important for survival of bacterial species in diverse environments (Pamp et al. 2008). They can directly alter cells' growth rate and gene transcription and can augment horizontal gene transfer (Hall et al. 2004). Some drugs like fluoroquinolones and tetracycline can only kill the more metabolically active cells found in the outer layers of biofilms (Costerton et al. 1999). Biofilms in bacteria are known to resist the environmental stresses like biocidal agents, UV damage, metal toxicity, and acid exposure (Fox 2013). They comprise a spatiotemporal heterogeneity making them 1000 times more resistant to antibiotics (Sharma et al. 2016b). Thus, there seems a significant need to develop antimicrobial peptides (AMPs) as prophylactic and therapeutic agents against drug-resistant bacteria and biofilms (Nell et al. 2006). Plethora of studies was conducted to assess action of peptides against multiple bacterial species. Machine learning tools were used to build six SVM and weka-based models trained on 80 biofilm-active AMPs and 88 QSPs (Ong et al. 2013). The positive and negative datasets consisted of 90 AMPs and 220 unique QSPs found to be active against bacterial biofilms. For an independent validation dataset, the best model produced an overall accuracy of 95.24%.

The majorities of selected ABPs were ≤18 residues in length with an improved antibiofilm activity, stability, and reduced toxicity (Keller and Surette 2006; Wang et al. 2011a). Majority of selected QSPs were longer pre-proteins possibly due to the high bioenergetic expense involved (Donaldson 2013). This study attempted to identify possible drug candidates for antibiofilm therapy for which machine learning predictions were applied on FDA-approved peptide drugs and AMPs. Twenty-nine FDA-approved peptide drugs were predicted as "biofilm-active." Moreover, ten "biofilm-inactive" peptides were predicted exclusively on amino acid composition with SVM and Weka NT5 BPP models. Five out of six models predicted Sinapultide (KL4), a 21-residue-long peptide designed to mimic C terminus of human lung surfactant protein-B (SP-B) as "biofilm-active." Eleven out of the 13 PSM peptides were predicted as "biofilm-active" by at least 2 and at most 6 models of dPABBs. Sinapultide closely resembles phenol-soluble modulins (PSMs) with physicochemical properties (Lebeaux et al. 2014) and was useful in promoting mucus clearance and may perhaps also be effective against bacterial biofilms infecting lungs of cystic fibrosis (Sasaki et al. 2010). For AMPs, six peptides were predicted as "biofilm-active" by all the six models consisting of Omiganan and Pexiganan acetate which was shown to be effective against catheter infections and bacterial middle ear infection, where biofilm involvement was reported (Lynn et al. 2004). The SVM whole AAC model predicted the antibiofilm activity of six (26 total) peptides, while the SVM model positively predicted two peptides. The Weka Whole AAC model correctly predicted 12 (total 26), while the Weka model based on 8 assumptions predicted 14 out of the 26 peptides as being biofilm-active. The Weka NT5 model was

able to achieve the best performance in terms of percentage of peptides correctly predicted. Biofilms are heterogeneous and dynamic structures, so any predictions regarding the activity of these peptides should be made in a species-specific manner. The dPABBs web server develops a prediction strategy for identification and optimization of unique antibiofilm peptides (De Jesus and Allen 2013).

Bioactive peptides within protein sequences are important targets of therapeutic product. Several in silico strategies pertaining to sequence and structure homology were used to detect novel bioactive peptides (Lata et al. 2010). Homology-based prediction proved to be extremely successful in identifying antimicrobial peptides (Thomas et al. 2010). Other machine learning prediction tools based on SVM (Fjell et al. 2007; Wang et al. 2011b), hidden Markov models (Mooney et al. 2012), sequence alignments, and feature selection (Mooney et al. 2013) were also effective. Some useful tools like PeptideRanker are general bioactive peptide predictors (Pollastri and McLysaght 2005). Recently, a new tool, PeptideLocator, was developed which combined many classes of bioactive peptides that are functionally distinct, trained with bidirectional recursive neural networks (BRNN) to predict number of structural features of protein (Mooney et al. 2006). BRNN was used to learn the mapping between input and output formula. BRNN was also used for predicting secondary structure (Barrett and Udani 2011) and structural motifs (Qian et al. 1995). PeptideLocator can predict the probability score between 0 and 1 of certain residue being part of a bioactive peptide. The closer the probability is to 1, more confident PeptideLocator is that the residue is part of a bioactive peptide. PeptideLocator has been shown to comprise specificity, sensitivity, and accuracy of over 80%, showing a strong correlation between predicted and observed classifications. It uses features like secondary structure, solvent accessibility, and structural motifs for identification of bioactive peptide within a protein sequence. Solvent accessibility feature was found to contribute most to the accuracy of BRNN, and around 90% of bioactive peptide residues were predicted correctly. However, the percentage of nonbioactive peptide residues incorrectly predicted as bioactive was also found to be high. Further, PeptideLocator was used to predict bioactive peptides in other classes (antifreeze, antimicrobial, cytokines and growth factor, peptide hormones, toxins, and venom) where the accuracy was found to be around 86%. Authors also employed PeptideLocator with 661 unique protein sequences for searching proteins with peptide regions with y residues over a threshold x. Using a threshold of 0.8, at least 80% of the residues were predicted over 0.8 threshold, and predicted peptides functions range from alpha-amylase inhibitors (Amano et al. 1998) and sheep lactoferrin fragment (Thomas et al. 2010) to wheat allergen peptide associated with Bakers asthma (Gupta et al. 2016). PeptideLocator was also found to correctly predict *Pisum sativum* bioactive peptide from defensin-like protein which was experimentally validated from CAMP database (Pérez-Pérez et al. 2017). PeptideLocator can, thus, accurately identify peptide regions in protein sequences if they are bioactive and can be effectively used in conjunction with other tools.

A protein-binding microarrays and a two-layered bioinformatics approach to show that LuxR binds a 21-bp consensus operator with dyad symmetry were also used. In vitro and in vivo analyses of two promoters directly regulated by LuxR

allowed identifying those bases that are critical for LuxR binding. Together, the in silico and biochemical results enabled to scan the genome and identify novel targets of LuxR in *V. harveyi* and thus expand the understanding of QS regulon (Hwang et al. 2016). Some groups constructed a collection of screening systems, QS inhibitor (QSI) selectors, which enables to identify a number of novel QSIs among natural and synthetic compound libraries. The two most active were garlic extract and 4-nitro-pyridine-N-oxide (4-NPO). GeneChip-based transcriptome analysis revealed that garlic extract and 4-NPO had specificity for QS-controlled virulence genes in *P. aeruginosa*. These two QSIs also significantly reduced *P. aeruginosa* biofilm tolerance to tobramycin treatment as well as virulence in a *C. elegans* pathogenesis model (Pérez-Pérez et al. 2014).

Novel molecular tools were constructed, which allow for in situ detection of N-acyl homoserine lactone (AHL)-mediated QS in *P. aeruginosa* biofilms. The reporter responds to AHL activation of LasR by expression of an unstable version of green-fluorescent protein (Gfp). Gfp-based reporter technology was applied for nondestructive, single-cell-level detection of QS in laboratory-based *P. aeruginosa* biofilms (Engebrecht and Silverman 1987). Structure-based virtual screening was also used in a search for putative QS inhibitors from a database comprising approved drugs and natural compounds. The database was built from compounds which showed structural similarities to previously report QS inhibitors, the ligand of *P. aeruginosa* QS receptor LasR, and a QS receptor agonist. Six top-ranking compounds, all recognized drugs, were identified and tested for quorum-sensing-inhibitory activity (Mihăşan 2010a).

Some groups evaluated SVM and random forest (RF) to extract sequence-based features and to identify unique sequence motifs (Mohammed Zaghlool Saeed Al-Khayyat and Ammar Ghanem Ameen Al-Dabbagh 2016b). The sequence-based features were used to construct SVM prediction models with sequence motifs information and hybrid models. This technique is robust, cost-effective, and efficient to identify biofilm inhibiting peptides (BIPs). A tenfold cross-validation and performance evaluation was performed with validation dataset for predicting BIPs. Distribution of amino acids in BIPs and non-BIPs was done by amino acid compositional analysis. BIPs were found to enclose more of positively charged amino acids and aromatic amino acids, whereas non-BIPs were rich in negatively charged amino acids. A dipeptide bias analysis found 183 (total of 400) dipeptides differentially present in BIPs and non-BIPs. Most abundant dipeptides in BIPs were positively charged, while non-BIPs were negatively charged. Both compositional and dipeptide analysis gave similar results. Most of the positive hydrophobic motifs were found to be most abundant in BIPs. These different amino acid sequence-based features were used for classification of peptides into BIPs or non-BIPs using SVM and RF. A web server was designed for peptide prediction which can take multiple peptide sequences, and SVM model can predict if the query peptide possessed any biofilm inhibitory activity. The results are reported in tabular format with prediction scores. A module named protein scan can pass multiple peptides through the prediction pipeline to predict peptides that can potentially inhibit biofilm formation and do a virtual screening to provide results in the tabular format. Other

modules, peptide mapping can experimentally validate biofilm inhibitory peptides on the query sequence, while the similarity search module can perform smith-waterman homology search of query sequence against experimentally validated BIPs. The SVM-based models were shown to display good performance compared to RF-based models, such technique of utilizing sequence data for predicting biofilm inhibiting peptides is very effective (Gasteiger et al. 2005).

Investigation of mode of action and classification of antibiotic agents (ceftazidime, patulin, and epigallocatechin gallate; EGCG) on *P. aeruginosa* biofilm using Raman spectroscopy with multivariate analysis, including support vector machine (SVM) and principal component analysis (PCA), were implemented. This method allows for quantitative, label-free, noninvasive, and rapid monitoring of biochemical changes in intricate biofilm matrices with high sensitivity and specificity (Cheng et al. 2005). Bacterial foraging algorithm mimicking bacterial behavior was introduced by Passino. However, his work did not implement an important bacterial behavior regulating division so-called dasiaquorum-sensingpsila. The QS is a chemical communication including producing, releasing, detecting, and responding to small hormone-like molecules termed AIs. Some groups proposed an optimization algorithm based on the bacterial QS (Kabsch and Sander 1983).

6.1.3.2 Network Analysis and Visualization

Various techniques were used for network analysis and visualization of QS data in different organisms. A network of potential anti-quorum-sensing agents for *P. aeruginosa* was created with information from biomedical ontologies and curated databases (Pompeani et al. 2008). A bioinformatics framework with 110 scientific articles and corresponding 1004 annotations were included in this network. Computational workflows to create comprehensive knowledge map can retrieve and integrate information and can uncover links and lead to new insights into QS-centric therapeutics. Some groups previously applied network approaches to study antibiotic resistance in *P. aeruginosa*, while others researchers attempted to extract information types and apply it to the retrieval and curation of research articles in *P. aeruginosa* QS (Rasmussen et al. 2005). The regulatory networks for QS system can be represented as key genes and their immediate neighbors. The QS in *P. aeruginosa* regulates in hierarchical manner, LasI/LasR system regulates RhlI/RhlR and PqsABCDE/PqsR systems, while AmbBCDE/IqsR system regulates PqsABCDE/PqsR (Pompeani et al. 2008).

A manual curation to do a comprehensive annotation of interactions between QS and agent entities and to comprehend inhibitory and noninhibitory interactions was studied (Hentzer et al. 2002). Visualization of agent-QS interactions can be performed on publicly accessible server http://pcquorum.org. Subnetworks can be analyzed through heat maps with access to interaction type, organism, strain, mode of growth, method, and PubMed reference. Details such as synonyms of antimicrobial agents, genes, proteins, and links to major biological databases such as CHEBI, PubChem, CHEMBL, and Uniprot are provided. These authors constructed a network with 1004 annotated agent–QS interactions categorized into antibiotics, antifungals, AMP, disinfectants, and natural and synthetic products. Targets are

categorized as genes, proteins, virulence factors, and virulence mechanisms. QS interactions related to biofilms and key virulence mechanism were also studied with the corresponding regulatory subnetworks. QS targets can be efficiently studied using network topological and related parametric measures as exemplified in unearthing the potential ones for multidrug-resistant *P. mirabilis (*Pawar et al. 2018*)*.

6.1.3.3 Prediction and Docking Techniques for Studying QS Structures

In *V. fischeri*, LuxI is an important component of QS-signaling pathway (Pawar et al. 2018). Homology modeling is a good way of predicting docking sites and a three-dimensional structure of LuxI and other QS components (Mihăşan 2010b). The automated docking program DOCK 5.3.0 is also applied for screening of quorum-sensing inhibitors (QSIs) of *P. aeruginosa* from a database containing 51 active components of Traditional Chinese Medicines with antibacterial activity. Five potential QSIs were revealed by the computer-based virtual screening (Mihăşan 2010b). Homology modeling is a method of structure prediction based on amino acid sequence similarity to closely related known structures (Mihăşan 2010b). Several groups attempted to utilize such techniques of homology modeling using Phyre2 and GalaxyWEB server (Kelley and Sternberg 2009). Evaluation is usually done by ERRAT, ANOLEA, QMEAN6, and Procheck scores (Kelley and Sternberg 2009). Models generated by this technique produced QMEAN6 score of 0.732, ERRAT score of 98.9%, and a better ANOLEA scores (Kelley and Sternberg 2009). ProtParam tool of ExPASy server characterizes physiochemical properties (molecular weight, amino acid composition, theoretical isoelectric point, extinction coefficient, and instability index) of LuxI (Al-Khayyat and Al-Dabbagh 2016). SSpro8 of SCRATCH was used to predict secondary and disordered regions, and structures are determined using Kabsch and Sanders method (Magnan and Baldi 2014). Effective motif identification can be done with Pfam and Prosite databases (Hwang et al. 2016). Homology modeling, refinement, and evaluation of 3D structure can be done by PHYRE2 (Kelley and Sternberg 2009). PROSA web tool can determine Z score, which measures deviation of total energy of structure found in native proteins (Wiederstein and Sippl 2007). Docking of compounds can be performed using CLC Drug Discovery workbench 2.0. The Z-scores of all the models generated are found to be similar to the normal values commonly found in native structures (Benkert et al. 2009). Alternate models can also be generated by a scoring function (Benkert et al. 2009). QMEAN6 scoring function uses two distance-dependent interaction potentials, a torsion angle potential, a solvation potential, the agreement of the predicted and the calculated secondary structures, and solvent accessibility prediction (Benkert et al. 2009). This study shows how certain sites of action can be exploited in ligand design to inhibit QS with homologous systems (Benkert et al. 2009).

Some groups presented a qualitative model of *P. aeruginosa* QS network, including interactions between Las and Rhl modules, the signaling molecule PQS, and the regulatory proteins Mvfr and VfR. Simulations exemplify the model to reproduce natural network behavior and suggest QS responses to pharmacological interference (Alessandro and Martin 2008). The origin of anti-quorum-sensing (QS) activities

for several members of a recently synthesized and in vitro tested class of lactone and thiolactone-based inhibitors is also computationally investigated. Docking and molecular dynamic (MD) simulations and binding free energy calculations were carried out to reveal the exact binding and inhibitory profiles of these compounds (Marawan et al. 2013).

6.1.3.4 Ultrahigh-Throughput Screening

Ultrahigh-throughput screening approaches were utilized for screening around 200,000 compounds for inhibitors of LasR-dependent gene expression (Ute et al. 2006b). Using such techniques, two compounds (tetrazole and V-06-018) with 50% inhibitory concentration (IC50) of 30 nM and 10 μM were identified. Both these inhibitors were identified as general inhibitors of QS and inhibited production of two QS-dependent virulence factors, elastase and pyocyanin. Application of machine learning algorithms also led to the development of quantitative activity–composition relationships classification models that allowed to direct point out those essential oil chemical components more involved in the inhibition of biofilm production. The action of selected essential oils on sessile phenotype makes them particularly interesting for possible applications such as prevention of bacterial contamination in the community and in healthcare environments in order to prevent human infections (Ute et al. 2006b). Phylogenetic overlaps between aromatics-degrading bacteria and acyl-homoserine-lactone (AHL) or AI-based QS bacteria are evident in literatures; however, the diversity of bacteria with both activities had never been thinly described. In silico searching in NCBI genome database revealed that more than 11% of investigated population harbored both aromatic ring-hydroxylating-dioxygenase (RHD) gene and AHL/AI-synthetase gene (Huang et al. 2013).

Some approaches to identify QS inhibitors use chemical synthesis of compounds modeled on the natural acyl-HSL signals, characterization of natural products, and screening for naturally occurring enzymes such as lactonases and acylases (Ute et al. 2006b). Screening a large library of synthetic molecules using nanowell technology is a newer approach for targeting QS inhibitors (Ute et al. 2006b). In this experiment, authors screened for inhibitors of *P. aeruginosa* by growing in the presence of 3OC12-HSL as any compound/inhibitor interfering with acyl-HSL reception would reduce the fluorescence. This method is capable of screening only strong inhibitors with lower endogenous signal of about 30-fold. A limited number (20) of compounds were identified, which may be because of substantial permeability barrier of small molecules. Based on potency and chemical stability, two QS inhibitors, V-06-018 and PD12, were selected. Both inhibitors resembled native *P. aeruginosa* LasI-generated signaling molecule. A transcriptome profiling showed that these inhibitors of rsaL-yfp expression served as general QS inhibitors. Gene expression profiles of cells grown without added signal or inhibitor and cells grown in the presence of 0.3 μM 3OC12-HSL were studied (Ute et al. 2006b). Further, influence of inhibitors on QS gene expression in the wild-type *P. aeruginosa* strain revealed expression of more quorum-controlled genes. Out of 293 induced genes, V-06-018 inhibited expression of 129, while PD12 inhibited expression of 7 indicating that V-06-018 functioned as a better inhibitor of QS than did PD12. This is a stringent

screen where the inhibitor should enter cells and interfere with 3OC12-HSL reception. The results of this screening technique can be compared with other natural product screening techniques identifying other novel inhibitors (furanone inhibitor and 4-nitro-pyridine-N-oxide) (Ute et al. 2006b). The inhibitors selected from this technique also inhibited virulence factors in a wild-type strain, which is more stringent evaluation of inhibitor efficacy. Such screening techniques present promising scaffolds for developing additional compounds for inhibiting QS.

6.2 Conclusion

QS controls expression of more than 300 genes important for virulence and normal biofilm development. Bioinformatic techniques were applied in form of prediction with docking and ultra-high-throughput screening for studying QS structures. Machine learning approaches like SVM and RF to extract sequence-based features and to identify unique sequence motifs were utilized extensively. SVM can extract physicochemical indices where QSPs were seen to prefer secondary structure conformations. Machine learning tools can also be used to build SVM and Weka-based models. Other machine learning prediction tools based on hidden Markov models, sequence alignments, and feature selection were also effective. Further, homology modeling was an effective way of predicting docking sites and three-dimensional structure of LuxI and other QS components. Some novel in silico approaches for predicting and designing antibiofilm peptides brought new insights into targeting QS. Furthermore, newer ultrahigh-throughput screening techniques also unveil interesting approaches in targeting QS in *P. aeruginosa*. All these existing and newer in silico approaches are promising ways in targeting QS in different bacterial species.

Acknowledgments The author acknowledges the support of Sunway University, Selangor, Malaysia, for providing the computational facilities and wishes to thank Rohit Mishra for the valuable contribution in gathering the articles for the concept provided.

Author Contributions CL conceived the concepts, planned, and designed the article. SP primarily wrote the manuscript. This was further revamped by CL followed by PVB for final checking.

References

Alessandro V, Martin F (2008) Modeling the quorum sensing regulatory network of human-pathogenic Pseudomonas aeruginosa. Biotechnol Prog. https://doi.org/10.1021/bp0343231

Al-Khayyat MZ, Al-Dabbagh AG (2016) In silico prediction and docking of tertiary structure of LuxI, an inducer synthase of Vibrio fischeri. Rep Biochem Mol Biol 4(2):66–75

Amano M et al (1998) Identification of the major allergens in wheat flour responsible for baker's asthma. Biochem J 330(Pt. 3):1229–1234

Baker-Austin C, Potrykus J, Wexler M, Bond PL, Dopson M (2010) Biofilm development in the extremely acidophilic archaeon Ferroplasma. acidarmanus Fer1. Extremophiles 14:485–491. https://doi.org/10.1007/s00792-010-0328-1

Barrett M, Udani J (2011) A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): a review of clinical studies on weight loss and glycemic control. Nutr J 10:24

Benkert P, Kunzli M, Schwede T (2009) QMEAN server for protein model quality estimation. Nucleic Acids Res 37:W510–W514

Bodor A, Elxnat B, Thiel V, Schulz S, Wagner-Dobler I (2008) Potential for luxS related signalling in marine bacteria of autoinducer-2 in the genus *Shewanella*. BMC Microbiol 8:1–9. https://doi.org/10.1186/1471-2180-8-1

Cheng J, Randall A, Sweredoski M, Baldi P (2005) SCRATCH: a protein structure and structural feature prediction server. Nucl Acids Res 33:W72–W76

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322

DasSarma S, DasSarma P (2006) Encyclopedia of life sciences. Wiley, London. Halophiles

De Jesus AJ, Allen TW (2013) The role of tryptophan side chains in membrane protein anchoring and hydrophobic mismatch. Biochim Biophys Acta 1828(2):864–876. Pmid:22989724

Donaldson S (2013) Effect of Lucinactant on mucus clearance in cystic fibrosis lung disease, https://clinicaltrials.gov/ct2/show/study/NCT00934362?sect=X01256

Dong YH, Wang LY, Zhang LH (2007) Quorum-quenching microbial infections: mechanisms and implications. Philos Trans R Soc Lond Ser B Biol Sci 362(1483):1201–1211. Pmid:17360274

Engebrecht J, Silverman M (1987) Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. Nucl Acids Res 15:10455–10467

Fjell C et al (2007) AMPer: a database and an automated discovery tool for antimicrobial peptides. Bioinformatics 23:1148–1155

Fox JL (2013) Antimicrobial peptides stage a comeback. Nat Biotechnol 31:379–382. https://doi.org/10.1038/nbt.2572

Garsin DA (2004) Microbiology. Peptide signals sense and destroy target cells. Science 306(5705):2202–2203. pmid:15619588

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A (2005) Protein identification and analysis tools on the ExPASy server. In: Walker JM (ed) The proteomics protocols handbook. Humana Press, Totowa, pp 571–607

Giacometti A, Cirioni O, Gov Y, Ghiselli R, Del Prete MS, Mocchegiani F, Saba V, Orlando F, Scalise G, Balaban N, Dell'Acqua G (2003) RNA III inhibiting peptide inhibits in vivo biofilm formation by drug-resistant Staphylococcus aureus. Antimicrob Agents Chemother 47(6):1979–1983

Guo X, Zhang G, Liu X, Ma K, Dong X (2011) Detection of the quorum sensing signals in methanogenic archaea. Wei Sheng Wu Xue Bao 51(9):1200–1204. Pmid:22126075

Gupta S, Sharma AK, Jaiswal SK, Sharma VK (2016) Prediction of biofilm inhibiting peptides: an in silico approach. Front Microbiol. https://doi.org/10.3389/fmicb.2016.00949

Haag AF, Kerscher B, Dall'Angelo S, Sani M, Longhi R, Baloban M (2012) Role of cysteine residues and disulfide bonds in the activity of a legume root nodule-specific, cysteine-rich peptide. J Biol Chem 287(14):10791–10798. Pmid:22351783

Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Micro 2:95–108

Havarstein LS, Hakenbeck R, Gaustad P (1997) Natural competence in the genus *Streptococcus*: evidence that streptococci can change phenotype by interspecies recombinational exchanges. J Bacteriol 179(21):6589–6594. Pmid:9352904

Hentzer M, Givskov M (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 112(9):1300–1307

Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22(15):3803–3815

Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB (2002) Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Microbiology 148(Pt 1):87–102

Huang Y, Zeng Y, Yu Z, Zhang J, Feng HL, Lin X (2013) In silico and experimental methods revealed highly diverse bacteria with quorum sensing and aromatics biodegradation systems--a potential broad application on bioremediation. Bioresour Technol 148:311–316

Hwang S, Kim CY, Ji S-G, Go J, Kim H, Yang S, Kim HJ, Cho A, Yoon SS, Lee I (2016) Network-assisted investigation of virulence and antibiotic-resistance systems in Pseudomonas aeruginosa. Sci Rep 6:26223

Jimenez JC, Federle MJ (2014) Quorum sensing in group a Streptococcus. Front Cell Infect Microbiol 4:127. Pmid:25309879

Kabsch W, Sander C (1983) Dictionary of protein secondary structure: pattern recognition of hydrogen bonded and geometrical features. Biopolymers 22(12):2577–2637

Keller L, Surette MG (2006) Communication in bacteria: an ecological and evolutionary perspective. Nat Rev Microbiol 4:249–258. https://doi.org/10.1038/nrmicro1383

Kelley LA, Sternberg MJ (2009) Protein structure prediction on the web: a case study using the Phyre server. Nat Protocols 4(3):363–371

Kolodkin-Gal I, Hazan R, Gaathon A, Carmeli S, Engelberg-Kulka H (2007) A linear pentapeptide is a quorum-sensing factor required for mazEF-mediated cell death in *Escherichia coli*. Science 318(5850):652–655. Pmid:17962566

Krämer R, Jung K (2010) Bacterial signaling. Wiley-VCH/John Wiley, Weinheim/Chichester

Kumar S, Kolodkin-Gal I, Engelberg-Kulka H (2013) Novel quorum-sensing peptides mediating interspecies bacterial cell death. MBio 4(3):e00314–e00313. pmid:23736285

Lahiri C (2018) Quorum sensing complexity of the gut enterobacteria Escherichia coli and Salmonella enterica. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 233–248

Lata S et al (2010) AntiBP2: improved version of antibacterial peptide prediction. BMC Bioinform 11(Suppl. 1):S19

Lebeaux D, Ghigo JM, Beloin C (2014) Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 78:510–543. https://doi.org/10.1128/MMBR.00013-14

Lynn D et al (2004) Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 56:170–177

Magnan CN, Baldi P (2014) SSpro/ACCpro 5: almost perfect prediction of protein secondary structure and relative solvent accessibility using profiles, machine learning and structural similarity. Bioinformatics (Oxf) 30(18):2592–2597. https://doi.org/10.1093/bioinformatics/btu352

Manefield M, de Nys R, Naresh K, Roger R, Givskov M, Peter S, Kjelleberg S (1999) Evidence that halogenated furanones from Delisea pulchra inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology 145(2):283–291

Marawan A, Stefanie B, Feng W (2013) Enzo a. in silico investigation of lactone and thiolactone inhibitors in bacterial quorum sensing using molecular modeling. Biomolecules arXiv 1305:3691

Medigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin P, Cheung F, Cruveiller S, D'Amico S, Duillo A et al (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium Pseudoalteromonas. haloplanktis TAC125. Genome Res 15:1325–1335. https://doi.org/10.1101/gr.4126905

Michiels J, Dirix G, Vanderleyden J, Xi C (2001) Processing and export of peptide pheromones and bacteriocins in Gram-negative bacteria. Trend Microbiol 9(4):164–168. Pmid:11286880

Mihăşan M (2010a) Basic protein structure prediction for the biologist: a review. Arch Biol Sci Belgrade 62(4):857–871

Mihăşan M (2010b) Basic protein structure prediction for the biologist: a review. Arch Biol Sci 62(4):857–871

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199. Pmid:11544353

Mohammed Zaghlool Saeed Al-Khayyat, Ammar Ghanem Ameen Al-Dabbagh (2016a) In silico prediction and docking of tertiary structure of LuxI, an inducer synthase of *Vibrio fischeri*. Rep Biochem Mol Biol 4(2)

Mohammed Zaghlool Saeed Al-Khayyat, Ammar Ghanem Ameen Al-Dabbagh (2016b) In silico prediction and docking of tertiary structure of LuxI, an inducer synthase of Vibrio fischeri. Rep Biochem Mol Biol 4(2)

Montgomery K, Charlesworth J, LeBard R, Visscher P, Burns B (2013) Quorum sensing in extreme environments. Life 3(1):131–148

Mooney C et al (2006) Protein structural motif prediction in multidimensional ø-ψ space leads to improved secondary structure prediction. J Comput Biol 13:1489–1502

Mooney C et al (2012) Towards the improved discovery and design of functional peptides: common features of diverse classes permit generalized prediction of bioactivity. PLoS One 7:E45012

Mooney C, Haslam NJ, Holton TA, Pollastri G, Shields DC (2013) PeptideLocator: prediction of bioactive peptides in protein sequences. Bioinformatics 29(9):1120–1126

Nakayama J, Tanaka E, Kariyama R, Nagata K, Nishiguchi K, Mitsuhata R et al (2007) Siamycin attenuates fsr quorum sensing mediated by a gelatinase biosynthesis-activating pheromone in Enterococcus faecalis. J Bacteriol 189(4):1358–1365. Pmid:17071762

Nakayama J, Uemura Y, Nishiguchi K, Yoshimura N, Igarashi Y, Sonomoto K (2009) Ambuic acid inhibits the biosynthesis of cyclic peptide quormones in gram-positive bacteria. Antimicrob Agents Chemother 53(2):580–586. Pmid:19015326

Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 104(1):313–322. pmid:5473898

Nell MJ et al (2006) Development of novel LL-37 derived antimicrobial peptides with LPS and LTA neutralizing and antimicrobial activities for therapeutic application. Peptides 27:649–660. https://doi.org/10.1016/j.peptides.2005.09.016

Nichols J, Johnson M, Chou C, Kelly R (2009) Temperature, not LuxS, mediates AI-2 formation in hydrothermal habitats. FEMS Microbiol Ecol 68:173–181. https://doi.org/10.1111/j.1574-6941.2009.00662.x

Nishiguchi K, Nagata K, Tanokura M, Sonomoto K, Nakayama J (2009) Structure-activity relationship of gelatinase biosynthesis-activating pheromone of *Enterococcus faecalis*. J Bacteriol 191(2):641–650. pmid:18996993

Ong ZY, Gao SJ, Yang YY (2013) Short synthetic β-sheet forming peptide Amphiphiles as broad Spectrum antimicrobials with Antibiofilm and endotoxin neutralizing capabilities. Adv Funct Mater 23:3682–3692. https://doi.org/10.1002/adfm.201202850

Paggi RA, Martone CB, Fuqua C, De Castro RE (2003) Detection of quorum sensing signals in the haloalkaliphilic archaeon *Natronococcus occultus*. FEMS Microbiol Lett 221(1):49–52. Pmid:12694909

Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T (2008) Tolerance to the antimicrobial peptide colistin in Pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol 68:223–240. https://doi.org/10.1111/j.1365-2958.2008.06152.x

Park J, Jagasia R, Kaufmann GF, Mathison JC, Ruiz DI, Moss JA et al (2007) Infection control by antibody disruption of bacterial quorum sensing signaling. Chem Biol 14(10):1119–1127. Pmid:17961824

Pawar S, Lahiri C (2018) Quorum sensing: an imperative longevity weapon in bacteria. Afr J Microbiol Res 12(4):96–104

Pawar S, Ashraf MI, Mujawar S, Mishra R, Lahiri C (2018) In silico identification of the indispensable quorum sensing proteins of multidrug resistant *Proteus mirabilis*. Front Cell Infect Microbiol 8

Pérez-Pérez M, Glez-Peña D, Fdez-Riverola F, Marky LA (2014) A lightweight web tracking tool for document annotation. Adv Intell Syst Comput 8th Int Conf Pract Appl Comput Biol Bioinforma PACBB 294:269–276

Pérez-Pérez M, Jorge P, Pérez Rodríguez G, Pereira MO, Lourenço A (2017) Quorum sensing inhibition in Pseudomonas aeruginosa biofilms: new insights through network mining. Biofouling 33(2)

Persson T, Hansen TH, Rasmussen TB, Skindersø ME, Givskov M, Nielsen J (2005) Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. Org Biomol Chem 3(2):253–262

Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP et al (1999) Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 96(20):11229–11234. Pmid:10500159

Pollastri G, McLysaght A (2005) Porter: a new, accurate server for protein secondary structure prediction. Bioinformatics 21:1719–1720

Pompeani AJ, Irgon JJ, Berger MF, Bulyk ML, Wingreen NS, Bassler BL (2008) The Vibrio harveyi master quorum-sensing regulator, LuxR, a TetR-type protein is both an activator and a repressor: DNA recognition and binding specificity at target promoters. Mol Microbiol 70(1):76–88. https://doi.org/10.1111/j.1365-2958.2008.06389.x

Qian Z et al (1995) Isolation and characterization of sheep lactoferrin, an inhibitor of platelet aggregation and comparison with human lactoferrin. Biochim Biophys Acta 1243:25–32

Rajput A, Gupta AK, Kumar M (2015) Prediction and analysis of quorum sensing peptides based on sequence features. PLoS One. https://doi.org/10.1371/journal.pone.0120066

Rasmussen TB, Givskov MC (2006) Quorum sensing inhibitors: a bargain of effects. Microbiology 152(4):895–904

Rasmussen TB, Bjarnsholt T, Skindersoe ME, Hentzer M, Kristoffersen P, Köte M et al (2005) Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. J Bacteriol 187(5):1799–1814. https://doi.org/10.1128/JB.187.5.1799-1814.2005

Rasmussen A, Rasmussen T, Edwards MD, Schauer D, Schumann U, Miller S (2007) The role of tryptophan residues in the function and stability of the mechanosensitive channel MscS from Escherichia coli. Biochemistry 46(38):10899–10908. Pmid:17718516

Samanta U, Chakrabarti P (2001) Assessing the role of tryptophan residues in the binding site. Protein Eng 14(1):7–15. Pmid:11287674

Sasaki K et al (2010) A peptidomics strategy for discovering endogenous bioactive peptides. J Proteome Res 9:5047

Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154

Schaadt NS (2013) Computational systems biology methods for functional classification of membrane proteins and modeling of quorum sensing in *Pseudomonas aeruginosa*

Schauder S, Bassler BL (2001) The languages of bacteria. Genes Dev 15(12):1468–1480. Pmid:11410527

Sharma A, Gupta P, Kumar R, Bhardwaj A (2016a) dPABBs: a novel in silico approach for predicting and designing anti-biofilm peptides scientific reports. https://doi.org/10.1038/srep21839

Sharma A, Gupta P, Kumar R, Bhardwaj A (2016b) dPABBs: a novel in silico approach for predicting and designing anti-biofilm peptides. Sci Rep 6:Article number: 21839. https://doi.org/10.1038/srep21839

Syvitski RT, Tian XL, Sampara K, Salman A, Lee SF, Jakeman DL (2007) Structure-activity analysis of quorum-sensing signaling peptides from *Streptococcus mutans*. J Bacteriol 189(4):1441–1450. Pmid:16936029

Thoendel M, Horswill AR (2010) Biosynthesis of peptide signals in gram-positive bacteria. Adv Appl Microbiol 71:91–112. Pmid:20378052

Thomas S et al (2010) CAMP: a useful resource for research on antimicrobial peptides. Nucl Acids Res 38(Suppl. 1):D774

Tian X, Syvitski RT, Liu T, Livingstone N, Jakeman DL, Li YH (2009) A method for structure–activity analysis of quorum-sensing signaling peptides from naturally transformable streptococci. Biol Proc 11(1):207

Tomasz A (1965) Control of the competent state in pneumococcus by a hormone-like cell product: an example for a new type of regulatory mechanism in bacteria. Nature 208(5006):155–159. Pmid::5884251

Ute M, Schuster M, Heim R, Singh A, Olson ER, Peter Greenberg E (2006a) Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen. Antimicrob Agent Chemother 50(11):3674–3679

Ute M, Schuster M, Heim R, Singh A, Olson ER, Peter Greenberg E (2006b) Novel Pseudomonas aeruginosa quorum-sensing inhibitors identified in an ultra-high-throughput screen. Antimicrob Agents Chemother 50(11):3674–3679

Vattem DA, Mihalik K, Crixell SH, McLean RJ (2007) Dietary phytochemicals as quorum sensing inhibitors. Fitoterapia 78(4):302–310

Wang R et al (2011a) Staphylococcus epidermidis surfactant peptides promote biofilm maturation and dissemination of biofilm-associated infection in mice. J Clin Invest 121:238–248. https://doi.org/10.1172/JCI42520

Wang P et al (2011b) Prediction of antimicrobial peptides based on sequence alignment and feature selection methods. PLoS One 6:e18476

Wiederstein M, Sippl MJ (2007) ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 35:W407–W410

Wynendaele E, Bronselaer A, Nielandt J, D'Hondt M, Stalmans S, Bracke N et al (2013) Quorumpeps database: chemical space, microbial origin and functionality of quorum sensing peptides. Nucleic Acids Res 41(Database issue):D655–D659. pmid:23180797

Zhang G, Zhang F, Ding G, Li J, Guo X, Zhu J, Zhou L, Cai S, Liu X, Luo Y et al (2012) Acyl homoserine lactone-based quorum sensing in a methanogenic archaeon. ISME J 6:1–9. https://doi.org/10.1038/ismej.2011.71

Part II

Role of Quorum Sensing in Medicine

7 Significance of Quorum Sensing and Biofilm Formation in Medicine and Veterinary Sciences

Prudhvi Lal Bhukya, Renuka Nawadkar, Pallaval Veera Bramhachari, and Ganugula Mohana Sheela

Abstract

Quorum sensing (QS) is a coordination of a group of organisms to exhibit a specific action. It is an acquired social behavior presented to perform either symbiotic or pathogenic activity; however, most of the cases in the absence of QS, the decision to execute certain actions has not been performed. Therefore, QS is also termed as "collective decisions"; it is induced and executed by signaling molecules when the signaling molecule crosses a certain threshold. QS phenomenon is shown by many bacteria and fungi and yeast; recently, viruses also have shown to communicate via QS. In modern research era, study of QS is of most interest for the majority of human healthcare as well as animal health reasons. In general, a key approach perceived is inhibition of QS in case of infections or biofilm formation. Inhibition of QS can reduce the initiation of disease and its severity. Hence, inhibition of QS is of significant interest in human and animal healthcare for developing diagnostic and therapeutic tool. Inhibition of QS is an emerging tool to perform the antimicrobial activity by using targeting agents at either three different levels: production, spread, or acceptance of the signal. The current review primarily empha-

All Authors Prudhvi Lal Bhukya, Renuka Nawadkar, Pallaval Veera Bramhachari and Ganugula Mohana Sheela have equally contributed

P. L. Bhukya
Hepatitis Division, National Institute of Virology, Pune, India

Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

R. Nawadkar
Department of Pathogenesis and Cellular Response, National Centre for Cell Science, Pune, Maharashtra, India

P. V. Bramhachari · G. M. Sheela (✉)
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
e-mail: mohana.sheelag@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_7

sizes diverse mechanisms of QS, its inhibition, recent challenges and advances in the field, and its clinical implications in human and animal healthcare.

Keywords
Quorum sensing · Inhibitors of quorum sensing · Antibacterial · Veterinary pathogens

7.1 Introduction

Quorum sensing (QS) is a coordination of a group of organisms to exhibit a specific action (Fuqua et al. 1994). Mechanically, QS is a regulated gene expression by a group of organisms in response to sensing population density. Gene expression is generally dependent on signaling molecule concentration present in the surrounding environment. QS is an acquired social behavior presented to perform a specific group activity like bioluminescence, biofilm formation, and virulence factor expression. However, most of the cases in the absence of quorum sensing, the decision to execute certain actions has not been performed (Boyen et al. 2009). Therefore, QS is also termed as "collective decisions"; it is induced and executed by signaling molecules also known as autoinducers. QS phenomenon is shown by many bacteria and fungi and yeast; recently, viruses also have shown to carry out communication via quorum sensing (Sprague and Winans 2006; Albuquerque and Casadevall 2012; Erez et al. 2017).

Initially, QS was observed in two marine species *V. fischeri* and *V. harveyi* by Hastings and Nealson that at a high density of cells, the induction of luciferase enzyme is dependent on the presence of autoinducer (Nealson and Hastings 1979). Communication systems analogous with other organisms were also observed in production of a competence inducing heat-resistant molecule in *S. faecalis* required for conjugation (Dunny et al. 1978) and in *M. xanthus* for development of fruiting body (Dworkin and Kaiser 1985). Recently QS, in particular, is of interest because a number of virulent genes are under regulation of QS cascade like cholera caused by *V. cholerae* (Miller et al. 2002), periodontitis caused by *A. actinomycetemcomitans* (Fong et al. 2001), banana black rot caused by *X. campestris* (Barber et al. 1997), and pneumonia caused by *K. pneumonia* (Russo et al. 2011). However, the clinical significance of bacterial biofilm in diseases was first established in device-related infections (Donlan and Costerton, 2002; Hall-Stoodley and Stoodley 2009). Biofilms share several common features: (i) cells are held together by exopolysaccharides (EPS), (ii) biofilms respond to extracellular signals, (iii) biofilms guard the pathogenic microbes from a wide assortment of environmental stresses, viz., predators, immune system, and antibiotics (Lemon et al., 2008). Bearing in mind the widespread participation of biofilms in infections and diseases in human, biofilms are likely responsible for various infections in veterinary medicine (Clutterbuck et al. 2007). Here, we emphasize the recent understanding on bacterial biofilms, from human and veterinary subjects. This review is deliberated to cover the topics on biofilm formation and strategies for QS inhibition in animal pathogens and to augment consciousness about the potential impact of biofilms on the treatment options.

7.2 Mechanism of Quorum Sensing

QS is carried out with the diffusible signal molecule which subsequently activates downstream signaling for initiation of gene expression for collective decision. In general, there are three classes of QS. Gram-negative bacteria use acyl homoserine lactone (AHL), while gram-positive bacteria use small peptide molecule as a signaling molecule. AI-2 (*LuxS*-encoded autoinducer) is common in both gram-positive and gram-negative bacteria.

7.3 QS in Gram-Negative Bacteria

Up to present, more than 25 gram-negative bacteria species have been identified to communicate via QS. Generally, QS in gram-negative bacteria differs from gram-positive bacteria by two ways, (a) type of QS signaling molecule (also called as autoinducer molecule) and (b) recognition system used by two groups which are different from each other. In gram-negative organisms, QS system is very basic and simple; it consists of signaling molecule and its receptor; at a minimum, it has basic homologs of *V. fischeri* QS system known as LuxL and LuxR (Miller et al 2001). LuxL gene produces autoinducer molecules in these organisms which are usually N-acetyl-homoserine lactones (AHLS); these are made up of homoserine lactone ring attached to acyl chain of varying length of about 4–18 carbons and variation in saturation of carbon chain (LaSarre and Federle 2013). While LuxR family proteins are a receptor for AHLs, this interaction followed by a regulated response to DNA binding regulates target genes response. In addition to that, the AHL concentration is determined by diffusion extra- and intracellularly once it crosses the critical threshold signaling response is operated (Fig. 7.1) (Tay and Yew 2013).

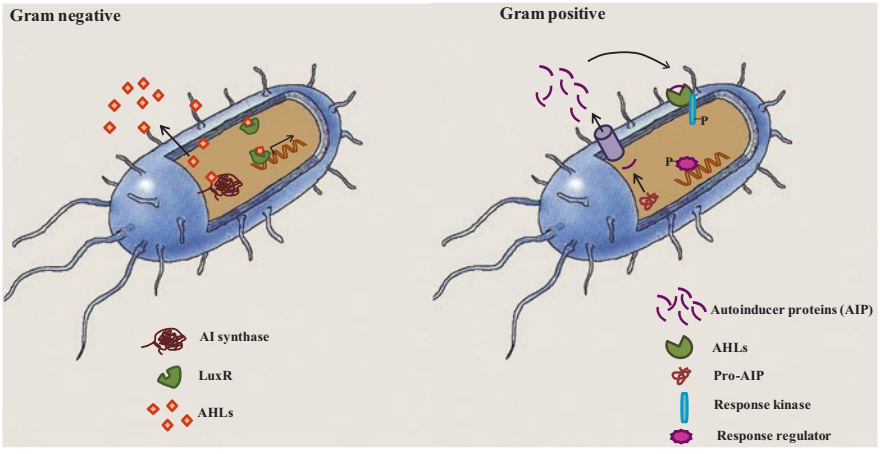


Fig. 7.1 General mechanism of QS in gram-negative and gram-positive organisms

7.4 QS in Gram-Positive Bacteria

Density-dependent regulation of certain responses and cell-to-cell communication are also established in gram-positive organisms. They instead of AHLs use secretary peptides as autoinducers. Precursor peptide chain is cleaved to produce a signal, and it is transported extracellularly via ABC transporters (LaSarre and Federle 2013). Peptides before extracellular transfer undergo different posttranslational processing or cyclization. Usually, detection of this peptide occurs by the extracellular receptor, histidine kinase, which autophosphorylates and transfers phosphate to response regulator molecule, which possesses DNA-binding capacity upon phosphorylation and finally regulates the response (Fig. 7.1).

7.5 QS in Both Gram-Positive and Gram-Negative Bacteria

Autoinducer-1 is another QS signal produced by many gram-positive and gram-negative species. Chemically, it is furanosyl borate diester part of the two-component system. It is produced by luxP and recognized by a protein having regulatory activity, LuxQ, while signal transduction is carried out by sensor kinase (LaSarre and Federle 2013). Strikingly, this QS system is common for luciferase operon expression observed in *V. harveyi* and *S. enterica*. The autoinducer AI-2 could serve as a "universal signal" for interspecies communication (Xavier and Bassler 2003).

7.6 Implications of QS in Human and Veterinary Pathogens

P. aeruginosa, *A. baumannii*, and *K. pneumonia* and *Enterobacter* spp. are well-known highly pathogenic bacteria and are the leading cause of several human nosocomial, urinary tract, respiratory tract, blood, and burn or wound infections (Lister et al. 2009). In *Enterobacter* spp., *E. coli* is a normal flora of GI tract where sometimes it acts as opportunistic pathogen also known for respiratory and urinary tract infection. *Enterobacter* spp. possess multiple mechanisms of QS, commonly used AHLs based in *E. coli* (Pinto et al. 2007; dos Reis Ponce et al. 2012), GS1-S-SdiA in *E. cloacae* (Shankar et al. 2013), and EAL-(SdiA-AL2) interaction, AI3. This biofilm-forming *Enterobacter* spp. always follows some communication or interaction among them (dos Reis Ponce et al. 2012) with *P. aeruginosa.* Moreover, virulent bacteria *K. pneumonia* enhances its virulence properties via AI-2, AHL, and siderophore-related QS molecule production and successfully mounts the virulence and pathogenesis (Zhu et al. 2011; Russo et al. 2011). *S. aureus*, *S. pneumonia*, *P. pseudintermedius,* and *Salmonella* are the highly pathogenic and virulent strains in veterinary therapeutics and cause various infections in rabbits, cows, dogs, cats, etc. *S. aureus* in rabbit is responsible for skin infection, mastitis, and internal abscessation (Vancraeynest et al. 2006), while in bovine, it causes skin and mammary gland

infection (Buzzola et al. 2007). *P. pseudintermedius* causes mastitis, otitis, dermatitis, and hemorrhagic pneumonia, while *Salmonella* mostly causes gastrointestinal complications. Accessory control regulator has highly studied QS among gram-positive organisms (Novick 2003), and it is a regulator of many virulent genes, such as hemolysins, enterotoxins, different proteins, and toxins. Strikingly, *Pseudomonas* sp. regulates its virulence factor by Las and Rh1QS mechanism (Girard and Bloemberg 2008). However, *Salmonella* QS system is composed of LuxR homolog and *SdiA* (Walters and Sperandio 2006).

Antibiotic resistance (AR) is a devastating problem if we take a closer look at the response of virulent organisms to existing antibiotics; the rate at which these microbes are developing resistant is quite alarming (Dellit et al. 2007). It is pertinent that the selection pressures induced due to the incorporation of drugs into the environment while targeting, these drugs are deemed as vital targets to bacterial metabolism. AR genes can spread rapidly to biofilms, may perhaps endure longer in the biofilms, and act as reservoirs for AR genes. Consequently, the exploitation of antibiotics to treat biofilm-associated bacterial infections are expected to hasten the expansion and spread of AR in bacteria (Ceri et al. 2010). This practice has lost effectively of many potential drugs; however, total numbers of available drugs for the treatment of such pathogenic organisms are limited now (Fernandes 2006; Dellit et al. 2007). However, the field of therapeutics is a burning need for designing novel antibacterial therapies with the aim to impair bacterial pathogenesis while preventing the generation of new drug-resistant strains. QS inhibition-based approach can be one of such antivirulence or antipathogenesis approach rather than providing bactericidal effects. The current review article, however, summarizes the current knowledge about the development of new strategies and anti-quorum sensing approaches for the treatment of human and veterinary diseases.

QS systems are conscientious for the expression of many bacterial virulence factors; hence, disruption or blocking of density-dependent communication between virulent pathogens is an effective way to interrupt cooperatively. Knowledge earned from last 45 years about the social behavior of pathogenic organisms during induction and progression of the disease is used to inhibit QS at different levels (LaSarre and Federle 2013). Additionally, QS inhibition unlikely affects the crucial processes of bacterial growth. Hence, disruption of QS, therefore, does not exert antibiotic-associated selection pressure (Rasko and Sperandio 2010). If QS is an essential task in bacterial life cycle, then there may be possibilities of development of anti-QS drug-resistant strains, keeping mind this could help us for promoting long-term efficacy of anti-QS drugs (LaSarre and Federle 2013). It is noteworthy that for some organisms, living in mixed consortia would provide protection; if these efforts are blocked, theoretically bacteria would be unable to mount an attack; on the other hand, bacteria become susceptible for any given antibiotic range. Hence, the study of QS in pathogenic organisms and the discovery of new anti-QS drugs will undeniably enhance the availability of drugs useful for human and veterinary treatment.

7.7 QS Inhibition in In Vitro

Ideally, QS inhibitor should be small in size, chemically stable, narrow range of action, and nontoxic to eukaryotes. There are several strategies to prevent and inhibit biofilm formation. These strategies include the prevention of microbial attachment, prevention of microbial growth, disruption of cell-to-cell communication, inhibition of matrix synthesis, and disintegration of the biofilm matrix (Anderson and O'Toole 2008).

(a) *Inhibition of QS Inducer Production*

Quite a few studies have been performed yet; therefore, the field is open for exploration. Naturally occurring quorum sensing inhibitor (QSI) is in large number but due to low amount availability and associated toxic effects made us to search chemically synthesized QSI. QS signal analogs like 3-oxy-acyl carrier protein, butyryl-S-adenosylmethionine, L/D-S-adenosylhomocysteine, and sinefungin have the ability to block AHL synthase production. But in vivo studies have not been performed as they also affect amino acid and fatty acid biosynthesis. A molecule targeting AHL synthase has been recently studied by Chung et al. (2011).

Another potential target is Methylthioadenosine/s-adenosylhomocysteine nucleosidase enzyme (MTAN). This enzyme is conserved in bacterial species only and involved in the biosynthesis of AI-1 and AI-2. Analog of MTA could be used without affecting eukaryotic cellular metabolism. Furthermore plethora of studies have reported the ability of sulfur-free and sulfur-containing transition state analogs as potent inhibitors for *E. coli* MTAN and can be further studied for drug design to arise effective analogs (Scutera et al. 2014). Anthranilate is a precursor molecule for *pseudomonas* QS signal known as pseudomonas quinolone signal (PQS). Calfee et al. (2001) studied methyl anthranilate effectively which inhibits the production of PQS. However, *P. aeruginosa* PQS can activate the genes for both LasB elastase (lasB) and the C4-HSLsynthase (rhlI) in *P. aeruginosa.* This signal was also shown to be part of the quorum-sensing hierarchy in *P. aeruginosa.* N-(3-oxododecanoyl)-L-homoserine lactone and N-butyryl-L-homoserine lactone acts as intercellular signals. These two signaling systems can be effectively regulated by decreasing transcriptional activation of *las* and *pqs* genes (Zhou et al. 2013); together, these therapies may provide a new remedy for many *Pseudomonas* infections.

1. *Targeting QS Signaling Molecule*

Decreasing concentration of QS signaling molecule will automatically shut down QS and connected expression of virulent genes thereby pathogenesis. As QS signal is secreted out of the cells, they are very easy to target. Inactivation of the signal is achieved by various methods. Commonly, enzymatic degradation or antibody-mediated depletion is popular and easy for study.

(a) *Enzymes*

Broadly QSI enzymes are categorized into two types: oxidoreductases which reduce carbonyl to a hydroxyl group and AL-lactonase, AHL acylase, and paraoxonase which cleave the AHLs. Till date, three oxidoreductases have been identified. CYP102A1 from *Bacillus megaterium* can oxidize both acyl homoserine lactone and acyl homoserine. W2 from *Rhodococcus erythropolis* is the enzyme having a dual activity of oxidoreduction along with amylase activity (Uroz et al. 2005; Chowdhary et al. 2007). Recently identified BpiB09 through metagenomics screening can inactivate 3-oxo-c12-homoserine lactone (Bijtenhoorn et al. 2011). Practically, 20 AHL lactonases are known and can be used for disruption of QS signaling molecule. A good example is PvdQ enzyme belonging to nucleotide hydrolase super family. This enzyme produced by *P. aeruginosa* PA01 strain explains it might be a strategy to inhibit its own QS. Overexpression studies showed inhibitory activity (Papaioannou et al. 2009) and successive production of stable powder formulation which is further used in treatment purpose (Yang et al. 2005).

(b) *Antibodies*

QS signaling molecules are relatively smaller in size; therefore, eukaryote is unable to mount an antibody response; in return, these molecules can elicit apoptosis and modulation of NF-kB activity. In an earlier work on an antibody generation against synthetic 3-oxo-AHL, RS2 was found effective on long side chain AHL but not on short chain AHLs. Vaccination strategy for AHL-carrier conjugate in mice model for lung infection showed a positive result with increased progression of disease (Miyairi et al. 2006). Subsequently, the study by Park et al. highlighted generation of the immune response against a lethal challenge of *S. aureus* with the usage of a monoclonal antibody against (AIP)-4. This encouraged the descending investigators to generate the monoclonal antibody. Palliyil et al. generated a monoclonal antibody against *P. aeruginosa* by phage display library construct preparation and screened with 1000 times more affinity (Palliyil et al. 2014).

2. *Targeting Signal Detection/Receptor*

Targeting signal detection/receptor is done via either of two methods.

(a) *Natural Analogs*

Natural compounds are very complex in nature with differential actions, which sometimes is difficult to design in the lab. The only difficulty with natural compounds is to get in bulk (LaSarre and Federle 2013). In gram-negative organisms, widely used reporter strain for measuring inhibitory activity is *C. violaceum* and *V. fischeri* (Rasmussen et al. 2005; McClean et al. 1997).

(b) *Furanones*

Brominated furanones were the first recognized small natural molecules as quorum sensing inhibitors (QSI); other forms include halogenated and chlorinated furanones, with the only concern being toxicity to the hosts. They have been mainly isolated from diverse sources including plants, marine samples, algae, fungi, bacteria, etc. proven to have inhibitory activity against gram-negative and gram-positive organisms. The following furanone molecules, C-56 (Rasch et al. 2004; Wu et al. 2004), C-30 (Hentzer et al. (2002); DC-917 inhibits growth of mouse lung carcinoma), C-2 (Ren et al. 2004) are extensively studied in pathogenic organisms, viz., *P. aeruginosa*, *V. harveyi*, and *V. campbellii*. *In vivo* studies in a mouse model showed no any toxic effects; but still, these compounds need to undergo clinical trials.

(c) *Compounds Other Than Furanones*

Due to the aquatic environment, these compounds generally occur in low concentration from sponges, microalgae, coral-associated bacteria, and cyanobacteria (LaSarre and Federle 2013). They can act on AHL as well as on AL2-based organisms. *V. harveyi*, *C. violaceum*, and *E. coli* QS can effectively inhibit without hampering growth (Skindersoe et al. 2008; Teasdale et al. 2009; Dobretsov et al. 2010). Surprisingly, these compounds also have shown to inhibit the inflammatory responses of macrophages via inhibiting NF-kB signaling. Halogenated derivatives showed potent activity than original compound (Kravchenko et al. 2008) (Table 7.1).

(d) *Peptide-Based Inhibitors*

These compounds are produced by a diverse range of microorganisms including *Actinomycetes*. Siamycin, diketopiperazines, cyclo[L-Tyr-(L or D)-Pro], and cyclo(L-Phe-L-Pro) are the cyclic peptides identified to have anti-QS activity; they have been tested for inhibition of gelatinase, secretary protease, and arginase production from *E. faecalis* and arginase-producing organisms. These natural compounds need to be isolated and studied for the mechanism of action; it may open

Table 7.1 Synthetic QS inhibitor compounds and their targets

Category	Synthetic inhibitor	Target(s)
Synthase inhibitors	Compound 10	LuxS
	pCIPhT–DADMe–ImmA	MTAN
	JA-C8	TofI (LuxI family, *B. glumae*)
Receptor inhibitors	trAIP-II	AgrC
	CTL, CL	CviR
	Compounds 19 and 20	PqsR
	Itc-11, -12	LasR
	TP-5	LasR
	4606-423	LuxN, CviR

new avenues for the study of QS inhibition and its use as a diagnostic tool for human and animal diseases. Recently, one group has tested the anti-QS effectiveness of essential oils and a combination of it. Results showed appreciable anti-QS and biosurfactant activity; therefore, employing such compounds is practical in biofilm-forming pathogens as well (Mukherji and Prabhune 2014). Use of these quenching agents is done successfully in controlling some diseases. Harmful fish pathogen *A. hydrophila* infection is reduced upon carp feeding on AiiA or recombinant *Bacillus* which can able to produce AiiA (Chen et al. 2010). But still, for approved medicinal use, anti-QS medicines need to undergo extensive clinical trials.

(e) *Fatty Acid-Based Inhibitors*

Numerous examples of fatty acid-based QS inhibitors capable of meddling with biofilm formation have been reported in recent times. Davies and Marques (2009) depicted a small messenger fatty acid molecule produced by *P. aeruginosa*, cis-2-decenoic acid, competent of inhibiting biofilm development. One of the marine natural product derivative bromoageliferin also named TAGE (transbromoageliferin analog) depicted antibiofilm activity against *P. aeruginosa* (Huigens et al. 2008; Truchado et al. 2009) evidenced that chestnut honey and its aqueous extract were evidenced as QS inhibitors. These two compounds also appreciably reduced the biofilm formation in *Y. enterocolitica* and *A. hydrophila*. The cathelicidin LL-37 was revealed to hinder *F. novicida* biofilm formation at subinhibitory concentrations (Amer et al. 2010).

(f) *Synthetic Compounds*

Approach to synthetic compounds is a best-studied strategy for inhibiting the QS signals perceived by the bacteria. The synthetic analogs and antagonists act by blocking or destructing the receptor–ligands interaction and downstream signaling. Computer-aided drug designing screening of small molecules also increased the range of QS systems agonists and antagonist which destructs the QS signaling. Agonists and antagonists of AHL receptors, AL-2 antagonists, and peptide analogs are currently in use. Synthetic AHL analogs are synthesized from natural AHL molecules by modifying the length, saturations, and oxidation states of natural AHL compounds. Among different types of QS molecules, AHL receptors like Lux-R, LasR, and TraR interactions are most studied with synthetic analogs (LaSarre and Federle 2013) (Table 7.1).

7.8 Future Perspectives and Conclusions

Research on biofilms is therefore an area of extreme curiosity (Haussler and Parsek 2010) and thus gained appreciation in animal and public health. Researchers now have access to a wide battery of techniques together with 3D imaging, advanced fluorescent stains, confocal laser scanning microscopy (CLSM), and molecular

reporter gene technology to research on biofilms. During the recent developments, next-generation antimicrobial compounds, are of serious concern ought to the synergies between antibiotics and molecules (e.g., enzymes, biocides, surfactants, metals, or QS inhibitors) for the treatment of biofilms (Ceri et al. 2010). There is an impending prospective for augmented resistance to antibiotics, disinfectants, and host immune response. This interferes with the effective treatment of animals and human subjects. The persistence of antibiotic resistance genes within biofilms is an additional aspect that isn't a surprise, for the reason that it has a probable impact on animal and public health equally. Further research is also obligatory to expand successful disinfection protocols to get rid of biofilms from the human and veterinary environments, since biofilms can serve as a reservoir for any given infectious agents (Clutterbuck et al. 2007). Ever since the technological advancements increased the rate of discovery of new QS signaling organism, molecules, receptors, and understanding of mechanism behind it. But the field is still evolving, while the targeting QS restricts the virulence properties of pathogenic organisms without affecting growth; study of anti-QS strategies is of much interest. Use of many anti-QS approaches in different gram-negative and gram-positive reporter strains showed effective results in in vitro and in few in vitro case studies. The field needs further computational studies to develop better compounds and clinical studies for testing the effectiveness of anti-QS compounds for disease treatment. In future disease, therapy will be common to use anti-QS drugs in combination to microbicidal drugs in veterinary and human medicine world.

Acknowledgments The authors are grateful to Krishna University, Machilipatnam, NIV Pune and NCCS Pune for the support extended.

Conflict of Interest The authors declare that they have no competing interests.

References

Albuquerque P, Casadevall A (2012) Quorum sensing in fungi–a review. Med Mycol 50(4):337–345

Amer LS, Bishop BM, van Hoek ML (2010) Antimicrobial and antibiofilm activity of cathelicidins and short, synthetic peptides against Francisella. Biochem Biophys Res Commun 396:246

Anderson GG, O'toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. In: Bacterial biofilms. Springer, Berlin, Heidelberg, pp 85–105

Barber CE et al (1997) A novel regulatory system required for pathogenicity of Xanthomonas campestris is mediated by a small diffusible signal molecule. Mol Microbiol 24(3):555–566

Bijtenhoorn P et al (2011) A novel metagenomic short-chain dehydrogenase/reductase attenuates Pseudomonas aeruginosa biofilm formation and virulence on Caenorhabditis elegans. PLoS One 6(10):e26278

Boyen F et al (2009) Quorum sensing in veterinary pathogens: mechanisms, clinical importance and future perspectives. Vet Microbiol 135(3–4):187–195

Buzzola FR et al (2007) Differential abilities of capsulated and noncapsulated Staphylococcus aureus isolates from diverse agr groups to invade mammary epithelial cells. Infect Immun 75(2):886–891

Calfee MW, Coleman JP, Pesci EC (2001) Interference with Pseudomonas quinolone signal synthesis inhibits virulence factor expression by Pseudomonas aeruginosa. Proc Natl Acad Sci 98(20):11633–11637
Ceri H, Olson ME, Turner RJ (2010) Needed, new paradigms in antibiotic development. Expert Opin Pharmacother 11:1233–1237
Chen R et al (2010) High yield expression of an AHL-lactonase from Bacillus sp. B546 in Pichia pastoris and its application to reduce Aeromonas hydrophila mortality in aquaculture. Microb Cell Factories 9(1):39
Chowdhary PK et al (2007) Bacillus megaterium CYP102A1 oxidation of acyl homoserine lactones and acyl homoserines. Biochemistry 46(50):14429–14437
Chung J et al (2011) Small-molecule inhibitor binding to an N-acyl-homoserine lactone synthase. Proc Natl Acad Sci 108(29):12089–12094
Clutterbuck AL, Woods EJ, Knottenbelt DC, Clegg PD, Cochrane CA, Percival SL (2007) Biofilms and their relevance to veterinary medicine. Vet Microbiol 121:1–17
Davies DG, Marques CNH (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 191:1393–1403
Dellit TH et al (2007) Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. Clin Infect Dis 44(2):159–177
Dobretsov S et al (2010) Malyngolide from the cyanobacterium Lyngbya majuscula interferes with quorum sensing circuitry. Environ Microbiol Rep 2(6):739–744
Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
dos Reis Ponce A et al (2012) AiiA quorum-sensing quenching controls proteolytic activity and biofilm formation by Enterobacter cloacae. Curr Microbiol 65(6):758–763
Dunny GM, Brown BL, Clewell DB (1978) Induced cell aggregation and mating in Streptococcus faecalis: evidence for a bacterial sex pheromone. Proc Natl Acad Sci 75(7):3479–3483
Dworkin M, Kaiser D (1985) Cell interactions in myxobacterial growth and development. Science 230(4721):18–24
Erez Z et al (2017) Communication between viruses guides lysis–lysogeny decisions. Nature 541(7638):488
Fernandes P (2006) Antibacterial discovery and development—the failure of success? Nat Biotechnol 24(12):1497
Fong KP et al (2001) Intra- and interspecies regulation of gene expression by Actinobacillus actinomycetemcomitans LuxS. Infect Immun 69(12):7625–7634
Fuqua WC, Winans SC, Peter Greenberg E (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176(2):269
Girard G, Bloemberg GV (2008) Central role of quorum sensing in regulating the production of pathogenicity factors in Pseudomonas aeruginosa. Future Microbiol 3:97–106
Hall-Stoodley L, Stoodley P (2009) Evolving concepts in biofilm infections. Cell Microbiol 11:1034–1043
Haussler S, Parsek MR (2010) Biofilms 2009: new perspectives at the heart of surface-associated microbial communities. J Bacteriol 192:2941–2949
Hentzer M et al (2002) Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Microbiology 148(1):87–102
Huigens RW III, Ma L, Gambino C, Moeller PDR, Basso A, Cavanagh J, Wozniak DJ, Melander C (2008) Control of bacterial biofilms with marine alkaloid derivatives. Mol BioSyst 4:614–621
Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31(2):224–245
Kravchenko VV et al (2008) Modulation of gene expression via disruption of NF-κB signaling by a bacterial small molecule. Science 321(5886):259–263
LaSarre B, Federle MJ (2013) Exploiting quorum sensing to confuse bacterial pathogens. Microbiol Mol Biol Rev 77(1):73–111
Lemon KP, Earl AM, Vlamakis HC, Aguilar C, Kolter R (2008) Biofilm development with an emphasis on Bacillus subtilis. Curr Top Microbiol Immunol 322:1–16

Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22(4):582–610

McClean KH et al (1997) Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology 143(12):3703–3711

Miller MB et al (2002) Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae. Cell 110(3):303–314

Miyairi S et al (2006) Immunization with 3-oxododecanoyl-L-homoserine lactone–protein conjugate protects mice from lethal Pseudomonas aeruginosa lung infection. J Med Microbiol 55(10):1381–1387

Mukherji R, Prabhune A (2014) Novel glycolipids synthesized using plant essential oils and their application in quorum sensing inhibition and as antibiofilm agents. Sci World J 2014:890709

Nealson KH, Hastings JW (1979) Bacterial bioluminescence: its control and ecological significance. Microbiol Rev 43(4):496

Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48(6):1429–1449

Palliyil S et al (2014) High-sensitivity monoclonal antibodies specific for homoserine lactones protect mice from lethal Pseudomonas aeruginosa infections. Appl Environ Microbiol 80(2):462–469

Papaioannou E et al (2009) Quorum-quenching acylase reduces the virulence of Pseudomonas aeruginosa in a Caenorhabditis elegans infection model. Antimicrob Agents Chemother 53(11):4891–4897

Pinto UM et al (2007) Detection of acylated homoserine lactones in gram-negative proteolytic psychrotrophic bacteria isolated from cooled raw milk. Food Control 18(10):1322–1327

Rasch M et al (2004) An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (Oncorhynchus mykiss, Walbaum). Syst Appl Microbiol 27(3):350–359

Rasko DA, Sperandio V (2010) Anti-virulence strategies to combat bacteria-mediated disease. Nat Rev Drug Discov 9(2):117

Rasmussen TB et al (2005) Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. J Bacteriol 187(5):1799–1814

Ren D et al (2004) Differential gene expression shows natural brominated furanones interfere with the autoinducer-2 bacterial signaling system of Escherichia coli. Biotechnol Bioeng 88(5):630–642

Russo TA et al (2011) Hypervirulent K. pneumoniae secretes more and more active iron-acquisition molecules than “classical” K. pneumoniae thereby enhancing its virulence. PLoS One 6(10):e26734

Scutera S, Zucca M, Savoia D (2014) Novel approaches for the design and discovery of quorum-sensing inhibitors. Expert Opin Drug Discovery 9(4):353–366

Shankar M et al (2013) Inactivation of the transcriptional regulator-encoding gene sdiA enhances rice root colonization and biofilm formation in Enterobacter cloacae GS1. J Bacteriol 195(1):39–45

Skindersoe ME et al (2008) Quorum sensing antagonism from marine organisms. Mar Biotechnol 10(1):56–63

Sprague GF, Winans SC (2006) Eukaryotes learn how to count: quorum sensing by yeast. Genes Dev 20(9):1045–1049

Tay S, Yew W (2013) Development of quorum-based anti-virulence therapeutics targeting Gram-negative bacterial pathogens. Int J Mol Sci 14(8):16570–16599

Teasdale ME et al (2009) Secondary metabolites produced by the marine bacterium Halobacillus salinus that inhibit quorum sensing-controlled phenotypes in gram-negative bacteria. Appl Environ Microbiol 75(3):567–572

Truchado P, Gil-Izquierdo A, Tomas-Barberan F, Allende A (2009) Inhibition by chestnut honey of N-acyl-L-homoserine lactones and biofilm formation in Erwinia carotovora, Yersinia enterocolitica, and Aeromonas hydrophila. J Agric Food Chem 57:11186–11193

Uroz S et al (2005) N-Acylhomoserine lactone quorum-sensing molecules are modified and degraded by Rhodococcus erythropolis W2 by both amidolytic and novel oxidoreductase activities. Microbiology 151(10):3313–3322

Vancraeynest D et al (2006) International dissemination of a high virulence rabbit Staphylococcus aureus clone. J Veterinary Med Ser B 53(9):418–422

Walters M, Sperandio V (2006) Quorum sensing in Escherichia coli and Salmonella. Int J Med Microbiol 296(2–3):125–131

Wu H et al (2004) Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in Pseudomonas aeruginosa lung infection in mice. J Antimicrob Chemother 53(6):1054–1061

Xavier KB, Bassler BL (2003) LuxS quorum sensing: more than just a numbers game. Curr Opin Microbiol 6(2):191–197

Yang F et al (2005) Quorum quenching enzyme activity is widely conserved in the sera of mammalian species. FEBS Lett 579(17):3713–3717

Zhou L et al (2013) Eugenol inhibits quorum sensing at sub-inhibitory concentrations. Biotechnol Lett 35(4):631–637

Zhu H et al (2011) A luxS-dependent transcript profile of cell-to-cell communication in Klebsiella pneumoniae. Mol BioSyst 7(11):3164–3168

8 Quorum Sensing and Multidrug Resistance Mechanism in *Helicobacter pylori*

Surekha Challa, Titash Dutta, Pallaval Veera Bramhachari, and Neelapu Nageswara Rao Reddy

Abstract

Antibiotics are integral components of medicine and therapy for majority of diseases. Despite the advancements in medical science, the therapeutic efficiency of major antibiotics is gradually decreasing due to increased antibiotic resistance in most of the bacterial species. The mechanisms involved in developing antibiotic resistance include modification of antibiotic molecules, reducing drug permeability, modification of target binding sites and biofilm formation. Nevertheless, reducing drug permeability and formation of biofilm are known to contribute antibiotic resistance in *Helicobacter pylori*. Further significant research is essential in improving the efficiency of eliminating bacterial infections. Therefore, this review contemplates on understanding the mechanisms involved in developing antibiotic resistance in pathogens and *H. pylori*.

Keywords

Antibiotics · *H. pylori* · Quorum sensing · MDR · Autoinducer-2(AI-2) · Efflux pumps

S. Challa · T. Dutta · N. N. Rao Reddy (✉)
Department of Biochemistry and Bioinformatics, Institute of Science, Gandhi Institute of Technology and Management (GITAM) (Deemed-to-be University), Visakhapatnam, Andhra Pradesh, India
e-mail: nrneelapu@gmail.com

P. V. Bramhachari
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_8

8.1 Introduction

Antibiotics gradually developed as indispensable components of modern medicine and therapy. They are used in majority of medical treatments ranging from surgery to cancer therapy. However, the therapeutic efficiency of the widely used antibiotics is under constant threat from the evolving antibiotic resistance in bacterial species. According to recent statistics claimed by World Health Organisation (WHO), antibiotic resistance significantly augmented the mortality rates and it is rated as the most potential threat to public health (World Health Organization 2014). Moreover, pathogens have evolved intricate resistance mechanisms with time and have therefore interfered in the mode of actions thereby rendering them ineffective. Therefore, it stands necessary to perceive the changes at the molecular level of antibiotics leading to resistance (Figs. 8.1 and 8.2). The different mechanisms providing resistance to antibiotics are (a) modification of the antibiotic molecule, (b) reduction in drug permeability, (c) modification of target binding sites and (d) formation of biofilm. Thus, the current review would provide insights in understanding the mechanism of antibiotic resistance in pathogens along with *H. pylori*.

The apparent process concerned with modification of the antibiotic molecules is either alteration or destruction. In the first case, structure of the antibiotic molecule is modified, whereas in the second case, the antibiotic molecule is destroyed. Few examples for resistance acquired via chemical alteration of antibiotic molecules include aminoglycoside and chloramphenicol catalysed by the aminoglycoside-modifying enzymes (AME) and chloramphenicol acetyltransferases (CAT), respectively (Munita and Arias 2016). However, antibiotic β-lactam is an example for antibiotic molecule which acquires resistance via destruction. The efficiency of antibiotic β-lactam is significantly affected with the emergent resistance shown in certain bacteria, like *Salmonella enterica* and *S. aureus* (Chuma et al. 2013; Bush 2013). Interestingly, the bacterial species have acquired genes coding enzyme β-lactamase which hydrolyses β-lactam ring of penicillins and cephalosporins. The enzyme hydrolysis destroys the antibiotic molecules either by using a Ser nucleophile, or by activation of water molecule mediated by a Zn^{2+} centre. Literature has reported antibiotic resistance to "cell wall inhibitors (e.g., β-lactams and glycopeptide agents), protein synthesis inhibitors (macrolides and tetracyclines), nucleic acid synthesis inhibitors (fluoroquinolones and rifampin), inhibitors of specific metabolic pathway (trimethoprim-sulfamethoxazole), and bacterial membrane structure denaturing agents (polymyxins and daptomycin)" (Tenover 2006). All these inhibitors tote up to resistance of antibiotics by modifying the structure of the antibiotic molecule.

Another important resistance mechanism includes decreasing the permeability to antibiotic molecules or the usage of efflux pumps for extruding the antibiotic molecules before it reaches target site, thereby mitigating its effect. Nevertheless, a rapid decrease in the permeability to antibiotic is possible when there is an alteration of porins leading to changes in the permeability of membrane. Resistance has been developed for a wide range of antimicrobial agents that include tetracyclines,

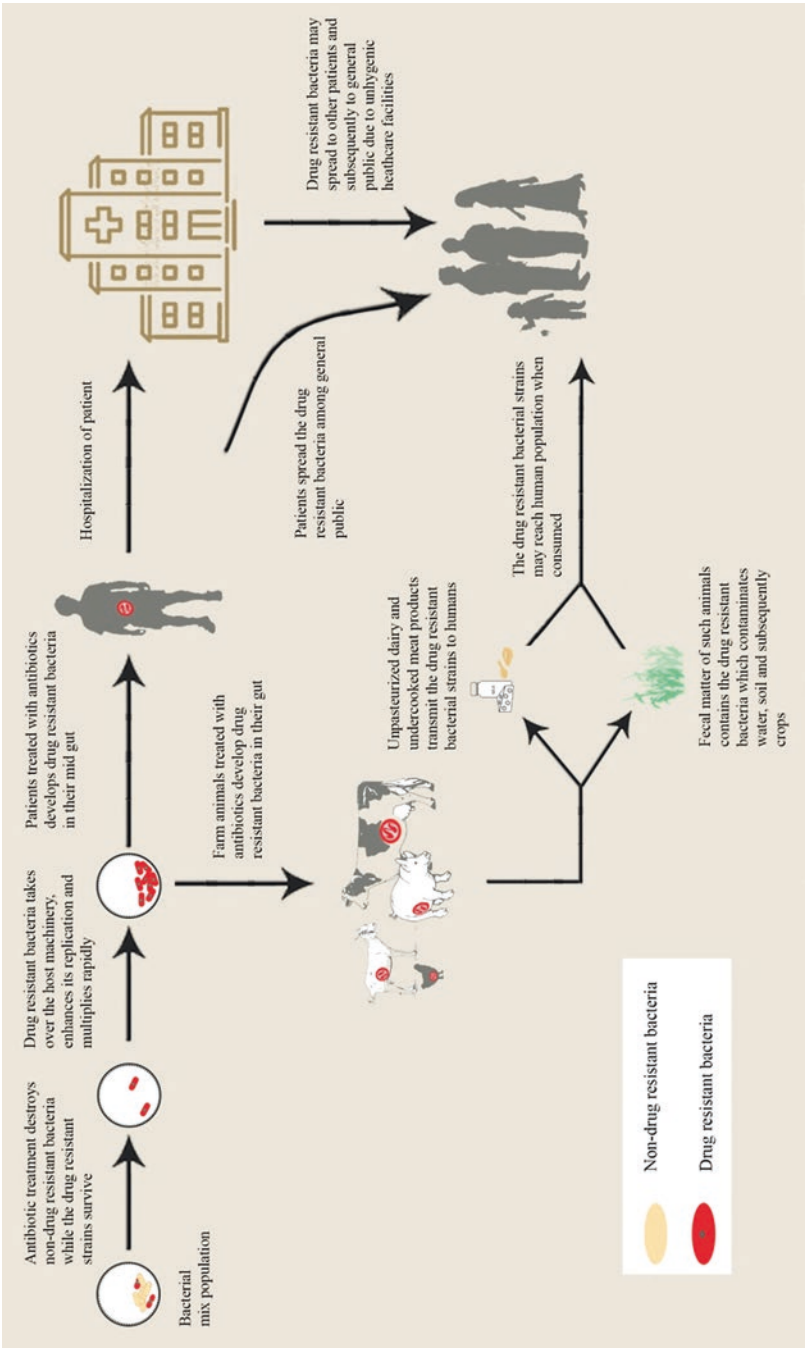


Fig. 8.1 Development and spread of antibiotic resistance

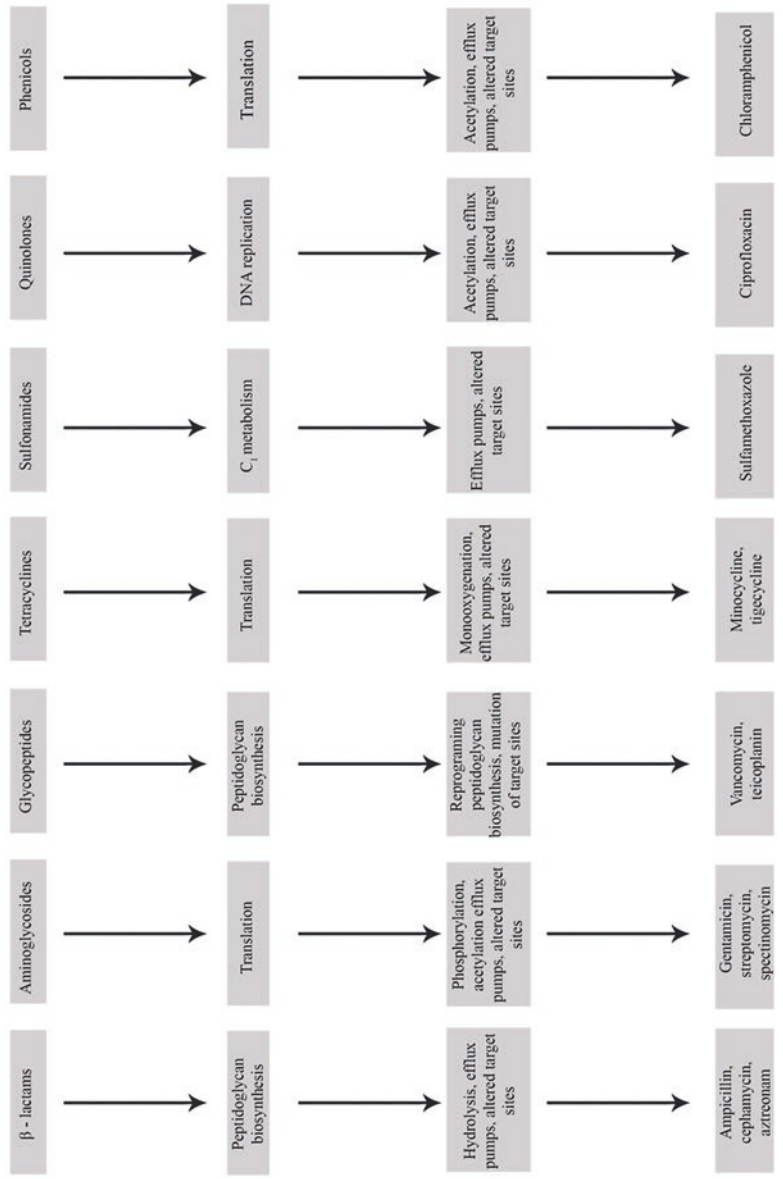


Fig. 8.2 Antibiotics, class, target sites and the mechanism of antibiotic resistance

β-lactams, fluoroquinolones and vancomycin. The decrease in permeability leading to antibiotic resistance is studied and documented in *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Salmonella* spp., *Neisseria gonorrhoeae* and *Klebsiella pneumonia* (Tenover 2006).

The "efflux pumps are present in Gram positive bacteria, Gram negative bacteria as well as in eukaryotic organisms. The pumps are equipped to either extrude specific antibiotic molecules or structurally dissimilar compounds (including antibiotics of different classes) leading to Multiple Drug Resistance (MDR)" (Webber and Piddock 2003). The efflux pumps are divided into five major families, namely, "major facilitator family (MF), multidrug and toxic efflux family (MATE), resistance-nodulation-division family (RND), small multi drug resistance family (SMS) and ATP binding cassette family (ABC)" (Webber and Piddock 2003). All these pumps employ proton motive force for extruding the antibiotic molecules, whereas the pumps of ABC family use ATP as its energy source for extruding the antibiotic molecules (Webber and Piddock 2003). Resistance is developed across-the-board of antimicrobial agents like β-lactams (some), chloramphenicol, fluoroquinolones and fusidic acid, novobiocin and tetracyclines. In addition, they also exude harmful compounds like bile salts, cationic dyes and disinfectants. The important efflux systems that lead to antibiotic resistance for many clinically important antimicrobial agents have been studied and documented in "*Campylobacter jejuni*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Salmonella typhimurium* and *Staphylococcus aureus*" (Webber and Piddock 2003; Lee et al. 2000). In-depth investigation is carried out to study the MDR in *P. aeruginosa* and *H. pylori*; vancomycin resistance in *S. aureus*; and cephalosporins resistance in *E. coli*.

Other mechanisms by which bacteria develop antibiotic resistance include changes in the binding sites of antibiotics. Alterations in the binding sites prevent binding of the antibacterial agents and its further effect (Munita and Arias 2016). The probable mechanisms responsible for change in the sites are (a) protection of the target site and (b) modification of the target site. Pathogens encode molecules or protein which dislodge, release and prevent rebinding of the antibiotic to protect the target and its binding site. The examples of resistance determinants studied and documented for protection of target site are quinolone resistance protein (Qnr), fusidic acid resistance proteins (FusB and FusC) and tetracycline resistance determinants Tet(M) and Tet(O). Modification of the target site is possible with the following mechanisms: "a) mutations in the genes encoding the target site (e.g. rifampin (RIF) resistance), b) enzymatic alterations in the binding site (e.g. addition of methyl groups), and c) replacement or bypass of the original target" (Munita and Arias 2016).

Biofilm formation (cell aggregates) is another mechanism by which bacteria develop antibiotic resistance. Bacteria in biofilm participate in multicellular lifestyle to survive even in unfavourable environmental conditions. The formation and maturation of biofilm involves stages which are irreversible and reversible; and are preserved amongst species with definite bacterial properties. Stages include bacterial adherence, biofilm maturation and dispersing. Bacterial adherence is influenced by motility, chemotaxis, adhesions and adhesive appendages. Biofilm maturation is maintained by the products shared; and dispersal is influenced by availability of

nutrients and oxygen, stress and toxic products (Sauer et al. 2004; Karatan and Watnick 2009; Hong et al. 2010; Rowe et al. 2010; Challa et al. 2018). Bacteria forming biofilm are "always exposed to sub-lethal doses of antibiotics and it was also suggested that in soft tissues like intestine, and lungs, bacteria might be exposed to sub-minimum inhibitory concentration (MIC) levels influencing the evolution of antibiotic resistance" (Cammarota et al. 2010). Bacterial cells in biofilm are less sensitive to many antibiotics and may develop antibiotic tolerance without any genetic changes (Song et al. 2016). "Biofilms exposed to sub-lethal concentration of antibiotics show several phenotypic changes such as biofilm structure, cell morphology, growth rate, induction of extracellular DNA and bacterial membrane vesicles. The inability of antibiotics to penetrate through the biofilm is one of the important factors for antibiotic tolerance. The rate of penetration of antibiotics into biofilm is dependent on bacterial species in the biofilm and the antibiotic used". The rate of penetration of antibiotics varies with the type of antibiotics used. Addition of norspermidine and norspermine improved antibiotic penetration and efficacy (Kolodkin-Gal et al. 2012; Siala et al. 2014). Various molecular mechanisms are employed by bacteria that lead to development of "biofilm-based antibiotic resistance and tolerance in pathogenic bacteria" (Hall and Mah 2017) (Table 8.1). In order to counter the growing bacterial resistance to antibiotics, the intricate mechanisms, which are involved, should be thoroughly studied to develop strategies aimed at increasing the efficacy and shelf-life of the antibiotic molecules.

8.2 *Helicobacter pylori* Pathogenesis and Immune Evasion

Helicobacter pylori was found in human adenoid tissue and tonsil, dental plaque, oral lesions, saliva and stomach. *H. pylori* was known for causing gastrointestinal disorders like gastritis, ulcers and gastric cancer (Neelapu et al. 2014; Neelapu 2018). Sometimes, *H. pylori* may trigger some diseases like laryngitis and glossitis, pharyngitis, otitis and sinusitis (Kurtaran et al. 2008). *H. pylori* "colonizes the stomach using chemoreceptor TlpB and enzyme urease (Sweeney et al. 2012; Wen et al. 2003). Toxin VacA, gastrin and cytokines of *H. pylori* destabilize the barrier of gastric mucosa (Neelapu et al. 2014). Later, *H. pylori* use adhesion molecules like AlpA, AlpB, BabA, HopZ, OipA and SabA and cohere to the stomach lining (Petersen and Krogfelt 2003; Ilver et al. 1998; Mahdavi et al. 2002; Moodley et al. 2009). Cytotoxin associated gene pathogenicity island (cag PAI) codes for T4SS system that helps in injecting CagA, VacA and peptidoglycan into the host bringing changes in the host cell…" (Yamaoka 2010). People infected with *H. pylori* showed an increase in activated dendritic cells (DCs) and macrophages in the gastric mucosa. B cells, DCs and macrophages forming the antigen-presenting cells (APC) internalize and process antigen. However, this antigen is presented via class II MHC molecules to CD4+ T cells leading to initiation of T cell response which is antigen-specific. Interleukins and tumor necrosis factor are produced by activated macrophages causing inflammation (gastritis). IL-6, IL-1β and IL-12 are the interleukins produced. Further, inflammation is prolonged and augmented leading to ulcers and

Table 8.1 Antibiotic resistance and tolerance in bacteria of biofilm

S. no.	Organisms	Tolerant genes	Resistance to antibiotics	References
1	*Pseudomonas aeruginosa*	*brlR*, *sags*, *ndvB*, *exaA*, *pqqC*, *erbR*, *PA1875-1877*, *tssC1*, *hcp1*, *PA0756-0757*, *PA2070*, *PA5033*, *pslABCDEFGHIJKLMNO*, *pelABCDEFG*, *relA*, *spot*	Tobramycin, norfloxacin, trimethoprim, tetracycline, kanamycin, chloramphenicol, gentamicin, ciprofloxacin, colistin, polymyxin B, ofloxacin, meropenem	Liao and Sauer (2012), Gupta et al. (2013, 2014), Liao et al (2013), Chambers et al. (2014), Petrova and Sauer (2011), Gupta et al. (2013, 2014), Mah et al. (2003), Sadovskaya et al. (2010), Beaudoin et al. (2012), Zhang and Mah (2008), Zhang et al. (2011, 2013), Billings et al. (2013), Colvin et al. (2012), Nguyen et al. (2011), and Khakimova et al. (2013).
2	*Escherichia coli*	*rapA*, *yafQ*	Penicillin G, norfloxacin, chloramphenicol Tobramycin, cefazolin	Lynch et al. (2007) and Harrison et al. (2009)
3	*Enterococcus faecalis*	*epaOX*, *epaI*, *gelE*, *fsrA*, *fsrC*	Gentamicin Daptomycin Linezolid	Dale et al. (2015)
4	*Streptococcus mutans*	*dltABCD*	Gentamicin	Nilsson et al. (2016)

gastric cancer (Neelapu et al. 2014; Neelapu 2018; Johnson and Ottemann 2018). *H. pylori* adapts a number of immune evasion strategies to protect itself from immune system of the host. The strategies adapted by *H. pylori* to evade immune system are hindering the perception of the innate immunity, averting the actual T cell response, modulating adaptive immunity and avoiding humoral response. Hindering the perception of the innate immunity is possible by evading the receptors of immune recognition, inhibiting phagocytosis and inhibiting the action of ROS and NO. Averting the actual T cell response is likely when proliferation and signalling of T cell is inhibited; and T cell response is skewed by *H. pylori* with respect to regulatory T cells. Functions of APC are modulated in adaptive immunity by inhibiting presentation of antigens and maturation of DC and its function, apoptosis of gastric epithelial cells and macrophages. The high genome diversity of *H. pylori* is also linked with evasion immune (Lina et al. 2014). Lewis et al. (2011) reported that

H. pylori uses macrophage arginase II to induce immune evasion. Apart from the mechanisms to evade immune system, *H. pylori* developed antibiotic resistance in individuals when initial treatment failed requiring additional rounds of antibiotics. Rising antibiotic resistance is due to efflux pumps or formation of biofilm or potential reinfection of *H. pylori* (Siddique et al. 2018). Therefore, understanding the efflux pumps or formation of biofilm in *H. pylori* infections would provide fundamental insight on the rising antibiotic resistance.

8.3 Drug Resistance in Pathogen *H. pylori*

Treating bacterial infections has become difficult as adaptability of bacteria and acquired resistance is increasing rapidly. MDR is common with pathogens in the gastrointestinal tract of human due to antibiotic treatment. *H. pylori* colonizing the stomach develops MDR by a very efficient method of gene transfer. *H. pylori* has developed resistance to proton-pump inhibitors (PPIs), clarithromycin, metronidazole, macrolide, amoxicillin, levofloxacin, etc. (Table 8.2). Triple therapy for *H. pylori* consists of clarithromycin, amoxicillin or metronidazole, proton-pump inhibitor. The combination has lost efficacy and the roles of rdxa, frxa and efflux pump were identified in metronidazole-resistant *H. pylori (*Lee et al. 2018*)*. The recommended second-line treatment for *H. pylori* is proton-pump inhibitors, levofloxacin and amoxicillin (Osaki et al. 2006).

MDR in *H. pylori* has significantly increased in the last decade and has affected the efficacy of several drug combinations (Torres et al. 2001; Gao et al. 2010; Sun et al. 2010; Bolor-Erdene et al. 2017). Torres et al. (2001) reported drug resistance to combination of drugs metronidazole, clarithromycin and amoxicillin. They quantified the resistance levels of 195 "*H. pylori* strains isolated from children and adults of Mexico" to a combination of three drugs, namely, metronidazole (80%), clarithromycin (24%) and amoxicillin (18%). Similar report on *H. pylori* strains in relation to MDR showed a sharp increase in the level of resistance to most of the drug combinations predominantly for metronidazole, clarithromycin and amoxicillin combinations (Gao et al. 2010). Similar "study was carried out to evaluate the resistance of *H. pylori* strains collected from patients in Shanghai to multiple drug combinations including metronidazole, clarithromycin, amoxicillin, furazolidone, levofloxcin and tetracycline" (Sun et al. 2010). Based on the minimal inhibitory concentration (MIC), *H. pylori* strains showed significant resistance to metronidazole/levofloxacin combination (43.1%) followed by metronidazole/clarithromycin combination (24.1%) (Sun et al. 2010). Another report showed MDR in *H. pylori* strains isolated from 320 patients residing in Mongolia for the drugs clarithromycin (35.5%), metronidazole (68.4%), erythromycin (28.2%), tetracycline (25%) and amoxicillin (23%), respectively (Torres et al. 2001).

In order to counter MDR and facilitate the eradication of *H. pylori* infections, many groups were successful in identifying new or alternative drug targets (Neelapu et al. 2013, 2015, 2016; Neelapu and Pavani 2013; Nammi et al. 2016, 2017; Pasupuleti et al. 2017) developing new drug combinations as well as targeting the

Table 8.2 Resistance to drugs or drug combinations used for treatment of *H. pylori* infections

S. no.	Antibiotic formulation	Resistance percent	References
1	Amoxicillin	28.3	Erdene et al. (2017)
2	Metronidazole	80	Torres et al. (2001)
3	Clarithromycin	24	Torres et al. (2001)
4	Amoxicillin	18	Torres et al. (2001)
5	Erythromycin	28.2	Erdene et al. (2017)
6	Clarithromycin + levofloxacin	1.6	Zullo et al. (2007)
7	Amoxicillin+ tetracycline	34.5	Erdene et al. (2017)
8	Metronidazole + clarithromycin + amoxicillin	8.7	Torres et al. (2001)
9	Clarithromycin + metronidazole + tetracycline	14.4	Erdene et al. (2017)
10	Proton-pump inhibitor + amoxicillin + metronidazole	57	Bardhan et al. (2001)
11	Ranitidine bismuth citrate + clarithromycin + metronidazole	66	Savarino et al. (1997)
12	Clarithromycin + metronidazole + tetracycline + erythromycin	6	Erdene et al. (2017)
13	Clarithromycin + metronidazole	4.9	Boyanova et al. (2009)
14	Clarithromycin + metronidazole + levofloxacin	15	Ndip et al. (2008)
15	Clarithromycin + metronidazole + rifabutin	0.08	Wueppenhorst et al. (2011)
16	Clarithromycin + metronidazole + levofloxacin + rifabutin	0.7	Wueppenhorst et al. (2011)
17	Clarithromycin + metronidazole + tetracycline + erythromycin + nitrofurantoin	5	Erdene et al. (2017)

candidate genes involved in suppressing the MDR. A novel drug combination of fluoroquinolones has been developed and was found to be effective in suppressing the MDR in susceptible *H. pylori* strains. Levofloxacin and moxifloxacin drugs, which belong to the class of fluoroquinolones, are used in combination with amoxicillin. They serve as proton-pump inhibitors (PPI) thereby preventing the extrusion of antibiotic molecules and allowing them to attach to their target site and perform a similar action. Similarly, combination of rifabutin and furazolidone is successful in reducing MDR in several *H. pylori* strains.

Several groups have identified the candidate genes involved in suppressing the MDR and targeted them (Liu et al. 2008; Huang et al. 2015). Liu et al. (2008) reported candidate genes involved in suppressing the MDR by observing that upregulation of the *hefA* gene in *H. pylori* increased MDR (Liu et al. 2008). When "*H. pylori* strains were treated with Chinese herbs namely emodin, baicalin, schizandrin and berberine", significant reduction in the MIC of tetracycline and amoxicillin drug combination (2 fold) was observed which was also correlated with decrease in mRNA levels of the *hefA* gene. Similarly, upregulation of another candidate gene for efflux pumps (*hp1165*) reduced MDR for tetracycline and amoxillicin drug combinations (Huang et al. 2015). Thus, these findings opened a novel facet to combat MDR in *H. pylori* strains by targeting specific genes encoding for efflux pumps.

8.4 Establishment of *H. pylori* Biofilm in Gastric Mucosa of Human

H. pylori persist as biofilm both in environment and gastric mucosa of human (Surekha and Neelapu 2018). *H. pylori* may exist and survive as biofilm in water distribution systems such as pond, river water, shallow ground water, sewage and well water in both developed and developing countries (Surekha and Neelapu 2018; Watson et al. 2004; Hegarty et al. 1999; Horiuchi et al. 2001; Imanishi et al. 2003; Lu et al. 2002; Moreno et al. 2003). Thus, the drinking water can be an important source for *H. pylori* infection. Carron et al. (2006) used "endoscopy, scanning electron microscopy to study the biopsy samples and established that *H. pylori* existed as biofilm in the gastric mucosa of human". Coticchia et al. (2006) reported "the presence and density of *H. pylori* biofilms in the gastric mucosa of human". Patients with peptic ulcer disease (i.e. urease positive for *H. pylori*) reported biofilms with 97.3%, whereas urease-negative patients reported biofilm with 1.64% for *H. pylori* establishing the fact that mature biofilms were present in the human gastric mucosa. Cammarota et al. (2010) reported that patients with more than four failures during therapy showed biofilms in the gastric mucosa, whereas biofilm ceased to exist in patients when the microbe was eliminated. Most of the "samples from *H. pylori*-positive patients showed coccid bacteria congregated in a microbial biofilm" (Cammarota et al. 2010). Cellini et al. (2008) harnessed the potential of SEM and identified the dominant S-shaped morphotype of *H. pylori* along with coccid aggregate in the biopsies of patients.

8.5 Quorum Sensing in *H. pylori*

H. pylori try to persist in the adverse environmental factors like immune system action, low pH, oxidative stress and antimicrobial substances by forming a protective structure biofilm. The cells in the biofilm have a communication system known as quorum sensing (QS) (Mohana Sheela et al. 2018; Neelapu et al. 2018; Whiteley et al. 2017; Gohil et al. 2018). The signalling molecules which are part of the communication system are known as autoinducers (AIs) (Sperandio et al. 2003; Zhao et al. 2018). AIs at a threshold concentration trigger signal transduction cascade. Signalling alters "various gene expressions related to biofilm formation, motility, secretion system, sporulation, and virulence factors" (Fuqua et al. 1994). AI system "plays an important role in formation of bacterial biofilm", and AI-1, AI-2, oligopeptides and diffusible signal factors (DSF) are the QS molecules which were well characterized (Fuqua et al. 2001). "N-acyl-L-homoserine lactone (N-AHL) or AI-1 signalling molecule" is species specific and is utilized by gram-negative bacteria for their activity (Fuqua et al. 2001). LuxS gene encodes furanosyl borate diesters (AI-2 signalling molecule) and "is utilized by both gram-positive and gram-negative bacterial species". Gram-positive bacteria also produce oligopeptides with a species-specific action. Autoinducer-2 is "designated as AI-2 and is produced from the precursor *S*-adenosylhomocysteine (SAH). Two enzymes namely, 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN) and LuxS (metalloenzyme) are required for this synthesis reaction. Interestingly these sequential reactions generate an intermediate 4,5-dihydroxy-2,3-pentanedione (DPD) which undergoes" conformational rearrangement to produce cyclic furanones that are classified as AI-2. The small size of AI-2 molecules facilitates their transport and diffusion through bacterial cell membranes and their extracellular accumulation. The new class of "quorum-sensing molecules which are involved in quorum-sensing activity are diffusible signal factors (DSFs) (Zhou et al. 2015). DSFs are a class of fatty acid derivatives that are present in gram negative bacteria and are involved in morphological transformation of *H. pylori*" (Krzyżek and Gościniak 2018).

H. pylori movement is triggered due to changes in environmental cues and "it uses a two component signal-transduction system for chemotaxis" (Zhou et al. 2015). It was identified that in *H. pylori* quorum sensing network is initiated by the chemoreceptor TlpB which helps in signal perception of the AI-2 as a chemorepellent. AI-2 and DSF of quorum sensing system were reported in *H. pylori* (Zhou et al. 2015). LuxS gene produces extracellular signalling molecules similar to AI-2 in *H. pylori* (Forsyth and Cover 2000; Joyce et al. 2000; Lee et al. 2006). Maximum production of "AI-2 is observed during mid-exponential phase of growth" (Doherty et al. 2010). The LuxS plays a pivotal role in methyl cycle and cell-to-cell signalling (Doherty et al. 2010). Another course of action for LuxS is controlling motility by modifying transcription and biosynthesis of flagella (Rader et al. 2011; Shen et al. 2010). Cole et al. (2004) described the relation between biofilm formation and luxS in *H. pylori*. It is noteworthy that LuxS mutants of SD3 and SD4 strains showed better formation of biofilm by twofold than the parental strains. However, Osaki et al. (2006) reported lower motility in luxS-deficient mutant TK1402 strain than

parental strains. In addition, luxS mutants in a Mongolian gerbil model showed evidence of reduced infection rate than parent strains. Thus, luxS produces AI-2 initiating formation of biofilm thereby increasing bacteria survival in the biofilm. DSFs are the second class of signalling molecules in the quorum sensing system that are used by *H. pylori* to communicate between two microbes in the biofilm. DSFs control autoaggregative behaviour, formation of biofilm and "the transition of microorganisms into the coccoid form" (Krzyżek and Gościniak 2018). Strikingly, DSF stimulates the transition of "*H. pylori* into a sedentary state" (resistant coccoid form) initiating the formation of biofilm. Thus, inhibition of quorum sensing system (AI-2 and DSF) in *H. pylori* leads to decrease in virulence.

8.6 Quorum Quenching in *H. pylori*

Interfering with the mechanism of quorum sensing and formation of biofilm is known as quorum quenching, and the inhibitors interfering with quorum signalling are known as quorum sensing inhibitors (Gohil et al. 2018). Quorum quenching is possible when an enzyme degrades or inhibits signalling molecule, blocks quorum signal generated and blocks reception of quorum signal. The quorum sensing inhibitors belong to two broad categories: molecules which mimic quorum sensing signals and enzyme inhibitors. It is interesting to note that first category molecules mimic the quorum sensing signals to block the reception of the signal or generated signal, whereas the second category are enzyme inhibitors. Some of the quorum quenching molecules are listed in Table 8.3. Though quorum quenching molecules are identified in other bacteria, studies should consider on identifying quorum quenching molecules in *H. pylori* and also establish the process of quorum quenching. Thus, these findings will help to develop novel methods for eradication of *H. pylori* pathogenic infections.

8.7 Conclusion and Future Directions

H. pylori infection results in acute transmissible disease ranging from sores, ulcers, to potentially gastric cancer if left untreated. Hitherto the treatments pertaining to *H. pylori* infections require a combination of two or multiple antibiotics. Despite the advancements in drug delivery systems as well as development of novel drug molecules, treatment of *H. pylori* infections are not effective due to increased bacterial resistance towards the antibiotics used for treatment. MDR in *H. pylori* has been associated with the upregulation of candidate genes of efflux pumps, namely, *hefA* and hp1165. Moreover, quorum sensing molecule AI-2 also plays a significant role in developing *H. pylori* infections. Increased antibiotic resistance to *H. pylori* has also been linked to decline in efficacy of the antibiotics used for treating various diseases. Therefore, studies encompassing development of novel antibiotic combinations, downregulation of mRNA levels of candidate genes encoding for efflux pumps as well as understanding the cues that regulate the motility and

Table 8.3 Quorum quenching molecules along with mechanism and target

S. no.	Inhibitors	Class of quorum quenching molecule	Mechanism	Target	References
1	t 5Z-4-bromo-5-bromomethylene-3-butyl-2(5H)-furanone	Halogenated furanones	Blocks reception of quorum signal	Acyl homoserine lactone (AHL) signals	Ren et al. (2001)
2	Synthetic AI peptides (AIPs)	Synthetic AI peptides (AIPs)	Blocks reception of quorum signal	Autoinducer Peptides (AIP) signals	Basavaraju et al. (2016)
3	Triclosan	Enzyme inhibitor	Enzyme degrades or inhibits signalling molecule	Reduces production of AHL by inhibiting enoyl-acyl carrier protein (ACP) reductase involved in acyl-ACP synthesis and intermediate AHL biosynthesis	Basavaraju et al. (2016)
4	Closantel	Enzyme inhibitor	Enzyme degrades or inhibits signalling molecule	Inhibits two-component system histidine kinase sensor	Basavaraju et al. (2016)
5	AHL lactonases	Enzyme inhibitor	Enzyme degrades or inhibits signalling molecule	AHL lactonases hydrolyse the lactone ring in the homoserine moiety of AHLs	Dong et al. (2007) and Biradar and Devi (2011)
6	AHL acylase	Enzyme inhibitor	Enzyme degrades or inhibits signalling molecule	Degradation of AHL signals by hydrolysing the amide bond of AHLs	Leadbetter and Greenberg (2000)

chemotaxis of *H. pylori* inside the gastrointestinal tract are important. Moreover, studies should also contemplate on unravelling the relation between antibiotic resistance and geographical location. These findings will help to develop novel methods for eradication of *H. pylori* pathogenic infections.

Acknowledgements CS, NNR and TD are grateful to GITAM (Deemed to be University) for providing necessary facilities to carry out the research work and for extending constant support. PVBC is thankful to Krishna University Machilipatnam. TD is thankful for financial support in the form of DST Inspire Fellowship (IF 160964), Department of Science and Technology, New Delhi. The authors are also thankful to Dr Neha Merchant, Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, USA, for scientific and language editing.

Authors Contribution CS and NNRR initiated the review; manuscript was written by all authors CS, TD, PVBC and NNRR and revised by CS, PVBC and NNRR.

Conflict of Interest Statement The authors declare that there is no potential conflict of interest.

References

Bardhan KD, Morton D, Perry MJ, Sanders DS, Morris P, Rowland A, Thompson M, Mitchell TR, Roberts PM (2001) Ranitidine bismuth citrate with clarithromycin alone or with metronidazole for the eradication of *Helicobacter pylori*. Aliment Pharmacol Ther 15:1199–1204

Basavaraju M, Sisnity VS, Palaparthy R, Addanki PK (2016) Quorum quenching: signal jamming in dental plaque biofilms. J Dent Sci 11:349–352

Beaudoin T, Zhang L, Hinz AJ, Parr CJ, Mah TF (2012) The biofilm-specific antibiotic resistance gene ndvB is important for expression of ethanol oxidation genes in *Pseudomonas aeruginosa* biofilms. J Bacteriol 194:3128–3136

Billings N, Millan MR, Caldara M, Rusconi R, Tarasova Y, Stocker R, Ribbeck K (2013) The extracellular matrix component Psl provides fast-acting antibiotic defense in *Pseudomonas aeruginosa* biofilms. PLoS Pathog 9:1003526

Biradar B, Devi P (2011) Quorum sensing in plaque biofilm: challenges and future aspects. J Contemp Dent Pract 12:479e85

Bolor-Erdene M, Namdag B, Yamaoka Y, Jav S (2017) Antibiotic resistance of *Helicobacter pylori* in Mongolia. J Infect Dev Countries 11:887–894

Boyanova L, Ilieva J, Gergova G, Spassova Z, Nikolov R, Davidkov L, Evstatiev I, Kamburov V, Katsarov N, Mitov I (2009) Evaluation of clinical and socio- demographic risk factors for antibacterial resistance of *Helicobacter pylori* in Bulgaria. J Med Microbiol 58:94–100

Bush K (2013) Proliferation and significance of clinically relevant β-lactamases. Ann N Y Acad Sci 1277:84–90

Cammarota G, Branca G, Ardito F, Sanguinetti M, Ianiro G, Cianci R, Torelli R, Masala G, Gasbarrini A, Fadda G, Landolfi R (2010) Biofilm demolition and antibiotic treatment to eradicate resistant *Helicobacter pylori*: a clinical trial. Clin Gastroenterol Hepatol 8:817–820

Carron MA, Tran VR, Sugawa C, Coticchia JM (2006) Identification of *Helicobacter pylori* biofilms in human gastric mucosa. J Gastrointest Surg 10:712–717

Cellini L, Grande R, Di Campli E, Traini T, Di Giulio M, Nicola Lannutti S, Lattanzio R (2008) Dynamic colonization of *Helicobacter pylori* in human gastric mucosa. Scand J Gastroenterol 43:178–185

Challa S, Mohana Sheela G, Neelapu NRR (2018) Understanding the bacterial biofilm resistance to antibiotics and immune evasion. In: Bramha Chari PV (ed) Implication of quorum sensing system in biofilm formation and virulence. Springer Nature, Cham, pp 369–381

Chambers JR, Liao J, Schurr MJ, Sauer K (2014) BrlR from *Pseudomonas aeruginosa* is ac-di-GMP-responsive transcription factor. Mol Microbiol 92:471–487

Chuma T, Miyasako D, Hesham D, Takayama T, Nakamoto Y, Shahada F, Akiba M, Okamoto K (2013) Chronological change of resistance to β-lactams in *Salmonella enterica serovar infantis* isolated from broilers in Japan. Front Microbiol 4:113

Cole SP, Harwood J, Lee R, She R, Guiney DG (2004) Characterization of monospecies biofilm formation by *Helicobacter pylori*. J Bacteriol 186:3124–3132

Colvin KM, Irie Y, Tart CS, Urbano R, Whitney JC, Ryder C, Howell PL, Wozniak DJ, Parsek MR (2012) The Pel and Psl polysaccharides provide *Pseudomonas aeruginosa* structural redundancy within the biofilm matrix. Environ Microbiol 14:1913–1928

Coticchia JM, Sugawa C, Tran VR, Gurrola J, Kowalski E, Carron MA (2006) Presence and density of *Helicobacter pylori* biofilms in human gastric mucosa in patients with peptic ulcer disease. J Gastrointest Surg 10:883–889

Dale JL, Cagnazzo J, Phan CQ, Barnes AM, Dunny GM (2015) Multiple roles for *Enterococcus faecalis* glycosyltransferases in biofilm-associated antibiotic resistance, cell envelope integrity, and conjugative transfer. Antimicrob Agents Chemother 59:4094–4105

Doherty NC, Shen F, Halliday NM, Barrett DA, Hardie KR, Winzer K, Atherton JC (2010) In *Helicobacter pylori*, LuxS is a key enzyme in cysteine provision through a reverse transsulfuration pathway. J Bacteriol 192:1184–1192

Dong YH, Wang LH, Zhang LH (2007) Quorum quenching microbial infections: mechanisms and implications. Philos Trans R Soc Lond Biol Sci 362:1201e11

Forsyth MH, Cover TL (2000) Intercellular communication in *Helicobacter pylori*: LuxS is essential for the production of an extracellular signaling molecule. Infect Immun 68:3193–3199

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Gao W, Cheng H, Hu F, Li J, Wang L, Yang G, Xu L, Zheng X (2010) The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China. Helicobacter 15:460–466

Gohil N, Ramírez-García R, Panchasara H, Patel S, Bhattacharjee G, Singh V (2018) Book review: quorum sensing vs. quorum quenching: a battle with no end insight. Front Cell Infect Microbiol 8:106

Gupta K, Marques CN, Petrova OE, Sauer K (2013) Antimicrobial tolerance of *Pseudomonas aeruginosa* biofilms is activated during an early developmental stage and requires the two-component hybrid SagS. J Bacteriol 195:4975–4987

Gupta K, Liao J, Petrova OE, Cherny KE, Sauer K (2014) Elevated levels of the second messenger c-di-GMP contribute to antimicrobial resistance of *Pseudomonas aeruginosa*. Mol Microbiol 92:488–506

Hall CW, Mah TF (2017) Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 41:276–301

Harrison JJ, Wade WD, Akierman S, Vacchi-Suzzi C, Stremick CA, Turner RJ, Ceri H (2009) The chromosomal toxin gene yafQ is a determinant of multidrug tolerance for *Escherichia coli* growing in a biofilm. Antimicrob Agents Chemother 53:2253–2258

Hegarty JP, Dowd MT, Baker KH (1999) Occurrence of *Helicobacter pylori* in surface water in the United States. J Appl Microbiol 87:697–701

Hong SH, Lee J, Wood TK (2010) Engineering global regulator Hha of *Escherichia coli* to control biofilm dispersal. Microb Biotechnol 3:717–728

Horiuchi T, Ohkusa T, Watanabe M, Kobayashi D, Miwa H, Eishi Y (2001) *Helicobacter pylori* DNA in drinking water in Japan. Microbiol Immunol 45:515–519

Huang YQ, Huang GR, Wu MH, Tang HY, Huang ZS, Zhou XH, Yu WQ, Su JW, Mo XQ, Chen BP, Zhao LJ (2015) Inhibitory effects of emodin, baicalin, schizandrin and berberine on hefA gene: treatment of *Helicobacter pylori*-induced multidrug resistance. World J Gastroenterol 21:4225

Ilver D, Arnqvist A, Ögren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T (1998) *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279:373–377

Imanishi Y, Ogata T, Matsuzuka A, Tasaki T, Fujioka T, Akashi M, Makino Y, Nishizono A (2003) Possibility for the presence of *Helicobacter pylor*i in drinking well water. Kansenshogakuzasshi Jpn J Infect Dis *77*:18–23

Johnson KS, Ottemann KM (2018) Colonization, localization, and inflammation: the roles of *H. pylori* chemotaxis *invivo*. Curr Opin Microbiol 41:51–57

Joyce EA, Bassler BL, Wright A (2000) Evidence for a signaling system *in Helicobacter pylori*: detection of aluxS-encoded autoinducer. J Bacteriol 182:3638–3643

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73(2):310–347

Khakimova M, Ahlgren HG, Harrison JJ, English AM, Nguyen D (2013) The stringent response controls catalases in *Pseudomonas aeruginosa* and is required for hydrogen peroxide and antibiotic tolerance. J Bacteriol 195:2011–2020

Kolodkin-Gal I, Cao S, Chai L, Böttcher T, Kolter R, Clardy J, Losick R (2012) A self-produced trigger for biofilm disassembly that targets exopolysaccharide. Cell 149(3):684–692

Krzyżek P, Gościniak G (2018) A proposed role for diffusible signal factors in the biofilm formation and morphological transformation of *Helicobacter pylori*. Turk J Gastroenterol 29:7–12

Kurtaran H, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, Akcay A, Kanbay M (2008) Role of *Helicobacter pylori* in pathogenesis of upper respiratory system diseases. J Natl Med Assoc 100:1224

Leadbetter JR, Greenberg EP (2000) Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. J Bacteriol 182:6921e6

Lee A, Mao W, Warren MS, Mistry A, Hoshino K, Okumura R, Ishida H, Lomovskaya O (2000) Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. J Bacteriol 182:3142–3150

Lee WK, Ogura K, Loh JT, Cover TL, Berg DE (2006) Quantitative effect of luxS gene inactivation on the fitness of *Helicobacter pylori*. Appl Environ Microbiol 72:6615–6622

Lee SM, Kim N, Kwon YH, Nam RH, Kim JM, Park JY, Lee YS, Lee DH (2018) Rdxa, frxa, and efflux pump in metronidazole-resistant *Helicobacter pylori*: their relation to clinical outcomes. J Gastroenterol Hepatol 33:681–688

Lewis ND, Asim M, Barry DP, de Sablet T, Singh K, Piazuelo MB, Gobert AP, Chaturvedi R, Wilson KT (2011) Immune evasion by *Helicobacter pylori* is mediated by induction of macrophage arginase II. J Immunol 186(6):3632–3641

Liao J, Sauer K (2012) The MerR-like transcriptional regulator BrlR contributes to *Pseudomonas aeruginosa* biofilm tolerance. J Bacteriol 194:4823–4836

Liao J, Schurr MJ, Sauer K (2013) The MerR-like regulator BrlR confers biofilm tolerance by activating multidrug efflux pumps in *Pseudomonas aeruginosa* biofilms. J Bacteriol 195:3352–3363

Lina TT, Alzahrani S, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE (2014) Immune evasion strategies used by *Helicobacter pylori*. World J Gastroenterol 20(36):12753–12766

Liu ZQ, Zheng PY, Yang PC (2008) Efflux pump gene hefA of *Helicobacter pylori* plays an important role in multidrug resistance. World J Gastroenterol 14:5217

Lu Y, Redlinger TE, Avitia R, Galindo A, Goodman K (2002) Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. Appl Environ Microbiol 68:1436–1439

Lynch SV, Dixon L, Benoit MR, Brodie EL, Keyhan M, Hu P, Ackerley DF, Andersen GL, Matin A (2007) Role of the rapA gene in controlling antibiotic resistance of *Escherichia coli* biofilms. Antimicrob Agents Chemother 51:3650–3658

Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'toole GA (2003) A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 426:306

Mahdavi J, Sondén B, Hurtig M, Olfat FO, Forsberg L, Roche N, Ångström J, Larsson T, Teneberg S, Karlsson KA, Altraja S (2002) *Helicobacter pylori Sab A* adhesin in persistent infection and chronic inflammation. Science 297:573–578

Mohana Sheela G, Prathyusha AMVN, Neelapu NRR, Bramha Chari PV (2018) Intra and interspecies communication in microbes: Living with complex and sociable neighbors. In: Bramha Chari PV (ed) Implication of quorum sensing system in biofilm formation and virulence. Springer Nature, Cham, pp 7–16

Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, Maady A, Bernhöft S, Thiberge JM, Phuanukoonnon S, Jobb G (2009) The peopling of the Pacific from a bacterial perspective. Science 323:527–530

Moreno Y, Botella S, Alonso JL, Ferrús MA, Hernández M, Hernández J (2003) Specific detection of *Arcobacter* and *Campylobacter* strains in water and sewage by PCR and fluorescent in situ hybridization. Appl Environ Microbiol 69:1181–1186

Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4:1–37

Nammi D, Srimath-Tirumala-Peddinti RCPK, Neelapu NRR (2016) Identification of drug targets in *Helicobacter pylori* by in silico analysis: Possible therapeutic implications for gastric cancer. Curr Cancer Drug Targets 16:79–98

Nammi D, Yarla NS, Chubarev VN, Tarasov VV, Barreto GE, Pasupulati CAM, Aliev G, Neelapu NRR (2017) A systematic in-silico analysis of *Helicobacter pylori* pathogenic islands for identification of novel drug target candidates. Curr Genome 18:450–465

Ndip RN, Malange Takang AE, Ojongokpoko JE, Luma HN, Malongue A, Akoachere JF, Ndip LM, MacMillan M, Weaver LT (2008) *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastroduodenal pathologies in Cameroon: current status of antibiogram. Tropical Med Int Health 13:848–854

Neelapu RR (2018) Role and regulation of transcriptional factors in gastric cancer. In: Role of transcription factors in gastrointestinal malignancies. Springer Nature, Hiedelberg.. ISBN 978-981-10-6727-3, pp 107–130

Neelapu NRR, Pavani T (2013) Identification of novel drug targets in HpB38, HpP12, HpG27, Hpshi470, HpSJM180 strains of *Helicobacter pylori*: an *insilico* approach for therapeutic intervention. Curr Drug Targets 14:601–611

Neelapu NRR, Srimath-Tirumala-Peddinti RCPK, Nammi D, Pasupuleti ACM (2013) New strategies and paradigm for drug target discovery: a special focus on infectious diseases tuberculosis, malaria, leishmaniasis, trypanosomiasis and gastritis. Infect Disord Drug Targets 13(5):352–364

Neelapu NRR, Nammi D, Pasupuleti ACM, Surekha C (2014) *Helicobacter pylori* induced gastric inflammation, ulcer, and cancer: a pathogenesis perspective. Interdiscip J Microinflammation 1:113

Neelapu NRR, Mutha NVR, Akula S (2015) Identification of potential drug targets in *Helicobacter pylori* strain HPAG1 by *in silico* genome Analysis. Infect Disord Drug Targets 15:106–117

Neelapu NRR, Nammi D, Pasupuleti AMC, Challa S (2016) Targets against *Helicobacter pylori* and other tumor-producing bacteria. In: New weapons to control bacterial growth. Springer, Cham, pp 239–279

Neelapu NRR, Titash D, Surekha C (2018) Quorum sensing and its role in *Agrobacterium* mediated gene transfer. In: Bramha Chari PV (ed) Implication of quorum sensing system in biofilm formation and virulence. Springer Nature, Cham, pp 259–275

Nguyen D, Joshi-Datar A, Lepine F, Bauerle E, Olakanmi O, Beer K, McKay G, Siehnel R, Schafhauser J, Wang Y, Britigan BE (2011) Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science 334:982–986

Nilsson M, Rybtke M, Givskov M, Høiby N, Twetman S, Tolker-Nielsen T (2016) The dlt genes play a role in antimicrobial tolerance of *Streptococcus mutans* biofilms. Int J Antimicrob Agents 48:298–304

Osaki T, Hanawa T, Manzoku T, Fukuda M, Kawakami H, Suzuki H, Yamaguchi H, Yan X, Taguchi H, Kurata S, Kamiya S (2006) Mutation of luxS affects motility and infectivity of *Helicobacter pylori* in gastric mucosa of a Mongolian gerbil model. J Med Microbiol 55:1477–1485

Pasupuleti AMP, Nammi D, Neelapu NRR (2017) Screening and identification of drug targets and vaccine candidates for *Helicobacter pylori* strain Hp26695. Int J Rec Sci Res 8(4):16384–16395

Petersen AM, Krogfelt KA (2003) *Helicobacter pylori:* an invading microorganism? A review. FEMS Med Microbiol Immunol 36:117–126

Petrova OE, Sauer K (2011) SagS contributes to the motile-sessile switch and acts in concert with BfiSR to enable *Pseudomonas aeruginosa* biofilm formation. J Bacteriol 193:6614–6628

Rader BA, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K (2011) *Helicobacter pylori* perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. Microbiology 157(9):2445–2455

Ren D, Sims JJ, Wood TK (2001) Inhibition of biofilm formation and swarming of *Escherichia coli* by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. Environ Microbiol 3:731–736

Rowe MC, Withers HL, Swift S (2010) Uropathogenic *Escherichia coli* forms biofilm aggregates under iron restriction that disperse upon the supply of iron. FEMS Microbiol Lett 307:102–109

Sadovskaya I, Vinogradov E, Li J, Hachani A, Kowalska K, Filloux A (2010) High-level antibiotic resistance in *Pseudomonas aeruginosa* biofilm: the ndvB gene is involved in the production of highly glycerol-phosphorylated β-(1→ 3)-glucans, which bind aminoglycosides. Glycobiology 20:895–904

Sauer K, Cullen MC, Rickard AH, Zeef LA, Davies DG, Gilbert P (2004) Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. J Bacteriol 186(21):7312–7326

Savarino V, Mansi C, Mele MR, Bisso G, Mela GS, Saggioro A, Caroli M, Vigneri S, Termini R, Olivieri A, Tosatto R, Celle G (1997) A new 1-week therapy for *Helicobacter pylori* eradication: ranitidine bismuth citrate plus two antibiotics. Aliment Pharmacol Ther 11(4):699–703

Shen F, Hobley L, Doherty N et al (2010) *Helicobacter pylori* autoinducer-2, but not LuxS/MccAB catalysed reverse transsulphuration, regulates motility through modulation of flagellar genetranscription. BMC Microbiol 10:210

Siala W, Mingeot-Leclercq M-P, Tulkens PM, Hallin M, Denis O, Van Bambeke F (2014) Comparison of the antibiotic activities of daptomycin, vancomycin, and the investigational fluoroquinolone delafloxacin against biofilms from *Staphylococcus aureus* clinical isolates. Antimicrob Agents Chemother 58(11):6385–6397

Siddique O, Ovalle A, Siddique AS, Moss SF (2018) *Helicobacter pylori* infection: an update for the internist in the age of increasing global antibiotic resistance. Am J Med. https://doi.org/10.1016/j.amjmed.2017.12.024

Song X, Ma Y, Fu J, Zhao A, Guo Z, Malakar PK et al (2016) Effect of temperature on pathogenic and non-pathogenic *Vibrio parahaemolyticus* biofilm formation. Food Control 73:485–491

Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB (2003) Bacteria–host communication: the language of hormones. Proc Natl Acad Sci U S A 100:8951–8956

Sun QJ, Liang X, Zheng Q, Gu WQ, Liu WZ, Xiao SD, Lu H (2010) Resistance of *Helicobacter pylori* to antibiotics from 2000 to 2009 in Shanghai. World J Gastroenterol 16:5118

Surekha C, Neelapu NRR (2018) Quorum sensing in *Helicobacter pylori*: role of biofilm and its implications for antibiotic resistance and immune evasion. In: Bramha Chari PV (ed) Implication of quorum sensing system in biofilm formation and virulence. Springer Nature, Cham, pp 361–381

Sweeney EG, Henderson JN, Goers J, Wreden C, Hicks KG, Foster JK, Parthasarathy R, Remington SJ, Guillemin K (2012) Structure and proposed mechanism for the pH-sensing *Helicobacter pylori* chemoreceptor TlpB. Structure 20:1177–1188

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control 34:3–10

Torres J, Camorlinga-Ponce M, Pérez-Pérez G, Madrazo-De la Garza A, Dehesa M, González-Valencia G, Muñoz O (2001) Increasing multidrug resistance in *Helicobacter pylori* strains isolated from children and adults in Mexico. J Clin Microbiol 39:2677–2680

Watson CL, Owen RJ, Said B, Lai S, Lee JV, Surman-Lee S, Nichols G (2004) Detection of *Helicobacter pylori* by PCR but not culture in water and biofilm samples from drinking water distribution systems in England. J Appl Microbiol 97:690–698

Webber MA, Piddock LJV (2003) The importance of efflux pumps in bacterial antibiotic resistance. J Antimicrob Chemother 51:9–11

Wen Y, Marcus EA, Matrubutham U, Gleeson MA, Scott DR, Sachs G (2003) Acid-adaptive genes of *Helicobacter pylori*. Infect Immun 71:5921–5939

Whiteley M, Diggle SP, Greenberg EP (2017) Progress in and promise of bacterial quorum sensing research. Nature 551:313

World Health Organization (2014) Antimicrobial resistance: global report on surveillance. World Health Organization.

Wuppenhorst N, Lenze F, Ross M, Kist M (2011) Isolation and eradication of a clinical isolate of *Helicobacter pylori* resistant to five antimicrobials in Germany. J Antimicrob Chemother 66:222–223

Yamaoka Y (2010) Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol 7:629

Zhang L, Mah TF (2008) Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J Bacteriol 190:4447–4452

Zhang L, Hinz AJ, Nadeau JP, Mah TF (2011) *Pseudomonas aeruginosa* tssC1 links type VI secretion and biofilm-specific antibiotic resistance. J Bacteriol 193:5510–5513

Zhang L, Fritsch M, Hammond L, Landreville R, Slatculescu C, Colavita A, Mah TF (2013) Identification of genes involved in *Pseudomonas aeruginosa* biofilm-specific resistance to antibiotics. PLoS One 8:61625

Zhao J, Quan C, Jin L, Chen M (2018) Production, detection and application perspectives of quorum sensing autoinducer-2 in bacteria. J Biotechnol. https://doi.org/10.1016/j.jbiotec.2018.01.009

Zhou L, Yu Y, Chen X, Diab AA, Ruan L, He J, Wang H, He YW (2015) The multiple DSF-family QS signals are synthesized from carbohydrate and branched-chain amino acids via the FAS elongation cycle. Sci Rep 5:13294

Zullo A, De Francesco V, Hassan C, Morini S, Vaira D (2007) The sequential therapy regimen for Helicobacter pylori eradication: a pooled-data analysis. Gut 56(10):1353–1357

Candida albicans Biofilm: Risks, Complications and Preventive Strategies

9

Prerna Pathak

Abstract

Candida albicans, a dimorphic opportunistic fungal pathogen, can cause a wide range of diseases in humans. Its ability to switch from commensal to pathogenic form is governed by several virulence attributes. The major feature which leads to pathogenicity is its ability to form biofilm. Biofilm formation is a highly regulated multistep process; there are several clinical implications associated with biofilm formation, as sessile cells exhibit high level of resistances against most of the antifungals, and it also protects the cells from harsh hostile environments. The implantable medical devices like catheter support biofilm mode of development by *Candida* which negatively impacts the health of immunocompromised individuals by causing recurrent infections. This review highlights the mechanism of *Candida albicans* biofilm formation and its clinical consequences, as well as currently available drugs against biofilm and alternative to drug therapy.

Keywords

Candida albicans · Biofilm · Hospital-acquired infections · Quorum sensing · Central venous catheters

9.1 Introduction

Candida albicans is a part of normal human microbiota; it is present as a commensal in oral cavity, genitourinary and gastrointestinal tracts of healthy individuals; however, a change in host immunity due to immunosuppressive treatment like

P. Pathak (✉)
Jawaharlal Nehru University, New Delhi, India
e-mail: prernapathak12@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_9

chemotherapy and organ transplantation allows it to switch to virulent form which in turn leads to candidiasis (Jackson et al. 2007; Wu et al. 2003; Achkar and Fries 2010; Rosenbach et al. 2010; Naglik et al. 2011). There are several factors which are responsible for its virulence; among them, biofilm formation plays the major role. Biofilms are highly organized communities of surface-attached organisms, wrapped within extracellular matrix (Costerton et al. 1995; Costerton et al. 1999; Donlan 2002). The complicated architecture, dense extracellular matrix and overexpression of drug efflux pumps make biofilm highly drug resistance (Fanning and Mitchell 2012; Finkel and Mitchell 2011). Bacterial biofilms are highly explored; however, very little is known about fungal biofilm. Biofilm formation is a complex process which is highly regulated by a series of transcription factors.

Among all the known *Candida* species, *C. albicans* is most frequently isolated from clinical samples; it is a major cause of hospital-acquired infections (HAI). The frequent use of intravascular devices like heart valves, pacemakers, urinary and venous catheters, dentures, etc. results in HAI as they are more susceptible to biofilm formation (Cauda 2009; Donlan and Costerton 2002; Kojic and Darouiche 2004; Seddiki et al. 2013). In spite of the availability of so many different classes of antifungals, the mortality rate of candidiasis in adults is almost 50% (Chander 2009; Andes et al. 2012).

9.2 *Candida albicans* Biofilm Formation

Biofilms are not just aggregation of cells; rather it is an extremely organized community of microorganism which regulates the inflow of nutrients and outflow of waste products. The four main stages of *C. albicans* biofilm formation are (Fig. 9.1)

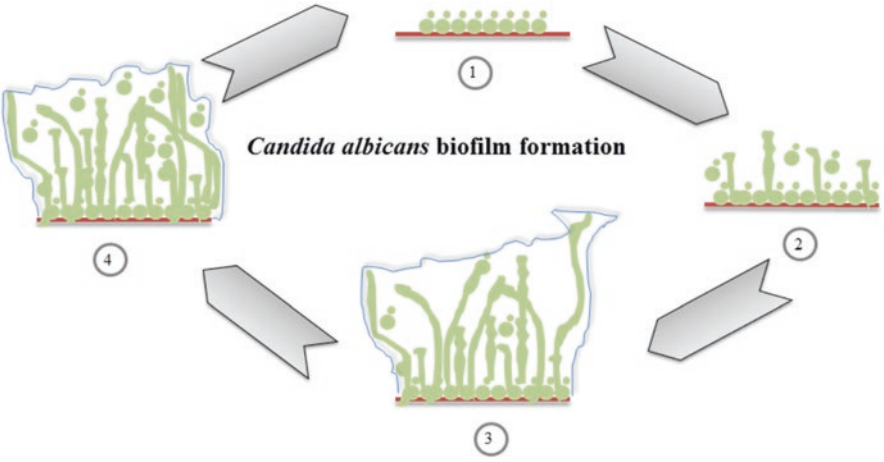


Fig. 9.1 Stages of *Candida albicans* biofilm formation. *1*. Adherence of yeast cells to a surface. *2*. Commencement of cell proliferation. *3*. Maturation which includes hyphae growth along with the production of extracellular matrix material. *4*. Dispersal of yeast cells from the biofilm to new site

(a) adherence of cells to the surface, (b) proliferation, (c) formation of hyphal cells and (d) dispersion of cells to new sites (Chandra et al. 2001; Douglas 2003; Hawser and Douglas 1994; Uppuluri et al. 2010; Verstrepen and Klis 2006).

9.3 Adherence

This is the initial step of biofilm formation; it is the ability of cells to adhere to each other or to the surface such as biotic and abiotic surfaces. Adhesins are the class of cell wall proteins that promotes this attachment; generally, these proteins comprise of GPI anchor, a peptide- or carbohydrate-binding domain (Nobile et al. 2006). Adhesins mainly belong to Als (agglutinin-like sequence) family consisting of 8 members *ALS1-7* and *ALS9*; among them, *ALS-3* plays a crucial role in biofilm formation as deletion of this protein causes biofilm defect (Chandra et al. 2001). The master regulator of biofilm formation is Bcr1, which is a transcription factor, and its downstream targets such as Als1, Als3 and Hwp1 (Nobile et al. 2006; de Groot et al. 2013). Another important adhesin in *C. albicans* is Hwp1 (hyphal wall protein); it is a mannoprotein which has a role in biofilm formation; apart from this, other members of hwp family which play a role in biofilm formation are Hwp2, Rbt1, Eap1 and Ywp1 (de Groot et al. 2013).

9.4 Hyphal Cells Formation

The next main step in biofilm formation is yeast-to-hyphal switching. Hyphae formation is the main factor which distinguishes *C. albicans* from other fungal species; hyphae play an imperative role in the architecture of *C. albicans* biofilm; they act as a scaffold for yeast cells. Hyphae formation is governed by a series of transcription factors like Efg1, Ndt80, Tec1 and Rob1 (de Groot et al. 2013; Nobile et al. 2012; Ramage et al. 2002); these transcription factors are crucial for biofilm development and its maintenance.

9.5 Production of Extracellular Matrix

Mature *C. albicans* biofilms are wrapped around by a layer of complex extracellular matrix (ECM), which provides a three-dimensional structure to the biofilm and makes cells partly immobilized (Schweizer et al. 2000). ECM acts a physical barrier and protects biofilms against phagocytosis and drug diffusion; meanwhile, it also acts as a scaffold and provides integrity to biofilm. The biochemical analysis of *C. albicans* biofilm reveals the presence of the following macromolecular components: 55% proteins, 25% carbohydrates, 15% lipids and 5% noncoding DNA (Flemming and Wingender 2010). Rml1 and Zap1 are the main transcription regulators of ECM production in *C. albicans* (Zarnowski et al. 2014).

9.6 Dispersion

The another important stage in the biofilm formation is dispersal of cells during and after biofilm formation; cells are dispersed to the new site and form biofilm, and this is the pathogen way to cause infection. Although the morphology of dispersed cells is similar to that of cells in planktonic growth mode, they have some unique features like increased adherence, enhanced virulence and increased efficiency of biofilm formation. Nrg1 and Ume6 are the two major transcriptional regulators of *C. albicans* biofilm dispersal (Nobile et al. 2009).

9.7 Risk Associated with *C. albicans* Biofilm

Among all the known *Candida* spp., *C. albicans* is the prominent causes of hospital-acquired infections; it is frequently isolated as an agent of bloodstream and urinary tract infections (Uppuluri et al. 2010; Calderone 2002). Majority of diseases caused by *C. albicans* are linked with its biofilm mode of growth (Ramage and Lopez-Ribot 2005); biofilms are frequently found on the surface of implantable medical devices like urinary and intravascular catheters (Crump and Collignon 2000; Maki and Tambyah 2001; Adair et al. 1999). The increased drug resistance caused by biofilm is the main reason of concern, the mechanism of this antifungal resistance is not fully known; however, it is believed that ECM acts as an impediment for cytotoxic drugs (Kojic and Darouiche 2004).

Catheter-associated biofilms are the major causes of mortality among the hospital-acquired infections. In the USA, around 5 million central venous catheters (CVC) are interpolated yearly (Darouiche 2001). In the USA, around 80,000 catheter-related infections occur annually which result up to 20,000 deaths (Mermel et al. 2001). Among all the inserted medical devices, CVC are the most infected surgical implants; it is used to administer nutrients and drugs. Catheter-related infections can occur anytime during its use – sometimes infection-causing organism are introduced from the hands of hospital staff or from the patient's skin; sometimes the infusion fluid itself is contaminated (Goldmann and Pier 1993). Apart from CVC, other medical implants are also subjected to infections like pacemakers, heart valves, joint prostheses, implantable cardioverter defibrillators (ICD), ventricular assist devices (VADs), penile prostheses, etc.

9.8 Preventive Measures for *Candida* Biofilm

Biofilm-based infections are difficult to treat as there is no drug available which specifically targets biofilm. Lack of knowledge behind the underlying mechanism of biofilm formation and its mechanism of dispersal makes its treatment difficult. *C. albicans* has minimum susceptibility to currently available drugs. The following approaches are in use nowadays against biofilm.

9.9 Lock Therapy Approach

According to the guidelines of Infectious Disease Society of America, this is the recommended approach for treating catheter-related infections (Carratalà 2002). In this therapy, high doses of antimicrobial agents (100–1000-fold the minimum inhibitory concentration) are installed into the catheter for hours to days (Teughels et al. 2006). There are reports available on the use of antifungal lock therapy; although it shows promising result, it is not a cost-effective approach as huge dosages are required to completely eradicate the fungal growth.

9.10 Surface Modification and Material Coating

Current research focuses on the use of different materials for surface coating and modification of surface to prevent biofilm formation. Fungicidal and fungistatic materials are used for coating the surfaces of medical devices; antifungals such as nystatin, cycloheximide and amphotericin B are used to coat the medical devices; cycloheximide shows potent reduction of *C. albicans* biofilm (Redding et al. 2009). Similarly, a thin film of polyelectrolyte containing an antifungal β-peptide shows 74% inhibitory action against *C. albicans* biofilm (Karlsson et al. 2010).

Surface of medical devices plays a decisive role in its stability, as well as its performance, like albumin adhesion prevents the attachment of microorganisms, while fibrinogen shows antagonistic effect (Anderson et al. 2008). Surface modification is a good approach against biofilms; miconazole, an antifungal, incorporates well on the modified surface and shows reduction in *C. albicans* biofilm formation. The modified form of silicon rubber and polyethylene shows efficient inhibitory action against *C. albicans* biofilm in vitro (Contreras-Garcia et al. 2011).

9.11 Plant Products

Plants are used for decades as a source of medicine by many countries. Several medicinal plants produce products which possess antimicrobial and antifungal properties. There are reports of plants having anti-candida activities like *Thymus villosus*, *Cinnamomum zeylanicum*, eucalyptus, ginger grass oil and lemongrass oil; however there is very little information available in this context (Soliman et al. 2017). Apart from the above-mentioned plants, pomegranate peel have certain compounds which show antifungal properties like Castgalagin, quercetin, punicalagin, kaempferol, catechin and granatin (Dahham et al. 2010).

9.12 Quorum Sensing Molecules (QSM)

Along with plant products, some quorum sensing molecules also show antifungal properties like farnesol, a sesquiterpene alcohol produced by *C. albicans* as a signalling molecule known to negatively regulate biofilm formation in vitro (Alem

et al. 2006). Tyrosol is a derivative of phenethyl alcohol known to activate the biofilm formation (Hogan et al. 2004). Homoserine lactone, a bacterial QSM, represses *C. albicans* filamentation (Park et al. 2014). Phenazine which is produced by *Pseudomonas* possesses anti-candida activity (Valle et al. 2006). A better understanding of quorum sensing mechanism could help in the identification of novel anti-biofilm molecules.

9.13 Conclusion and Future Perspective

In the current scenario, there is a rise in the number of people affected by *C. albicans*; with increase in infection, there is increase in the use of medical implants. The surface of implantable medical devices is frequently associated with biofilm; there is no concrete solution to *Candida* biofilm; however, increases in research over the decade brought insight into several strategies which target biofilm as well as some potent antifungal molecules. Future healthcare regime should include improved drug delivery system, combined antifungal approach and use of proper sterilization technique for biomaterials. This chapter brings light on therapies which are used currently to treat biofilm; there is still an urgent need to develop a better understanding about biofilm formation as well as its mechanism of interaction with biotic and abiotic surfaces.

Acknowledgements The author gratefully acknowledges the University Grant Commission, Government of India, for awarding Junior and Senior Research Fellowship.

Conflict of Interest The author has no conflict of interest.

References

Achkar JM, Fries BC (2010) Candida infections of the genitourinary tract. Clin Microbiol Rev 23(2):253–273

Adair CG, Gorman SP, Feron BM et al (1999) Implications of endotracheal tube biofilm ventilator-associated pneumonia. Intensive Care Med 25:1072–1076

Alem MAS, Oteef MDY, Flowers TH, Douglas LJ (2006) Production of tyrosol by Candida albicans biofilms and its role in quorum sensing and biofilm development. Eukaryot Cell 5(10):1770–1779

Anderson JM, Rodriguez A, Chang DT (2008) Foreign body reaction to biomaterials. Semin Immunol 20(2):86–100

Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH et al (2012) Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: A patient-level quantitative review of randomized trials. Clin Infect Dis 54(8):1110–1122

Calderone RA (2002) Introduction and historical perspectives. In: Calderone RA (ed) *Candida* and Candidiasis. ASM Press, Washington, DC, pp 3–13

Carratalà J (2002) The antibiotic-lock technique for therapy of 'highly needed' infected catheters. Clin Microbiol Infect 8(5):282–289

Cauda R (2009) Candidaemia in patients with an inserted medical device. Drugs 69(Suppl 1):33–38

Chander J (2009) Textbook of medical mycology, 2nd edn. Mehta Publisher, New Delhi
Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA (2001) Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol 183(18):5385–5394
Contreras-Garcia A, Bucioa E, Brackmanc G, Coenyec T, Concheirob A, Alvarez-Lorenzob C (2011) Biofilm inhibition and drug-eluting properties of novel DMAEMA-modified polyethylene and silicone rubber surfaces. *Biofouling* 27(2):123–135
Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745
Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322
Crump JA, Collignon PJ (2000) Intravascular catheter-associated infections. Eur J Clin Microbiol Infect Dis 19:1–8
Dahham SS, Ali MN, Tabassum H, khan M (2010) Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). Am Eurasian J Agric Environ Sci 9(3):273–281
Darouiche RO (2001) Device-associated infections: a macroproblem that starts with microadherence. Clin Infect Dis 33:1567–1572
de Groot PWJ, Bader O, de Boer AD, Weig M, Chauhan N (2013) Adhesins in human fungal pathogens: glue with plenty of stick. Eukaryot Cell 12:470–481. https://doi.org/10.1128/EC.00364-12
Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890
Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15(2):167–193
Douglas LJ (2003) Candida biofilms and their role in infection. Trends Microbiol 11(1):30–36
Fanning S, Mitchell AP (2012) Fungal biofilms. PLoS Pathog 8(4):e1002585
Finkel JS, Mitchell AP (2011) Genetic control of Candida albicans biofilm development. Nat Rev Microbiol 9(2):109–118
Flemming H, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633. https://doi.org/10.1038/nrmicro2415
Goldmann DA, Pier GB (1993) Pathogenesis of infections related to intravascular catheterization. Clin Microbiol Rev 6:176–192
Hawser SP, Douglas LJ (1994) Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun 62(3):915–921
Hogan DA, Vik A, Kolter R (2004) A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology. Mol Microbiol 54(5):1212–1223
Jackson BE, Wilhelmus KR, Mitchell BM (2007) Genetically regulated filamentation contributes to *Candida albicans* virulence during corneal infection. Microb Pathog 42(2–3):88–93
Karlsson AJ, Flessner RM, Gellman SH, Lynn DM, Palecek SP (2010) Polyelectrolytemultilayers fabricated from antifungal β-peptides: design of surfaces that exhibit antifungal activity against *Candida albicans*. Biomacromolecules 11(9):2321–2328
Kojic EM, Darouiche RO (2004) Candida infections of medical devices. Clin Microbiol Rev 17(2):255–267
Maki DG, Tambyah PA (2001) Engineering out the risk of infection with urinary catheters. Emerg Infect Dis 7:1–6
Mermel LA, Farr BM, Sherertz RJ, Raad II, O'Grady N, Harris JS, Craven DE (2001) Guidelines for the management of intravascular catheter-related infections. Infect Control Hosp Epidemiol 22:222–242
Naglik JR, Moyes DL, Wachtler B, Hube B (2011) *Candida albicans* interactions with epithelial cells and mucosal immunity. Microbes Infect 13(12–13):963–976
Nobile CJ, Andes DR, Nett JE, Smith FJ, Yue F, Phan QT et al (2006) Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation in vitro and in vivo. PLoS Pathog 2:e63. https://doi.org/10.1371/journal.ppat.0020063
Nobile CJ, Nett JE, Hernday AD, Homann OR, Deneault JS et al (2009) Biofilm matrix regulation by *Candida albicans* Zap1. PLoS Biol 7:e1000133

Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM et al (2012) A recently evolved transcriptional network controls biofilm development in *Candida albicans*. Cell 148:126–138

Park SJ, Han KH, Park JY, Choi SJ, Lee KH (2014) Influence of bacterial presence on biofilm formation of Candida albicans. Yonsei Med J 55(2):449–458

Ramage G, Lopez-Ribot JL (2005) Techniques for antifungal susceptibility testing of Candida albicans biofilms. Methods Mol Med 118:71–79

Ramage G, VandeWalle K, Lopez-Ribot JL, Wickes BL (2002) The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. FEMS Microbiol Lett 214:95–100

Redding S, Bhatt B, Rawls HR, Siegel G, Scott K, Lopez-Ribot J (2009) Inhibition of *Candida albicans* biofilm formation on denture material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107(5):669–672

Rosenbach A, Dignard D, Pierce JV, Whiteway M, Kumamoto CA (2010) Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. Eukaryot Cell 9(7):1075–1086

Schweizer A, Rupp S, Taylor BN, Rollinghoff M, Schroppel K (2000) The TEA/ATTS transcription factor CaTec1p regulates hyphal development and virulence in *Candida albicans*. Mol Microbiol 38:435–445

Seddiki SM, Boucherit-Otmani Z, Boucherit K, Badsi-Amir S, Taleb M, Kunkel D (2013) Assessment of the types of catheter infectivity caused by Candida species and their biofilm formation: first study in an intensive care unit in Algeria. Int J Gen Med 6:1–7

Soliman S, Alnajdy D, El-Keblawy AA, Mosa KA, Khoder G, Noreddin AM (2017) Plants' natural products as alternative promising anti-*Candida* drugs. Pharmacogn Rev 11(22):104–122

Teughels W, Van Assche N, Sliepen I, Quirynen M (2006) Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 17(Suppl 2):68–81

Uppuluri P, Chaturvedi AK, Srinivasan A, Banerjee M, Ramasubramaniam AK, Köhler JR et al (2010) Dispersion as an important step in the Candida albicans biofilm developmental cycle. PLoS Pathog 6(3):e1000828

Valle J, Da Re S, Henry M, Fontaine T, Balestrino D, Latour-Lambert P et al (2006) Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. Proc Natl Acad Sci U S A 103(33):12558–12563

Verstrepen KJ, Klis FM (2006) Flocculation, adhesion and biofilm formation in yeasts. Mol Microbiol 60:5–15. https://doi.org/10.1111/j.1365-2958.2006.05072.x

Wu TG, Mitchell BM, Carothers TS et al (2003) Molecular analysis of the pediatric ocular surface for fungi. Curr Eye Res 26(1):33–36

Zarnowski R, Westler WM, Lacmbouh GA, Marita JM, Bothe JR, Bernhardt J et al (2014) Novel entries in a fungal biofilm matrix encyclopedia. MBio 5:e1333–e1314. https://doi.org/10.1128/mBio.01333-14

Quorum Sensing and Biofilm Formation by Oral Pathogenic Microbes in the Dental Plaques: Implication for Health and Disease

10

Pallaval Veera Bramhachari, V. K. Shakeel Ahmed, Joseph Selvin and Saqib Hassan

Abstract

Periodontal disease is caused by bacteria in dental plaque, the sticky substance that forms on our teeth. Dental plaque has the properties of a biofilm which is comprised of several hundred different bacterial species. Bacteria in biofilms communicate through signalling molecules and use this "quorum sensing" system to optimize their virulence factors and survival. These bacteria respond differently to antibiotics and antimicrobials and more often show drug resistance. Microbial gene expression was found to alter markedly in biofilms. The cells of our immune system release substances to get rid of the bacteria that cause inflammation and damage to the gums, periodontal ligament or alveolar bone. This leads to swollen, bleeding gums, a sign of gingivitis which is the earliest stage of periodontal disease. Such damage from periodontal disease can also cause teeth to become loose apart from leading to other oral infections. Periodontal disease is a widespread and serious health problem in adult population worldwide. To treat such diseases, the expenditure is too high. Furthermore, periodontal disease was found to be linked to other systemic illnesses, such as heart disease and preterm births. The need of the hour is to understand the disease process so that we may be able to control periodontal disease and improve the health status of the

P. V. Bramhachari
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

V. K. S. Ahmed
Department of Orthodontics, Ragas Dental College and Hospital, Chennai, India

J. Selvin
Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India

S. Hassan (✉)
Department of Medicine, Imperial College London, London, UK
e-mail: s.hassan@imperial.ac.uk

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_10

adult population worldwide. It has been well established that periodontal disease can be caused by shifts in the microbial population of the dental biofilms. Therefore, understanding the molecular details of quorum sensing mechanisms and disrupting such processes may open a new avenue for controlling bacterial infections.

Keywords
Biofilm · Bacteria · Heart · Quorum sensing · Resistance

10.1 Introduction

Quorum sensing (QS) is a bacterial cell-to-cell communication system which helps in the regulation of gene expression in response to fluctuations in cell population density (Miller and Basler 2001). QS bacteria produce and release chemical signal molecules called autoinducers that increase in concentration as a function of their cell density (Romero et al. 2011). This process enables bacteria to communicate and to synchronize phenotypic expression of biofilm development, motility, bioluminescence and virulence (Tang and Zhang 2014; Kiran et al. 2017). With the recent advancements in research, it was revealed that bacteria have a complex chemical signalling system that enables them to communicate intraspecifically as well as interspecifically. QS is exhibited by many known human and plant bacterial species as well as in extremophiles such as *Natronococcus occultus*, *Halomonas* genus, *Thermotoga maritima* and *Acidithiobacillus ferrooxidans* (Johnson et al. 2005; Llamas et al. 2004; Paggi et al. 2003; Rivas et al. 2007). Both Gram-positive and Gram-negative bacteria possess QS activity. Some of the QS molecules which were studied in detail include the acylhomoserine lactones (AHL), autoinducer 2 (AI-2) and peptide signals; nonetheless, some other signal molecules also exist, such as indole and cholera AI (Nazzaro et al. 2013). Based on the signalling molecules and sensing mechanism, three major classes of QS were identified, and these include (i) Gram-negative LuxI/LuxR-like QS system that uses AHLs as signalling molecules (Fuqua et al. 1994); (ii) Gram-negative *V. harveyi*-like two-component signalling circuits that recognize three different signalling molecules, AHLs, FBD, and an uncharacterized Cal-1 molecule (Bassler et al. 1993; Henke and Bassler 2004); (iii) Gram-positive two-component signalling systems that use modified oligopeptides as AIs (Lazazzera and Grossman 1998).

Periodontitis is a worldwide prevalent oral disease which occurs due to the dysbiosis of the periodontal microbiome (Deng et al. 2018). Marsh (1994) suggested that oral diseases especially dental caries and periodontal disease should be considered as consequences of imbalance in oral microbial biofilms which is ecologically driven. Both of these diseases are not caused by classic microbial pathogens, but by the microorganisms residing in the oral microflora. Hence, most individuals harbour the microorganisms causing these diseases. A population of acid-tolerant and acid-producing strains such as *S. mutans* and *Lactobacilli* is favoured at a low pH

environment in case of dental caries caused by fermentation of carbohydrates. This increased acid formation assisted by acid-producing bacteria in turn leads to demineralization. In case of periodontal disease, mixed anaerobic microorganisms were found to be involved; periodontal disease occurs when there is alteration in plaque community which further leads to inflammation. Growth of periodontal pathogens is favoured by an increased flow of gingival crevicular fluid, increased nutrients and the rise in pH (Marsh 1994). In order to maintain good oral health and to prevent dental caries, gingivitis and periodontitis, it is very essential to control oral biofilms. However, it is not so easy to control oral biofilms by mechanical means, and there are difficult targets for chemically controlling this phenomenon (Socransky 2002). It was observed that only few accessible oral prophylactic agents' apart from chlorhexidine and fluorides comprise significant effects in checking the oral biofilms (Petersen and Scheie 1998; Wu and Savitt 2002; Scheie 2003). This low efficacy is attributed for the fact that microorganisms involved in the biofilm formations are complex and show intricate resistance. Keeping the recent studies in view, development of novel strategies to prevent and treat diseases caused by oral biofilms might benefit to understand microbial gene expression and regulation in oral biofilms.

10.2 Quorum Sensing: A Mode of Communication in Oral Biofilms

Biofilms represents a natural scenario for bacterial communication (Davey and O'Toole 2000; Kolenbrander et al. 2002). QS was identified in many bacterial species; however, there are many aspects related to this process which need to be explored further. It was reported that the molecular mechanisms of QS differ between Gram-positive and Gram-negative microorganisms (Håvarstein et al. 1996; Cvitkovitch 2001). The involvement of two-component signal transduction systems in oral biofilm formation was first evidenced in *S. gordonii* (Loo et al. 2000) (Table 10.1). Notably, it was observed that biofilm formation-deficient *S. gordonii* mutants were obtained through the disruption of *com*D, which encodes for the histidine kinase receptor and binds the competence-stimulating signal peptide (CSP). This QS peptide was described to be an inducer of competence for natural transformation (Håvarstein et al. 1996, 1997). Biofilm formation deficiency was observed in *S. mutans* on inactivation of the gene encoding the CSP and other competence-related genes like *com*C/*com*E (Bhagwat et al. 2001; Li et al. 2002; Yoshida and Kuramitsu 2002). Later, it was suggested that the competence system might play a fundamental role in sensing and eliciting responses to environmental stress conditions (Yother et al. 2002). In *S. mutans*, the competence-related signalling system was related to the ability to adapt acidic conditions (Li et al. 2001b) (Table 10.1). AI-like activity was found to be present in several putative microorganisms that cause periodontal disease (Frias et al. 2001). It is notable that *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* possess AI-2 activity (Frias et al. 2001). Presence of highly conserved homologues of the AI-2 synthase gene, *luxS*, was confirmed in *S. mutans*, *S. gordonii*, *P. gingivalis* and *A.*

Table 10.1 Signaling molecules in dental pathogens

Bacterium	Signal	Phenotype	References
Streptococcus mutans	CSP[a]	Biofilm formation, natural transformation, acid tolerance, cell separation	Li et al. (2001a, b, 2002), Petersen and Scheie (2000) and Yoshida and Kuramitsu (2002)
Streptococcus gordonii		Biofilm formation, natural transformation	Håvarstein et al. (1996, 1997) and Loo et al. (2000)
Streptococcus mitis		Natural transformation	Håvarstein et al. (1997) and Whatmore et al. (1999)
Streptococcus oralis		–	Håvarstein et al. (1997) and Whatmore et al. (1999)
Streptococcus sanguis		–	Håvarstein et al. (1997)
Streptococcus crista		–	Håvarstein et al. (1997)
Streptococcus anginosus		–	Håvarstein et al. (1997)
Streptococcus intermedius		–	Håvarstein et al. (1997)
Streptococcus mutans	AI-2[b]	Natural transformation, regulation of the stress response or homeostasis, biofilm structure	Yother et al. (2002) and Merritt et al. (2003)
Streptococcus gordonii		Mixed-species biofilm formation	McNab et al. (2003)
Porphyromonas gingivalis		Mixed-species biofilm formation, protease and hemagglutinin activities	Burgess et al. (2002), Chung et al. (2001) and McNab et al. (2003)
Fusobacterium nucleatum		Unknown	Frias et al. (2001)
Prevotella intermedia		Unknown	Frias et al. (2001)
Actinobacillus actinomycetemcomitans		Adaptation to iron-limited conditions, leukotoxin production	Fong et al. (2001, 2003) and Frias et al. (2001)
Streptococcus salivarius	SalA[c]	Bacteriocin production	Upton et al. (2001)

[a]CSP = Competence-stimulating peptide encoded by comC
[b]AI-2 = Autoinducer 2, hypothetically synthesized by luxS
[c]SalA, Salivaricin A, bacteriocin encoded by salA

actinomycetemcomitans through DNA sequence analysis (Wen and Burne 2002; Merritt et al. 2003; McNab et al. 2003; Chung et al. 2001; Frias et al. 2001; Burgess et al. 2002; Fong et al. 2001, 2003). Remarkably, it was observed that amount of single-species biofilm doesn't get influenced by the inactivation of homologous AI-2 synthase in *S. mutans* (Wen and Burne 2002; Merritt et al. 2003) and *S. gordonii* and (McNab et al. 2003). AI-2 response system helps in interspecies communication, thus exploring its role in mixed biofilm communities would be fascinating. Recently, it was reported by Szafrański et al. (2017) that the complete QS regulon of *S.mutans* was induced by *Aggregatibacter actinomycetemcomitans* a pathogen in oral cavity by an unknown mechanism which needed the presence of ComS, the synthase for the XIP prepeptide. *A. actinomycetemcomitans* was found to grow in a highly virulent form but it down regulated the genes essential for its escape from the host immune response in co-culture with *S.mutans.* The presence of active QS

regulon in vivo was observed through the expression of transformasome and mutacin genes of *S.mutans* in periodontal pockets. Polymicrobial communities harbouring *Streptococci* and *Aggregatibacter* spp. are conscientious for oral infectious diseases. Thus, the above-mentioned interactions may have a crucial role in the dysbiosis of such communities (Szafrański et al. 2017) (Table 10.1).

10.3 Bacterial Diversity in Oral Biofilms

Oral biofilms possess more than 1000 species of bacteria; among these, 50% of the species are culturable, while others can be identified only through molecular methods. Many of the unique bacterial species are found in the oral cavity (Jacob 2006). Oral bacteria have the ability to adhere to the solid surfaces of teeth. Oral biofilms are not uniform and tend to vary in community structure and dynamics from time to time. Surprisingly, it was found that there is a varying diversity of bacterial species in samples from different locations of the oral cavity (Auschill et al. 2004). Nevertheless, it was also observed that there is a difference in the bacterial viability at different sites in the oral cavity (Arweiler et al. 2004). It was observed that early dental plaque is dominated mostly by *Streptococcus* species (60–90%) and other species that are present include *Eikenella* spp., *Haemophilus* spp., *Prevotella* spp., *Capnocytophaga* spp., *Propionibacterium* spp. and *Veillonella* spp. (Nyvad and Kilian 1987). In healthy subjects, *Actinomyces* spp. was reported to be the predominant species after 2 h of biofilm formation and after 6 h of biofilm formation. *S. oralis* and *S. mitis* become the most prevalent bacteria. In a co-culturing experiment, *Actinomyces* spp. was found to form a mixed biofilm with *Lactobacillus* spp. thus promoting its growth (Filoche, Anderson and Sissons, Filoche et al. 2004). Bacterial adhesion factors (surface proteins) are used by bacteria in biofilms to adhere to surfaces and to each other through specific physical interactions. Salivary components, for example, proline-rich proteins (PRP), are crucial for the binding of bacteria. Some of the late colonizing species in oral biofilm include *A. actinomycetemcomitans*, *Prevotella intermedia*, *Eubacterium* spp., *Treponema* spp. and *Porphyromonas gingivalis* (Kolenbrander et al. 2002). *Fusobacterium nucleatum* serves as a link between early and late colonizers. It was reported that older biofilms are more often pathogenic, probably due to higher population of the late colonizers.

10.4 Bacterial Interaction in Oral Biofilms

With such a diverse bacterial population in the oral cavity, it was suggested that there is an interaction between bacteria which ensures their individual survival (Jacob 2006). In the absence of synergistic and competitive interactions, the survival of the bacterial species depends on their individual growth rates. As the growth rates differ from species to species, such a condition would lead to the survival of just a few species; however, the scenario is different in reality; many bacteria do coexist in the biofilms (Chesson 2000). By various mechanisms, bacteria cooperate

and compete which results in temporal changes in the composition of a biofilm, but they eventually form the climax community, and therefore significance of such a community in relation to health should not be underrated (Jacob 2006). Tissue pathology plays a crucial role in determining the shifts in the composition of oral biofilms, and this is in accordance with the ecological plaque hypothesis (Marsh 1994, 2003).

10.5 Oral Biofilms

Diverse biofilms harbour in the oral cavity. The substrates for the biofilms in the oral cavity include the natural dentition and dental prostheses such as dentures and implants.

10.5.1 Dental Plaque: The Most Common Biofilm

Even though the oral cavity is exposed to air, once the surface of the teeth is colonized by the bacteria the tooth surface may become anaerobic (Donlan and Costerton 2002). Dental plaque is a polymicrobial biofilm and causal agent for dental caries and periodontal disease. (Morse et al. 2018). Gingival crevice fluid (GCF) acts as a main source of nutrients (proteins and glycoproteins) for the development of plaque within the subgingival crevice. Once the oral biofilm develops, proteolytic enzymes are produced that directly damage soft and hard tissues; these enzymes also tend to interfere with the host defence mechanisms (Donlan and Costerton 2002).

10.5.2 Dentures

Candida albicans, a pathogenic fungus is usually associated with dentures. Denture wearers are thus at a risk of developing denture stomatitis, an inflammation of the oral tissues (Susewind et al. 2015). It was found that more than 65% of denture wearers have stomatitis. Stomatitis contributes to poor oral health and systemic diseases (Preshaw et al. 2011).

10.5.3 Implants

Dental implants are used to replace missing teeth, and these are synthetic structures made of titanium and plastic materials. Individuals with implants tend to develop infections and inflammation near the implant region. Some bacteria that are associated with implants adhere to the rough surfaces of implants and form a biofilm (Schaumann et al. 2014). Inflammation occurs due to the development of pathogenic biofilms on the implant surfaces.

10.6 The Oral Metagenome in Health and Disease

The human oral cavity is a complex ecosystem of microbes that harbours hundreds of bacterial species, and some of them are responsible for the development of oral diseases such as dental caries and periodontal disease. Belda-Ferre et al. (2012) for the first time described the metagenome of the oral cavity under health and diseased conditions. These researchers focused on supragingival dental plaque and cavities. The results of this study revealed that the cavities were not dominated by *S. mutans* which is otherwise the aetiological agent for dental caries to a certain extent it contained a complex community comprised of numerous bacterial species thus supporting the view that dental caries is a polymicrobial disease. Healthy individuals who never suffered from dental caries exhibited an overrepresentation of several functional categories such as genes for antimicrobial peptides and QS. Moreover, there was absence of *S. mutans* in healthy individuals, but they had other bacterial species in high numbers. Some of these dominant bacteria in healthy individuals were cultured and it was evidenced that these bacterial species inhibit the growth of cariogenic bacteria, hence, suggesting the use of such commensal bacteria as probiotics to promote oral health and check dental caries. One more study conducted by Liu et al. (2012) has revealed that oral microbiome of individuals suffering from periodontal disease was enriched in virulence factors and adapted to a parasitic lifestyle that takes advantage of the disrupted host homeostasis.

10.7 The Resistance of Biofilms to Antimicrobials: A Medical Challenge

Biofilms have been found to resist the traditional antimicrobial therapy. Biofilm-associated infections pose an immense challenge to the world of medicine as these are accountable for causing many chronic infections (Berger et al. 2018). Nonetheless, few diseases that are caused due to the biofilm formation on host's tissues include otitis media, cystic fibrosis, valve endocarditis and periodontal disease. With the increasing use of medical implants particularly dentures, the prevalence of biofilm-associated infections are drastically escalating (Dye et al. 2015). Elderly people who make use of dentures are at higher risk of developing biofilm-associated infections as they don't clean their dentures appropriately; hence, it leads to oral infections like denture stomatitis and sometimes other fatal systemic infections like chronic obstructive pulmonary disease (COPD) (Neppelenbroek 2015).

The increased antibiotic tolerance by the biofilms has been attributed to (i) the failure of antimicrobial agent to reach the target; (ii) degradation of enzymes in antibiotics in biofilm matrix; (iii) change in the pattern of gene expression; and (iv) persistence of the cells in mature biofilm (Carpentier 1999; Costerton et al. 1999; Costerton 2007). Signalling molecules which have been named as "alarmones" released from killed cells are thought to produce signals in biofilms communities so as to alert underlying deep cells to go into a state of resistance (Gilbert et al. 1993).

10.8 Control of Oral Biofilm Through Quorum Quenching (QQ)

QQ refers to all processes involved in the disruption of QS (Dong et al. 2001). QQ helps in the inhibition of extracellular signalling via:

(i) Avoiding the homoserine lactone (HSL) and decreasing the concentration of extracellular HSL by QQ enzymes called lactonases that are produced by different *Bacillus* species (coded by aiiA gene) degrading the signal molecules (Dong et al. 2002). There are two main classes of enzymes (AHL lactonases and AHL acylases) that cause degradation of signal molecules and act as useful tools in controlling the virulence of many pathogenic organisms (Dong et al. 2002; Czajkowski and Jafra 2009).
(ii) Use of competitive molecules (with homologous structure) in inhibiting the signal reception, such molecules are natural produced by *V. fischeri*, *A. tumefaciens*, *C. violaceum* and *A. salmonicida* or can be produced synthetically. Synthetic biology can be a good emerging application of QS circuits, wherein cells can be engineered with desirable functions (Jayaraman and Wood 2008). Some molecules that are uncompetitive with different chemical structure have also been found to interfere with HSL binding to the sensor protein.

Quorum sensing inhibitors/quorum quenchers (QSI) provide an ecological strategy to fight against the biofilm-associated infections via inhibition of intercellular signalling by QS. Some QSI have been found to be naturally produced by highly evolved plants. *Delisea pulchra*, an endemic species of algae that grows in Australia was found to produce halogenated furanones which thwart bacterial aggregation and thus checks biofilm formation (Manefield et al. 1999). A library of more than 200 furanones and furanone analogues were developed using synthetic approaches (on mouse models) with potent antipathogenic properties both in vitro and in vivo (De Nys et al. 2006). Lichens (symbionts) were found to produce metabolites (usnic acid, barbatic acid) with antimicrobial properties towards dental plaque-associated *S. mutans* as well as on whole plaque (Ghione et al. 1988; Grasso et al. 1989; Francolini et al. 2004; Chifiriuc et al. 2009). Some aquatic and terrestrial plants were also found to produce QSI (*Nimphaea* sp., *Allium sativum*, *Pisum sativum*, *Capsicum anuum*, etc.) (Hentzer and Givskov 2003). In animals, QSI were also identified as lactonases like molecules in mammalian serum and QSI with antiinfectious properties in epithelial cells (Yang et al. 2005). Antipathogenic compounds that inhibit QS pathways can be used as an alternative to antibiotics predominantly against the infections caused by resistant strains. Weakening the bacterial virulence by QSI than by antibiotics is an intelligent strategy and avoids risk of inducing resistance (Suga and Smith 2003; Costerton et al. 2007). Thus, novel QSI molecules need biosprospection in nature via screening different organisms from varied environments so as to find potent QSI to fight antimicrobial resistance in biofilms.

10.9 Conclusions and Future Perspectives

Biofilms have a huge impact on almost all aspects of our lives especially health. As we age, there are increased chances that we need hospitalization at some stage or the other, and we may have to use biomedical implants like dental implants, vascular grafts, contact lenses and orthopaedic implants. It has been observed that biofilms form on such implants, and these biofilms often contain pathogenic microorganisms that can pose a threat to human health. Biomedical technologies use disinfectant rinses or release antibiotics that have hardly helped in reducing such biofilm-associated infections and may possibly have contributed to the increase in the antibiotic resistance. During the last decade, there has been a lot of research on biofilms, and many targets have been identified for disrupting biofilms through the use of QSI/QQ and thus reducing infections. Further, advancements in understanding of biofilm formation may provide potential strategies to significantly reduce biofilm infections.

References

Arweiler NB, Hellwig E, Sculean A, Hein N, Auschill TM (2004) Individual vitality pattern of in situ dental biofilms at different locations in the oral cavity. Caries Res 38:442–447

Auschill TM, Hellwig E, Sculean A, Hein N, Arweiler NB (2004) Impact of the intraoral location on the rate of biofilm growth. Clin Oral Investig 8:97–101

Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Intercellular signalling in Vibrio harveyi: sequence and function of genes regulating expression of luminescence. Mol Microbiol 9(4):773–786

Belda-Ferre P, Alcaraz LD, Cabrera-Rubio R, Romero H, Simon-Soro A, Pignatelli M, Mira A (2012) The oral metagenome in health and disease. ISME J 6(1):46

Berger D, Rakhamimova A, Pollack A, Loewy Z (2018) Oral biofilms: development, control, and analysis. High-throughput 7(3):24

Bhagwat SP, Nary J, Burne RA (2001) Effects of mutating putative two-component systems on bioflm formation by Streptococcus mutans UA159. FEMS Microbiol Lett 205:225–230

Burgess NA, Kirke DF, Williams P, Winzer K, Hardie KR, Meyers NL et al (2002) LuxS-dependent quorum sensing in *Porphyromonas gingivalis* modulates protease and haemagglutinin activities but is not essential for virulence. Microbiology 148(Pt 3):763–772

Carpentier B (1999) Les biofilms (2). Bull Soc Fr Microbiol 14:105e11

Chesson P (2000) General theory of competitive coexistence in spatially varying environments. Theor Popul Biol 58:211–237

Chifiriuc MC, Ditu LM, Oprea E, Litescu S, Bucur M, Marutescu L et al (2009) In vitro study of the inhibitory activity of usnic acid on dental plaque biofilm. Roum Arch Microbiol Immunol 68(4):215–222

Chung WO, Park Y, Lamont RJ, McNab R, Barbieri B, Demuth DR (2001) Signaling system in *Porphyromonas gingivalis* based on a LuxS protein. J Bacteriol 183:3903–3909

Costerton JW (2007) Biofilms: the bacterial way to persist, abstracts book of the international symposium and the 43rd ESCMID post-graduate course- bacterial adaptation mechanisms: biofilms, hypermutability and antibiotic resistance. Springer, Singapore p 9

Costerton JW, Steward PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318e22

Costerton JW, Montanaro L, Arciola CR (2007) Bacterial communication in implants infections: a target for an intelligence war. Int J Artif Organs 30:757e63

Cvitkovitch DG (2001) Genetic competence and transformation in oral streptococci. Crit Rev Oral Biol Med 12:217–243
Czajkowski R, Jafra S (2009) Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules. Acta Biochim Pol 56:1e16
Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867
De Nys R, Givskov M, Kumar N, Kjelleberg S, Steinberg PD (2006) Furanones. In antifouling compounds. Springer, Berlin/Heidelberg, pp 55–86
Deng ZL, Sztajer H, Jarek M, Bhuju S, Wagner-Döbler I (2018) Worlds apart–transcriptome profiles of key oral microbes in the periodontal pocket compared to single laboratory culture reflect synergistic interactions. Front Microbiol 9:124
Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH (2001) Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 411(6839):813
Dong YH, Gusti AR, Zhang Q, Xu JL, Zhang LH (2002) Identification of quorum quenching N-Acyl homoserine lactonases from *Bacillus* species. Appl Environ Microbiol 68:1754e9
Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
Dye BA, Thornton-Evans G, Li X, Iafolla TJ (2015) Caries and tooth loss in adults in the United States, 2011–2012; Data Brief No. 197. National Center for Health Statistics, Hyattsville
Filoche SK, Anderson SA, Sissons CH (2004) Biofilm growth of *Lactobacillus* species is promoted by *Actinomyces species* and *Streptococcus mutans*. Oral Microbiol Immunol 19:322–326
Fong KP, Chung WO, Lamont RJ, Demuth DR (2001) Intra- and interspecies regulation of gene expression by *Actinobacillus actinomycetemcomitans* LuxS. Infect Immun 69:7625–7634
Fong KP, Gao L, Demuth DR (2003) luxS and arcB control aerobic growth of *Actinobacillus actinomycetemcomitans* under iron limitation. Infect Immun 71:298–308
Francolini P, Norris P, Piozzi A, Donelli G, Stoodley P (2004) Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agents Chemother 48(11):4360e5
Frias J, Olle E, Alsina M (2001) Periodontal pathogens produce quorum sensing signal molecules. Infect Immun 69:3431–3434
Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176(2):269
Ghione M, Parrello D, Grasso L (1988) Usnic acid revisited, its activity on oral flora. Chemioterapia 7(5):302–305
Gilbert P, Evans DJ, Brown MRW (1993). Formation and dispersal of bacterial biofilms *in vivo* and *in situ*, J Appl Bacteriol (Symp Suppl.). In: Quesnel LB, Gilbert P, Handley Pauline S (eds) Microbial cell envelopes: interactions and biofilms, vol 74. Blackwell Scientific Publications, Oxford, p 67S–78S
Grasso L, Ghirardi PE, Ghione M (1989) Usnic acid, a selective antimicrobial agent against *Streptococcus mutans*: a pilot clinical study. Curr Ther Res 10:67–70
Håvarstein LS, Gaustad P, Nes IF, Morrison DA (1996) Identification of the streptococcal competence-pheromone receptor. Mol Microbiol 21:863–869
Håvarstein LS, Hakenbeck R, Gaustad P (1997) Natural competence in the genus *Streptococcus*: evidence that *streptococci* can change pherotype by interspecies recombinational exchanges. J Bacteriol 179:6589–6594
Henke JM, Bassler BL (2004) QS regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. J Bacteriol 186:3794–3805
Hentzer M, Givskov M (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 112:1300
Jacob M (2006) Biofilms, a new approach to the microbiology of dental plaque. Odontology 94(1):1–9
Jayaraman A, Wood TH (2008) Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. Annu Rev Biomed Eng 10:145–167

Johnson MR et al (2005) Population density-dependent regulation of exopolysaccharide formation in the hyperthermophilic bacterium *Thermotoga maritima*. Mol Microbiol 55:664–674

Kiran GS, Hassan S, Sajayan A, Selvin J (2017) Quorum quenching compounds from natural sources. In bioresources and bioprocess in biotechnology. Springer, Singapore, pp 351–364

Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr (2002) Communication among oral bacteria. Microbiol Mol Biol Rev 66:486–505

Lazazzera BA, Grossman AD (1998) The ins and outs of peptide signaling. Trends Microbiol 6(7):288–294

Li YH, Lau PC, Lee JH, Ellen RP, Cvitkovitch DG (2001a) Natural genetic transformation of Streptococcus mutans growing in biofilms. J Bacteriol 183:897–908

Li YH, Hanna MN, Svensäter G, Ellen RP, Cvitkovitch DG (2001b) Cell density modulates acid adaptation in *Streptococcus mutans*: implications for survival in biofilms. J Bacteriol 183:6875–6884

Li YH, Tang N, Aspiras MB, Lau PC, Lee JH, Ellen RP et al (2002) A quorum-sensing signaling system essential for genetic competence in *Streptococcus mutans* is involved in biofilm formation. J Bacteriol 184:2699–2708

Liu B, Faller LL, Klitgord N, Mazumdar V, Ghodsi M, Sommer DD et al (2012) Deep sequencing of the oral microbiome reveals signatures of periodontal disease. PLoS One 7(6):e37919

Llamas I, Keshavan N, Gonzalez JE (2004) Use of *Sinorhizobium meliloti* as an indicator for specific detection of long-chain N-acyl homoserine lactones. Appl Environ Microbiol 70:3715–3723

Loo CY, Corliss DA, Ganeshkumar N (2000) *Streptococcus gordonii* biofilm formation: identification of genes that code for biofilm phenotypes. J Bacteriol 182:1374–1382

Manefield M, de Nys R, Naresh K, Roger R, Givskov M, Peter S, Kjelleberg S (1999) Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology 145(2):283–291

Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 8:263–271

Marsh PD (2003) Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 8:263–271

McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ (2003) LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. J Bacteriol 185:274–284

Merritt J, Qi F, Goodman SD, Anderson MH, Shi W (2003) Mutation of luxS affects biofilm formation in *Streptococcus mutans*. Infect Immun 71:1972–1979

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Ann Rev Microbiol 55(1):165–199

Morse DJ, Wilson MJ, Wei X, Lewis MAO, Bradshaw DJ, Murdoch C, Williams DW (2018) Denture-associated biofilm infection in three-dimensional Oral mucosal tissue models. J Med Microbiol 67:364–375

Nazzaro F, Fratianni F, Coppola R (2013) Quorum sensing and phyto-chemicals. Int J Mol Sci 56:12607–12619

Neppelenbroek KH (2015) The importance of daily removal of the denture biofilm for oral and systemic diseases prevention. J Appl Oral Sci 23:547–548

Nyvad BE, Kilian MO (1987) Microbiology of the early colonization of human enamel and root surfaces in vivo. Scand J Dent Res 95(5):369–380

Paggi RA, Martone CB, Fuqua C, de Castro RE (2003) Detection of quorum sensing signals in the haloalkaliphilic archaeon *Natronococcus occultus*. FEMS Microbiol Lett 221:49–52

Petersen FC, Scheie AA (1998) Chemical plaque control: a comparison of oral health care products. In: Busscher HJ, Evans LV (eds) Oral biofilms and plaque control. Harwood Academic Publisher, Amsterdam, pp 277–293

Petersen FC, Scheie AA (2000) Genetic transformation in *Streptococcus mutans* requires a peptide secretion-like apparatus. Oral Microbiol Immunol 15:329–334

Preshaw PM, Walls AWG, Jakubovics NS, Moynihan PJ, Jepson NJA, Loewy Z (2011) Association of removable partial denture use with oral and systemic health. J Dent 39:711–719

Rivas M, Seeger M, Jedlicki E, Holmes DS (2007) Second acylhomoserine lactone-producing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 73:3225–3231

Romero M, Martin-Cuadrado AB, Roca-Rivada A, Cabello AM, Otero A (2011) Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. FEMS Microbiol Ecol 75:205–217

Schaumann S, Staufenbiel I, Scherer R, Schilhabel M, Winkel A, Stumpp SN, Eberhard J, Stiesch M (2014) Pyrosequencing of supra- and subgingival biofilms from inflamed peri-implant and periodontal sites. BMC Oral Health 14:1–157

Scheie AA (2003) The role of antimicrobials. In: Fejerskov O, Kidd E (eds) Dental caries. The disease and its clinical management. Blackwell Munksgaard, Oxford, pp 179–189

Socransky S (2002) Dental biofilms: difficult therapeutic targets. Periodontol 2000(28):12–15

Suga H, Smith KM (2003) Molecular mechanisms of bacterial quorum sensing as a new drug target. Curr Op Chem Biol 7:586–591

Susewind S, Lang R, Hahnel S (2015) Biofilm formation and *Candida albicans* morphology on the surface of denture base materials. Mycoses 58:719–727

Szafrański SP, Deng ZL, Tomasch J, Jarek M, Bhuju S, Rohde M et al (2017) Quorum sensing of Streptococcus mutans is activated by *Aggregatibacter actinomycetemcomitans* and by the periodontal microbiome. BMC Genomics 18(1):238

Tang K, Zhang XH (2014) Quorum quenching agents: resources for antivirulence therapy. Mar Drugs 12(6):3245–3282

Upton M, Tagg JR, Wescombe P, Jenkinson HF (2001) Intra- and interspecies signaling between *Streptococcus salivarius* and *Streptococcus pyogenes* mediated by SalA and SalA1 lantibiotic peptides. J Bacteriol 183:3931–3938

Wen ZT, Burne RA (2002) Functional genomics approach to identifying genes required for biofilm development by *Streptococcus mutans*. Appl Environ Microbiol 68:1196–1203

Whatmore AM, Barcus VA, Downson CG (1999) Genetic diversity of the streptococcal competence (com) gene locus. J Bacteriol 75:3144–3154

Wu C, Savitt E (2002) Evaluation of the safety and efficacy of over the-counter oral hygiene products for the reduction and control of dental plaque and gingivitis. Periodontol 28:91–105

Yang F, Wang LH, Wang J, Dong YH, Yong H, Jiang Z (2005) Quorum quenching enzyme activity is widely conserved in the sera of mammalian species. FEBS Lett 579:3713e7

Yoshida A, Kuramitsu HK (2002) Multiple *Streptococcus mutans* genes are involved in biofilm formation. Appl Environ Microbiol 68:6283–6291

Yother J, Trieu-Cuot P, Klaenhammer TR, De Vos WM (2002) Genetics of *streptococci*, *lactococci*, and *enterococci*: review of the sixth international conference. J Bacteriol 184:6085–6092

Biofilm Formation on Ophthalmic Device-Related Infections: Insights on Clinical Implications

11

Kishore Kumar Godisela and Pallaval Veera Bramhachari

Abstract

Biofilms are structured communities of microorganisms which are encased within a self-produced matrix attached to surface of abiotic or biotic. There is growing evidence that bacterial biofilms play a major lead in a range of ocular infections. The presence of biofilms has been established on most indwelling ophthalmic devices such as intraocular lenses, scleral buckles, contact lenses and suture materials. Lack of poor lens hygiene leads to infections of soft lenses that are at high risk than other types of lenses. *Pseudomonas* spp. is gram-negative bacteria predominant on contact lenses. *Serratia* spp. and *Staphylococcus* spp. are the next dominant microorganisms in the eye. The biofilm of these organisms led to activation of various signalling cascades which cause permanent vision loss in humans. The strategy of preventing the microbial attachment and biofilm formation by utilizing single-cell repellent surfaces is the ideal choice. Natural and man-made anti-biofilm compounds have previously been discovered to address this problem. There is a large requirement for improvement of anti-biofilm formulations to control the post-surgery eye medical devices. The organoselenium polypropylene is the one which demonstrates the capacity to decrease biofilm formation. The utilization of organoselenium copolymer assumes an indispensable job in securing against contact focal point. Nisin poly-

All authors contributed equally

K. K. Godisela (✉)
SRR Government Arts & Science College, Karimnagar, Telangana, India

Government Degree & PG College for Men, Adilabad, Telangana, India
e-mail: kishorg818@gmail.com

P. V. Bramhachari
Department of Biotechnology, Krishna University,
Machilipatnam, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_11

propylene material showed to stop the biofilm formation of *S. epidermidis*. The review emphasizes on biofilm formation on ophthalmic devices and advanced developments in the anti-biofilm materials for better vision.

Keywords
Biofilm · Contact lens · Organoselenium polypropylene · Nisin polypropylene

11.1 Introduction

Most of the bacterial infections involve biofilms. Cluster of microorganisms that manufacture biofilms are found to be connected with the biotic and abiotic surfaces. Biofilms are single or multilayered (Karatan and Watnick 2009) and contain either consistent or heterogeneous populations of microorganism. These stay inside the framework of extracellular polymeric substances discharged by constituent populace of biofilms. They exhibit amazingly complicated multicellular behaviours that are coordinated by cell-to-cell signalling networks. Biofilms are an expanding issue of worry that is picking up significance as time passes. Biofilms also appear on therapeutic gadget surfaces, and spread of single and grouped cells suggests a tremendous danger of microorganism scattering among the host and intensified danger of contamination. Organisms within a biofilms are problematic to kill by conventional antimicrobial medical aid and might cause indolent infections. This chapter reviews the biofilm formation on varied ophthalmic devices, their role within the disease process and prevention ways.

The primary portrayal initiated by van Leeuwenhoek on restorative microbial biofilms outlined perceptions made on dental plaque. By observant that he may solely kill tiny proportion of the microorganisms adhering to his teeth, attributable to acknowledge natural resistance of microorganism during an exposure of a biocide, ethanoic acid (Hall-Stoodley et al. 2004). In fact, it took three additional centuries for Koch to explain the scientific theory for disease. The frequency of infections caused by biofilms has been thought to be from 65% to 80%. It has taken an additional century for the restorative significance of biofilms to be perceived in spite of its measurable contribution over 80% of bacterial contamination (Health 2007, November 12). Furthermore, the ibofilm examination procedures such as microwell plate assay, Calgary Device, confocal laser scanning microscopy, Bioflux device and atomic force microscopy have become progressively common in medicinal research (Berger et al. 2018).

11.2 Indwelling Devices and Biofilm Infections

One of the essential clinical diseases identified with biofilm formation was therapeutic device-related contaminations. Medical device-related infections create an enormous monetary burden on care services and are related to accrued patient morbidity and mortality (Donlan 2008). Healthcare-related diseases will happen in consideration homes, clinics or in a patient's home (van Kleef et al. 2013), with a predominance dimension of 6.4% and 1,000,000 cases detailed each year (HPA 2011). Among one million cases reported, an expected 60% of emergency clinic-related contaminations are due to biofilms that have formed on inhabiting devices (Darouiche 2004). Ophthalmic device-associated biofilm diseases in emergency clinic remain, by and large, a few days (Archibald and Gaynes 1997). Attributable to the maturing populace and the expanding assortment of implantable medical devices, contamination related to biofilms is feared to broaden. Many of the medical devices are contaminated with different types of biofilms (Marrie et al. 1982; Gristina and Costerton 1984, 1985; Webb et al. 1986). The occurrence of bacterial biofilms has been reported on several therapeutic devices such as intrauterine devices, prosthetic heart valves, urinary catheters, neurosurgical ventricular shunts, ventricular assist devices, intravascular catheters, prosthetic joint, coronary stents, cochlear implants and intraocular and contact lenses (Donlan and Costerton 2002; Bispo et al. 2015) (Fig. 11.1).

Biofilms on indwelling medical devices could be made out of gram-positive or gram-negative microorganisms or yeasts. Microbes usually detached from these devices include gram-positive *Staphylococcus epidermidis*, *Enterococcus faecalis*,

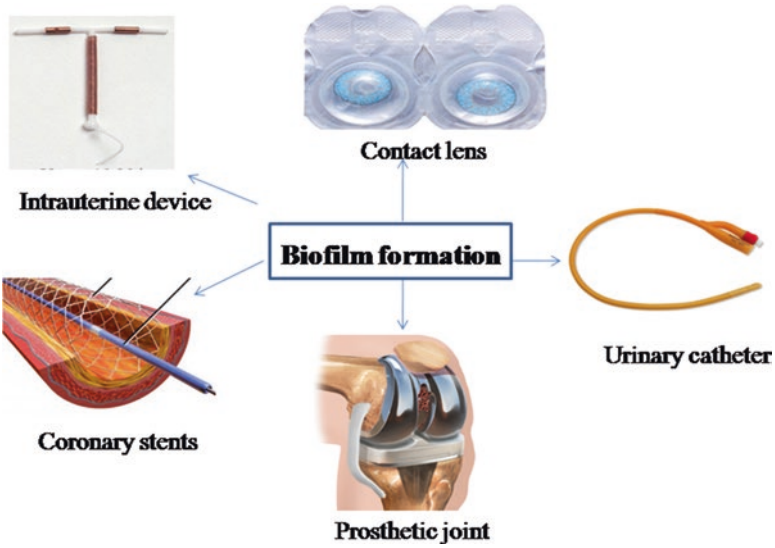


Fig. 11.1 The presence of bacterial biofilms has been reported on several medical devices such as intrauterine devices, urinary catheters, prosthetic joint, coronary stents and contact lenses

Table 11.1 Examples of vision-related infections to location and type of foreign body

Foreign body	Location	Infection	Organisms
Penetrating trauma, superficial	Eyelid area	Eyelid abscess	*Staphylococcus aureus*
Contact lens	Anterior segment	Keratitis	*Staphylococcus species*, *Pseudomonas species* and Fungi
Punctal plugs	Lacrimal duct	Conjunctivitis	*Pseudomonas aeruginosa*
Silicone sponge implants	Conjunctiva and sclera	Posterior segment	*Staphylococcus* sp.
Intraocular lens	Anterior segment	Pseudophalic endophthalmitis	*Staphylococcus* sp.
Penetrating trauma, deep	Crosses anterior to posterior segment	Endophthalmitis, orbital abscess	*Staphylococcus* sp., *Bacillus* sp. and *Fungi*

Staphylococcus aureus and *Streptococcus viridans*, and the gram-negative *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. This biofilms arrangement might be analysed by electron microscopy when upgraded by recolouring with ruthenium red (Springer and Roth 1973).

Table 11.1 represents the most common ocular-associated infections in human.

11.3 Factors Influencing Biofilm Formation on Ophthalmic Devices

Biofilm formation on indwelling therapeutic devices can be determined by several variables (biotic and abiotic factors). Biofilm arrangement is a multistep procedure and adhesion to ophthalmic gadgets varies between different genera/species/strains of microscopic organisms. Microorganisms ought to adhere to the exposed surfaces of the device sufficiently long to turn out to be irreversibly snared. The rate of cell connection relies upon the sum and sorts of cells inside the fluid from which the gadget is uncovered, the progression of fluid through the gadget, and the physico-chemical qualities of the surface. Segments in the fluid could adjust the surface properties and conjointly affect the rate of connection.

Once these cells irreversibly connect and fabricate extracellular polysaccharides to build up a biofilm, rate of development is affected by stream, surrounding temperature, and supplement creation of the medium and antimicrobial-medicate fixation. Common abiotic variables include pH scale, salt fixation, temperature, dampness, supplement availability, anti-microbial impacts and heavy metals (Fig. 11.2).

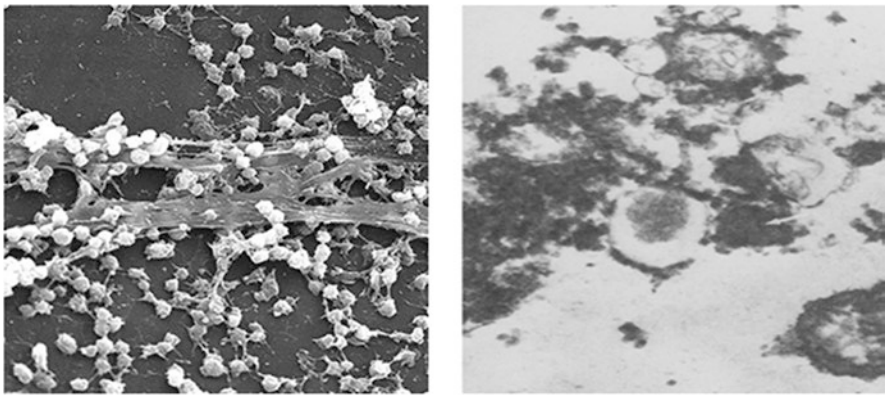


Fig. 11.2 TEM photomicrograph of an infected solid silicone buckling parts shows *Staphylococcus capitis* (**a**) sheltered in biofilms and biofilm formation of *Staphylococcus epidermidis* (**b**)

11.4 Biofilm Formation in Ophthalmological Devices

Biofilm formation on ophthalmic devices leads to various kinds of diseases like endophthalmitis, contact lens-associated keratitis and infectious crystalline keratopathy in humans. Many prosthetic device-associated ocular infections due to biofilm formation on biotic or abiotic materials fastened within the eye together with conjunctival infection and corrosion related to scleral buckles, endophthalmitis involving biofilms on intraocular lenses, keratoprosthesis and glaucoma drain implants and infectious crystalline keratopathy are reported (Zegans et al. 2005; Juarez-Verdayes et al. 2006; Ibanez et al. 2011).

11.5 Endophthalmitis

Plethora of reports state that clinical endophthalmitis treatment after cataract surgery is due to microorganisms intruding the site during surgery (Speaker et al. 1991; Sunderraj 1992). Staphylococcus epidermidis gram-positive bacteria conjure 76%–90% of the culture-positive cases of pseudo-endophthalmitis (Puliafito et al. 1982; Dickey et al. 1991). Improper handling of the surgical instruments can lead to rapid progression of the disease, which can cause an inflammation of the interior of the eye. Especially, cataract surgery and intraocular surgery with possible loss of vision and the eye itself are possible complications. Microorganisms adhere well to intraocular lenses due to electrostatic forces. During cataract surgery, simply wiping a lens around the wound will cause 26.7% of lenses to have viable organisms attached (Fisch et al. 1991).

Post-cataract endophthalmitis is primarily caused by gram-positive organisms from the microbiota of the eye surface. It is notable that Coagulase-negative Staphylococci (CoNS), especially epidermidis is caused by *Staphylococcus* (Benz et al. 2004; Schimel et al. 2013). Perhaps, there are two remedies available to manage endophthalmitis, and there is no single antibiotic treatment for all of the microbes isolated from eyes with endophthalmitis, and hence a combination of medical care is mostly counselled (Schimel et al. 2013). Hydrophobicity is consistently found to be a very important determinant of biofilm formation in different studies. Changing the surface to make it more hydrophilic may reduce early binding and robust staphylococcal biofilm development.

11.6 Contact Lens-Associated Keratitis

For patients, eye care practitioners and the contact lens industry, sight-threatening microbial keratitis associated with contact lens wear remains a serious concern. The use of contact lenses represents the most risk issue in developed countries for the event of microbial keratitis. Estimates of microbial keratitis in the United States suggested more than 30,000 cases annually (Pepose and Wilhelmus 1992). The incidence of contact lens-associated microbial keratitis has been shown to be impacted by the material of contact lens and conjointly by the damage schedule. Early epidemiological studies show a higher risk for the daily wear of soft contact lenses compared to the daily wear of rigid gas-permeable lenses (Cheng et al. 1999), and this risk was found higher for overnight wear soft contact lenses (Dart et al. 1991). Recently, a case-control study reported that silicone polymer hydrogel contact focal points and day-by-day expendable focal points were related with a higher rate of keratitis (Dart et al. 2008).

The increased threat of microbial keratitis in the contact lens is due to the lens that induces changes in the corneal epithelium that facilitate the movement of the organism to the ocular surface that would otherwise not be found during this niche and the limitation of natural clearance mechanisms (Fleiszig and Evans 2010; Willcox et al. 2010). There are two ways to infect the cornea and lead to keratitis with these contact lenses. One is the close interaction between the lens and the corneal epithelium that induces local changes, including hypercapnia and hypoxia, affecting the epithelium's ability to react to damage. This could result in compromising the exchange of tear fluids between the anterior and posterior sides of the lens, neutralizing the tear fluid's composition on the eye surface and limiting its antimicrobial properties (Fleiszig and Evans 2010; Willcox et al. 2010). However, the second approach provides a surface where microorganisms can fix and settle the surface as a biofilm, a source for microorganisms to develop into a previously damaged corneal epithelium (Willcox et al. 2010) (Fig. 11.3).

The capacity of microorganisms to stick to completely different contact lens materials has been established in vitro (Dutta et al. 2012) and is especially driven by surface hydrophobicity (Klotz et al. 1989; Bruinsma et al. 2001). *P. aeruginosa*, *S. aureus* and coagulase-negative *Staphylococci* (Tabbara et al. 2000) are three

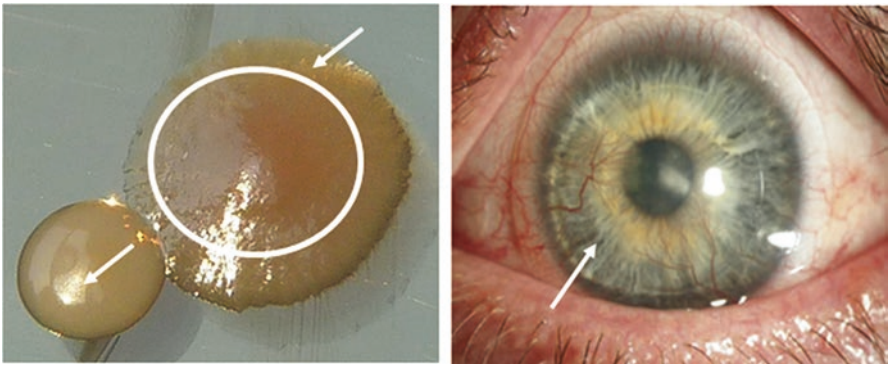


Fig. 11.3 Keratoconus patient with *P. aeruginosa* who developed an infection following hybrid contact lens wear

microorganisms predominant on contact lenses. It is a notable fact that, *P. aeruginosa* can easily stick to contact lenses, among any microorganisms tested so far, and this is presumably a reason that it is the most principal microbe that causes contact lens-associated keratitis (Hahn 1997). This bacterium produces many surface-associated adherence factors (adhesions) that promote epithelial cell attachment and contribute to the pathogen's virulence.

11.7 Scleral Buckle-Associated Infections

Scleral buckles are used in rhegmatogenic retinal detachments, where they are placed between the conjunctive and the sclera. Gram-positive cocci, especially coagulase-negative staphylococci, and nontuberculous mycobacterium often cause scleral buckle-associated infections (Smiddy et al. 1993; Pathengay et al. 2004). The presence of a biofilm within the explanted material was supposed to play a vital role in its pathogenesis due to the chronic evolution of this infection. From these buckle parts, more frequently gram-positive bacteria, and less frequently *Mycobacterium chelonae* and *Proteus mirabilis*, are found to grow (Holland et al. 1991). Scleral buckle infections tend to be relentless furthermore by being immune to antimicrobial treatment. Often, scleral buckle infections need exclusion of the buckling parts for resolution. Due to conjunctival erosion and infection, buckle materials are kept away from patients. The mechanism by which bacteria can persist and withstand antimicrobial treatment could also be the development of biofilms on scleral buckles. This mechanism would also describe the need to remove an infected buckle for infection resolution (Holland et al. 1991).

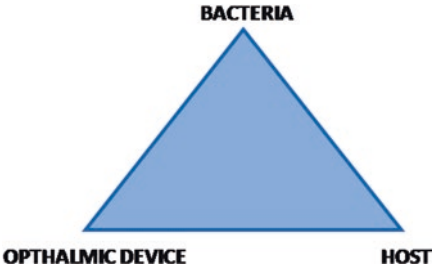


Fig. 11.4 The pathologic process of device-associated infections centred around the multifarious interaction of microorganism, device and host factors

11.8 Orbital Implants and Lacrimal Intubation Devices

Lacrimal cannulation devices together with lacrimal stents and Jones tubes are normally utilized during the dacryocystorhinostomy practice to treat nasolacrimal duct obstruction (NLDO), a general cause of epiphora (Eisenbeis et al. 2011; Bispo et al. 2015). As for alternative biomaterials fixed in the eyes, Jones tube as well as lacrimal stents could offer biofilm formation with an exterior. The bacterial biofilm formation on nasolacrimal stents could lead to prosthetic failure by occluding the stent (Ibanez et al. 2011). Bacterial colonization of the outer and inner surfaces of lacrimal stents was reported in some studies (Parsa et al. 2010) (Fig. 11.4).

The incidence of a polymicrobial biofilm was demonstrated by the evaluation of both Jones tube and silicone stent by scanning microscopy. Curiously, in the silicone stent biofilms, the authors have identified a variety of cell morphologies including fusiforms, short rod, cocci and spirochetes. Analysis of the interior surfaces of the silicone stent by confocal laser scanning microscopy unconcealed the presence of viable biofilms on the tube (Parsa et al. 2010). Biofilm formation on these polyurethane nasolacrimal stents has been connected with delayed failure of the device (Ibanez et al. 2011).

11.9 Conjunctival Plugs

Punctal plugs are used for the management of tear-deficient-kind dry eye. Conjunctival plugs are manufactured from hydrophobic acrylic, silicone, hydrogel and collagen. However, secondary complications might take place following implantation, including dacryocystitis, canaliculitis and acute conjunctivitis (Yokoi et al. 2000; Bourkiza and Lee 2012). Punctal plugs are usually used to treat dryness of the eye surface that does not respond to topical medication by occluding lacrimal ducts and blocking emptying of tears. It is vital that patients with punctal plugs are carefully observed. Examination of punctal aloof plugs from patients without clinical signs of infection revealed the presence of biofilms from microorganisms in 53% of the samples (Sugita et al. 2001). With regard to infection, many patients were asymptomatic, and the causal association between biofilms growing on punctal plugs and progress toward eye infection remains speculative. One case of conjunctivitis was associated with the formation of biofilm on a punctal plug (Yokoi et al.

2000). *S. haemolyticus* and *Candida tropicalis* are commonly growing biofilms on punctal plugs.

11.10 Outlook on Biofilm Prevention and Treatment Agents

Because biofilms are recognized for their great medical significance, efforts have been made to either stop their formation or get rid of them once they have been formed. There is a keen interest in ophthalmic materials capable of killing harmful microorganisms due to ever-increasing demand for healthy living. There are a few strategies to eradicate this biofilms on ophthalmic devices, which include preventing bacterial colonization on medical device surfaces by covalently attaching biocidal molecules and slowly releasing numerous antibiotics or modifying the external topology that may interfere with microbial adhesion.

The first two approaches would be much easier to achieve because they depend primarily on the coating of current ocular devices with obtainable biocidal molecules; the last is also more difficult because changes in the material topology could alter its optical clarity. Many medical procedures are currently used to treat device-related infections, including long-term antimicrobial strategies and antibiotic combinations and surgical revision. Unfortunately, these interventions carry the risk of re-infection and the development of antibiotic resistance, often at a higher rate. However, the application of non-adhesive and antimicrobial coatings has been researched and clinically tested as an alternative approach.

11.11 Investigational Strategies

There are some that have bactericidal potential and some that have adjunctive therapy potential among research strategies to control biofilms.

11.12 Biocidal Molecules or Inhibitors

The key to developing improved biomedical materials and devices, including infection-resistant medical implants, is effective management of biointerfacial interactions. Several of the medical devices are created from normal materials and do not seem to be antimicrobial, in order that they need modification. For example, device surfaces with modified chemicals such as polyethylene glycol and some other synthetic polymers are reported to repel (not kill) microorganisms (Ackart et al. 1975; Bridgett et al. 1992; Desai et al. 1992). These films were investigated for their resistance to bacterial adhesion. Alternatively, materials are often impregnated with antimicrobials, such as biocidal molecules, quaternary ammonium compounds, silver ions or iodine, which are slowly released into the surrounding solution over time and kill microorganisms (Fig. 11.5).

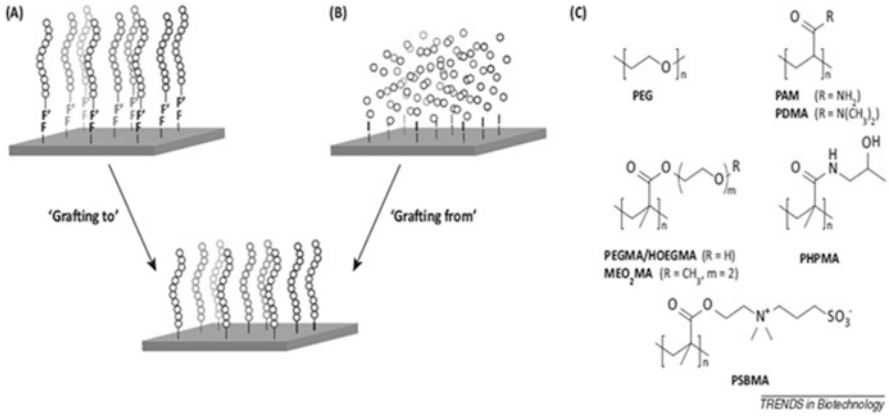


Fig. 11.5 Graft polymers can be established through the grafting-to technique (**a**), where preformed polymers are surface immobilized in a reaction between complementary functional groups. Grafting-from method (**b**) uses surface-immobilized initiators or chain transfer agents in a monomer solution that results in covalently immobilized graft polymer. Grafting-assisted materials (**c**)

The adhesion and colonization mechanism of bacteria on biomaterial surfaces are not fully understood; the influence of absorbable proteins was investigated. Biocidal compounds like polyethylene oxide (PEO) on the surfaces of polymeric biomaterials had shown larger protection from biofilm formation (Desai and Hubbell 1991). For instance, Desai et al. showed that incorporation of polyvinylpyrrolidone and polyethyl oxazoline on the surfaces of commonly used biomedical polymers, for example polyurethane, would provide a wide protection from various microorganisms' adherence.

Therefore, as an example for the prevention of biocides from the exploitation of surface colonization, a hundredfold reduction in cell counts when *S. aureus* is undisputed in previous studies. *S. aureus*, *E. coli* and *P. aeruginosa* were sprayed on glass slides covered with covalently fastened poly(4-vinyl-N-alkylpyridinium bromide) or N-hexylated polymer (Tiller et al. 2001). Inhibitory effects of 2,2′-dipyridyl and 1,2,3,4,6-penta-O-galloyl-b-D-glucopyranose in the contact lens on *S. aureus* were evidenced (Cho et al. 2015).

Antimicrobial peptides (AMPs) are another strategy for eradicating bacteria that form biofilms. AMPs' mechanism of action varies and involves disrupting bacterial cell membranes by creating hydrophilic channels, destabilizing the lipid bilayer and even changing membrane curvature. These mechanisms lead to penetration and death of bacterial cells. Quorum sensing (QS) is a form of chemical cell-to-cell communication used by microbes for cell-density-dependent signal transmission (Miller and Bassler 2001). Development of a peptide antagonist against fsr QS of *E. faecalis* derivatives would be useful to control the biofilm formation (Nakayama et al. 2013).

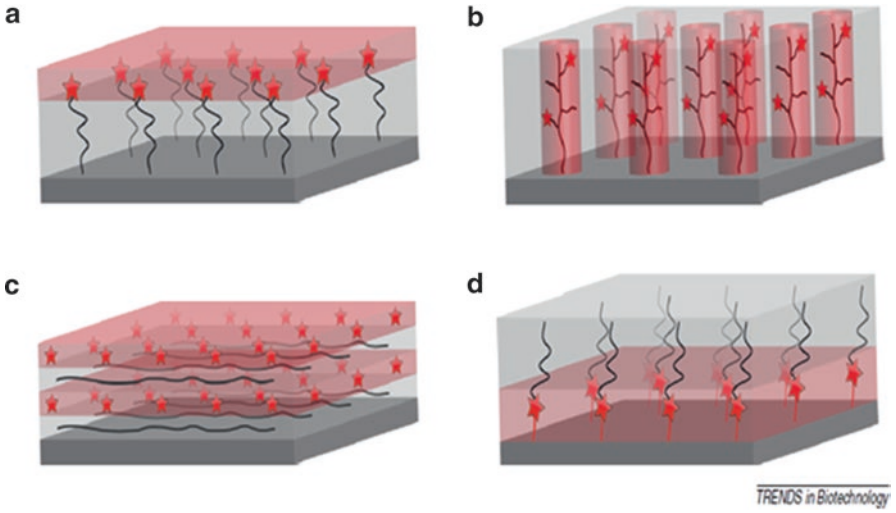


Fig. 11.6 Possible architecture for mixing low-fouling with antibacterial properties: (**a**) icing, (**b**) bottle brush, (**c**) multilayer and (**d**) castle

11.13 Antibiotic Molecules

When biofilms form after medical devices have been implanted, they carry the risk of device infection and may result in the patient suffering worse after treatment than before receiving medical treatment (Darouiche 2001). Bacterial colonization of the inward device may lead to both infection and failure of the device. Consistent with one study reported, surface coatings which will slowly discharge antibiotics, such as clarithromycin, rifampin and doxycycline, were capable of averting biofilm formation for up to 3 weeks (Rose et al. 2015). The intraocular lens designed to release norfloxacin was tested in rabbit model and in vitro to stop postoperative bacterial (Garty et al. 2011). Antimicrobial peptides coating on metal titanium have additionally been used fruitfully to prevent biofilm development and have the superimposed advantage that they are active against antibiotic-resistant strains (Kazemzadeh-Narbat et al. 2013). Gallium nitrate or silver also showed potential impact which will forestall the formation of biofilms. The advantages of visual exploitation systems that slowly unleash antimicrobial agents are that a number of regularly used ophthalmology antibiotics already have toxicity, penetration, and half-life. On the contrary, long exposure to these drugs could favour the selection of spontaneous resistant mutants and disturb the microbiome of the eye surface (Fig. 11.6).

11.14 Modifying the Exterior Topology of Ophthalmic Devices

Changes within the ophthalmic device material topology may change its optical clarity. It is always a pretty approach to change the external structure of eye devices to make it less adhesive for bacteria making an attempt to colonize. This would potentially eradicate the requirement for coating with biocide or antimicrobial agents that would be reserved for treatment and perioperative prophylaxis. Some polymers, such as dextran, polyacrylamide and polyethylene glycol, can form star-shaped, linear, and 'bottle brush' external nanostructures that impede the ability of the microbe to adhere to the substrate (May et al. 2014; Salwiczek et al. 2014).

In addition, nanotubes, nanopores and nanopillars made of anodized aluminium, polymethylmethacrylate and titanium dioxide were found to reduce microbial adhesion to coated surfaces (Desrousseaux et al. 2013).

11.15 ECM Degrading Enzymes

Synthetic biology involves engineering biological organisms through modular and generalizable victimization standard designs with the ultimate goal of developing useful solutions to real-world problems. To control the formation of biofilms on medical devices, few scientists are currently engaged in the extracellular matrix (ECM)-degrading enzymes. This can be an alternative strategy for microbial biofilm removal to stimulate microbial reversal to planktonic physiology. While the enzymes that degrade the ECM or the substratum can cost a clinical price, small signalling molecules that cause the expression of factors that stimulate biofilm dissimulation can be a viable alternative (Bispo et al. 2015). The use of phage endolysins as well as engineered phages expressing antibiofilm enzymes can also be promising choices in the site of infection for the eradication of bacterial biofilms (Lu and Collins 2007). Some cell signals, such as PQS, AI-2, C4HSL and AIP-I or their derivatives, are also of great therapeutic value (Dong et al. 2008; Kaplan 2010).

11.16 Conclusions and Future Directions

Since early descriptions quite three decades ago, our understanding of the formation and development of biofilms has advanced considerably. As medical interventions rely gradually on medical devices and prosthetic devices, a very important constraint is the requirement to prevent, decrease or eliminate microbial biofilms. Contact lenses and intraocular lenses have had an excellent impact on the restoration and recovery of vision within the field of eye care; however, eye infection prohibits their use. Strategies such as developing biofilm-active therapies and anti-biofilm surface coatings and improving surface topology to stop microbe adhesion are exciting avenues for future understanding to reduce the risk of visual infection associated with biofilm.

Acknowledgments Dr Kishore and Dr Bramhachari are grateful to thier academic institutions for the support extended.

Conflict of Interest The authors declares that they have no competing interests.

References

Ackart WB, Camp RL et al (1975) Antimicrobial polymers. J Biomed Mater Res 9(1):55–68
Archibald LK, Gaynes RP (1997) Hospital-acquired infections in the United States: the importance of inter-hospital comparisons. Infect Dis Clin North Am 11(2):245–255
Benz MS, Scott IU et al (2004) Endophthalmitis isolates and antibiotic sensitivities: a 6-year review of culture-proven cases. Am J Ophthalmol 137(1):38–42
Berger D, Rakhamimova A et al (2018) Oral biofilms: development, control, and analysis. High Throughput 7(3)
Bispo PJ, Haas W et al (2015) Biofilms in infections of the eye. Pathogens 4(1):111–136
Bourkiza R, Lee V (2012) A review of the complications of lacrimal occlusion with punctal and canalicular plugs. Orbit 31(2):86–93
Bridgett MJ, Davies MC et al (1992) Control of staphylococcal adhesion to polystyrene surfaces by polymer surface modification with surfactants. Biomaterials13(7):411–416
Bruinsma GM, van der Mei HC et al (2001) Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. Biomaterials 22(24): 3217–3224
Cheng KH, Leung SL et al (1999) Incidence of contact-lens-associated microbial keratitis and its related morbidity. Lancet 354(9174):181–185
Cho P, Shi GS et al (2015) Inhibitory effects of 2,2′-Dipyridyl and 1,2,3,4,6-Penta-O-Galloyl-b-D-Glucopyranose on biofilm formation in contact lens cases. Invest Ophthalmol Vis Sci 56(12):7053–7057
Darouiche RO (2001) Device-associated infections: a macroproblem that starts with microadherence. Clin Infect Dis 33(9):1567–1572
Darouiche RO (2004) Treatment of infections associated with surgical implants. N Engl J Med 350(14):1422–1429
Dart JK, Stapleton F et al (1991) Contact lenses and other risk factors in microbial keratitis. Lancet 338(8768):650–653
Dart JK, Radford CF et al (2008) Risk factors for microbial keratitis with contemporary contact lenses: a case-control study. Ophthalmology 115(10):1647–1654, 1654 e1–3
Desai NP, Hubbell JA (1991) Solution technique to incorporate polyethylene oxide and other water-soluble polymers into surfaces of polymeric biomaterials. Biomaterials 12(2):144–153
Desai NP, Hossainy SF et al (1992) Surface-immobilized polyethylene oxide for bacterial repellence. Biomaterials 13(7):417–420
Desrousseaux C, Sautou V et al (2013) Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation. J Hosp Infect 85(2):87–93
Dickey JB, Thompson KD et al (1991) Anterior chamber aspirate cultures after uncomplicated cataract surgery. Am J Ophthalmol 112(3):278–282
Dong YH, Zhang XF et al (2008) A novel two-component system BqsS-BqsR modulates quorum sensing-dependent biofilm decay in Pseudomonas aeruginosa. Commun Integr Biol 1(1):88–96
Donlan RM (2008) Biofilms on central venous catheters: is eradication possible? Curr Top Microbiol Immunol 322:133–161
Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15(2):167–193
Dutta D, Cole N et al (2012) Factors influencing bacterial adhesion to contact lenses. Mol Vis 18:14–21

Eisenbeis C, Neppert B et al (2011) Anatomic and subjective success of structured surgical treatment strategy in the management of chronic epiphora – a postoperative analysis of contentment. Klin Monatsbl Augenheilkd 227(11):879–886
Fisch A, Salvanet A et al (1991) Epidemiology of infective endophthalmitis in France. The French collaborative study group on endophthalmitis. Lancet 338(8779):1373–1376
Fleiszig SM, Evans DJ (2010) Pathogenesis of contact lens-associated microbial keratitis. Optom Vis Sci 87(4):225–232
Garty S, Shirakawa R et al (2011) Sustained antibiotic release from an intraocular lens-hydrogel assembly for cataract surgery. Invest Ophthalmol Vis Sci 52(9):6109–6116
Gristina AG, Costerton JW (1984) Bacteria-laden biofilms: a hazard to orthopaedic prostheses. Infect Surg 67A:264–273
Gristina AG, Costerton JW (1985) Bacterial adherence to biomaterials and tissue. J Bone Joint Surg Am 67(264–73):264
Hahn HP (1997) The type-4 pilus is the major virulence-associated adhesin of Pseudomonas aeruginosa--a review. Gene 192(1):99–108
Hall-Stoodley L, Costerton JW et al (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2(2):95–108
Health NIO (2007, November 12). Targeted research on oral microbial biofilms (DE98-006). http://grants.nih.gov/grants/guide/rfa-files/RFA-DE-98-006.html
Holland SP, Pulido JS et al (1991) Biofilm and scleral buckle-associated infections. A mechanism for persistence. Ophthalmology 98(6):933–938
HPA (2011) English national point prevalence survey on health care associated infections and antimicrobial use. Preliminary Data. Health Protection Agency, London
Ibanez A, Trinidad A et al (2011) Biofilm colonisation in nasolacrimal stents. B-ENT 7(1):7–10
Juarez-Verdayes MA, Reyes-Lopez MA et al (2006) Isolation, vancomycin resistance and biofilm production of Staphylococcus epidermidis from patients with conjunctivitis, corneal ulcers, and endophthalmitis. Rev Latinoam Microbiol 48(3–4):238–246
Kaplan JB (2010) Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res 89(3):205–218
Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol 73(2):310–347
Kazemzadeh-Narbat M, Lai BF et al (2013) Multilayered coating on titanium for controlled release of antimicrobial peptides for the prevention of implant-associated infections. Biomaterials 34(24):5969–5977
Klotz SA, Butrus SI et al (1989) The contribution of bacterial surface hydrophobicity to the process of adherence of Pseudomonas aeruginosa to hydrophilic contact lenses. Curr Eye Res 8(2):195–202
Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci U S A 104(27):11197–11202
Marrie TJ, Nelligan J, Costerton JW (1982) A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 66:1339–1343
May RM, Hoffman MG et al (2014) Micro-patterned surfaces reduce bacterial colonization and biofilm formation in vitro: potential for enhancing endotracheal tube designs. Clin Transl Med 3:8
Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199
Nakayama J, Yokohata R et al (2013) Development of a peptide antagonist against fsr quorum sensing of Enterococcus faecalis. ACS Chem Biol 8(4):804–811
Parsa K, Schaudinn C et al (2010) Demonstration of bacterial biofilms in culture-negative silicone stent and jones tube. Ophthal Plast Reconstr Surg 26(6):426–430
Pathengay A, Karosekar S et al (2004) Microbiologic spectrum and susceptibility of isolates in scleral buckle infection in India. Am J Ophthalmol 138(4):663–664
Pepose JS, Wilhelmus KR (1992) Divergent approaches to the management of corneal ulcers. Am J Ophthalmol 114(5):630–632

Puliafito CA, Baker AS et al (1982) Infectious endophthalmitis. Review of 36 cases. Ophthalmology 89(8):921–929

Rose WE, Otto DP et al (2015) Prevention of biofilm formation by methacrylate-based copolymer films loaded with rifampin, clarithromycin, doxycycline alone or in combination. Pharm Res 32(1):61–73

Salwiczek M, Qu Y et al (2014) Emerging rules for effective antimicrobial coatings. Trends Biotechnol 32(2):82–90

Schimel AM, Miller D et al (2013) Endophthalmitis isolates and antibiotic susceptibilities: a 10-year review of culture-proven cases. Am J Ophthalmol 156(1):50–52. e1

Smiddy WE, Miller D et al (1993) Scleral buckle removal following retinal reattachment surgery: clinical and microbiologic aspects. Ophthalmic Surg 24(7):440–445

Speaker MG, Milch FA et al (1991) Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology 98(5):639–649; discussion 650

Springer EL, Roth IL (1973) The ultrastructure of the capsules of Diplococcus pneumoniae and Klebsiella pneumoniae stained with ruthenium red. J Gen Microbiol 74(1):21–31

Sugita J, Yokoi N et al (2001) The detection of bacteria and bacterial biofilms in punctal plug holes. Cornea 20(4):362–365

Sunderraj, P. (1992). "Anterior chamber aspirate cultures after uncomplicated cataract surgery." Am J Ophthalmol 113(2): 221–2

Tabbara KF, El-Sheikh HF et al (2000) Extended wear contact lens related bacterial keratitis. Br J Ophthalmol 84(3):327–328

Tiller JC, Liao CJ et al (2001) Designing surfaces that kill bacteria on contact. Proc Natl Acad Sci U S A 98(11):5981–5985

van Kleef E, Robotham JV et al (2013) Modelling the transmission of healthcare associated infections: a systematic review. BMC Infect Dis 13:294

Webb LX, Meyers RT, Cordell AR, Hobgood AR, Costerton JW, Gristina AG (1986) Inhibition of bacterial adhesion by antibacterial surface pretreatment of vascular prostheses. J Vasc Surg 4:16–21

Willcox MD, Carnt N et al (2010) Contact lens case contamination during daily wear of silicone hydrogels. Optom Vis Sci 87(7):456–464

Yokoi N, Okada K et al (2000) Acute conjunctivitis associated with biofilm formation on a punctal plug. Jpn J Ophthalmol 44(5):559–560

Zegans ME, Shanks RM et al (2005) Bacterial biofilms and ocular infections. Ocul Surf 3(2):73–80

Part III

Role of Quorum Sensing in Agriculture

Quorum Sensing: Communication Complexity for Resilience of Plant-Microbe Interaction

12

K. Archana, K. Sathi Reddy, P. Ravinder, M. Yahya Khan, and Hameeda Bee

Abstract

Microorganisms employ a precised communication pattern among themselves in order to coordinate between various processes during their growth. Both unicellular and multicellular microbes are found to show cell density-driven gene expression. This phenomenon of density-dependent cell regulation used for survival, prevalence and colonization of specific host is generally termed as quorum sensing (QS). Microorganisms respond to this stimulus once the signalling molecule reaches its threshold concentration. Since they are found to be able to regulate their own production, they are termed as autoinducers (quorum sensing molecules). These molecules function by sensing their own population with respect to their density and distribution pattern in the prevailing environment. Hence, microorganisms use such environmental sensing mechanisms to get adapted as well as for their survival in the existing conditions in their habitat, thereby maintaining healthy cell population. The autoinducers occur widespread in several microorganisms and differ from each other in their molecular structures. Acyl homoserine lactones (AHL), Autoinducer (AI), i.e., AI-2, AI-3 and quinolones are the common QS signalling molecules in Gram-negative bacteria, while cyclic peptides, AI-2 and butyrolactones are observed as signalling molecules in Gram-positive bacteria. In the case of actinomycetes, small diffusible molecules called autoregulators, A-factor and 2-iso-octanoyl-(3*R*)-hydroxymethyl-γ-butyrolactone act as QS signalling molecules. Understanding the connection between genomes, gene expression and the molecules in complex environment is considered to be a tough task. Increasing interest towards studying the underlying mechanisms has led to the development of various model

K. Archana · K. Sathi Reddy · P. Ravinder · M. Yahya Khan · H. Bee (✉)
Department of Microbiology, UCS, Osmania University, Hyderabad, Telangana, India

Kalam Biotech Pvt. Ltd, Hyderabad, India
e-mail: drhami2009@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_12

systems. Among them, plant-microbe symbiotic system is considered to be the best one to study the inter-kingdom molecular cross-talk. During the process of evolution, plants started to respond to the external stimuli in different and more specific ways. One such way includes production of AHL-like molecules to regulate the QS of plant-associated microorganisms. In view of this, the present chapter will be focused on quorum sensing molecules and their role in plant-microbe interaction.

Keywords
Quorum sensing · Inter-kingdom signalling · Autoinducer · AHL · Lipoxygenase · Diffusible signal factor · Oxylipins · Gram-positive bacteria · Gram-negative bacteria · Fungi

12.1 Introduction

Rhizosphere is a complex ecosystem consisting of nutrient-rich soil which is highly influenced by the root exudates and associated microorganisms. It is densely populated with varied microorganisms such as bacteria, fungi, nematodes, protists and invertebrates. Plant root exudate is mainly attributed of primary metabolites including carbohydrates, amino acids, organic acids, enzymes and secondary metabolites such as alkaloids, terpenoids and phenolics that are responsible for shaping, interfering and signalling with the rhizosphere microflora (rhizomicrobiome). Exudation of these metabolites results in the benefit of plant by attracting and promoting the colonization of beneficial microorganisms in the rhizosphere, while combating the pathogenic ones (Mendes et al. 2013). Shaping and recruitment of rhizomicrobiome are regarded to occur through two processes. The first one involves stimulation of microbial multiplication by the root exudates. This process is considered as an active way of building up the selective rhizospheric microbial communities from the diverse microorganisms present in the soil through the processes that support, attenuate or inhibit the microbial growth and activity. However, the contribution of plant root secretions in determination of the rhizospheric microbial population cannot be merely termed as signalling. The second method involves detection of low molecular weight substances produced by plants or microbes and stimulation of cellular response which triggers a cascade of reactions and brings about the transcription of the gene in response to a particular compound. This process is believed to perfectly suit the definition of signalling. This phenomenon of signalling is divided into three categories which represent the major signalling mechanisms occurring in the rhizosphere:

1. Microbial intra-species and inter-species signalling that occurs mainly through quorum sensing (QS) signalling molecules which helps in the establishment of

the respective microbial community as well as brings about synchronization in their behaviour.
2. Signalling occurring from plant towards microorganisms through small molecules secreted by plants, which is observed in specialized symbiotic association, resulting in shaping plant-microbiome to maintain phenotypic plasticity.
3. Signalling initiated from microorganisms towards plants through microbial-produced compounds that affects gene expression and defence mechanisms in plant.

12.2 Quorum Sensing: Microbial Signalling Among Intra-species and Inter-species Inhabiting Rhizosphere

Microorganisms communicate and bring about coordination in their behaviour by responding to the stimulus generated by the quorum sensing molecules or autoinducers which are considered as cell density-dependent entities. Microorganisms are found to produce these autoinducers during their entire growth phase, but they are thought to actively respond towards such signals only at certain growth phase which in turn is highly species specific. This growth phase is determined by the cell density of microorganism. Microorganisms utilize quorum sensing mechanism to control wide variety of processes which include synchronized gene regulation and expression required for active colonization, symbiosis and host-bacterial virulence (González et al. 2017).

12.3 Quorum Sensing as Signalling Mechanism in Gram-Negative Bacteria

Quorum sensing has been the current subject of extensive investigation in microbiological research. The signalling molecules produced by the microorganisms are classified into different chemical classes and microbes may use more than one quorum sensing system. Quorum sensing phenomenon for the first time was studied in the marine bacterium *Vibrio fischeri*. A chemical compound produced by this bacterium was found to be responsible for their bioluminescence and the same was identified as, N-(3-oxo)-hexanoyl-L-homoserine lactone (3 oxo C6-HSL). Several quorum sensing signalling molecules of bacterial origin have been isolated and identified. Acyl homoserine lactone (AHL) is considered as a universal signalling molecule in the case of Gram-negative bacteria (Galloway et al. 2011). A wide variety of proteobacterial rhizosphere isolates including *Pseudomonas chlororaphis*, *Pseudomonas syringae*, *Burkholderia*, *Serratia*, *Erwinia* and *Ralstonia* are found to produce as well as respond to such QS signalling molecules. Structurally, AHL is composed of long acyl chain attached to homoserine lactonic ring (Ahmad et al. 2008). Different AHL molecules are found to differ in the acyl chain length and its

degree of saturation along with the occurrence or absence of hydroxyl or keto group on the C_3 carbon atom. *LuxR* family of proteins are reported to be involved in the synthesis of these signal molecules. AHLs have also been found to be involved in inter-kingdom signalling and influence regulation of gene expression and induction of systemic resistance in plants, thereby stimulating the growth and development. Recently, pyrones and dialkyl resorcinols found to be produced by a number of Gram-negative bacteria have been identified as signalling molecules. These are recognized by *LuxR* proteins and closely resemble *LuxR* family. DSF family (diffusible signal factor) is another class of QS signals in Gram-negative bacteria including *Burkholderia* spp. and *Stenotrophomonas maltophilia*. The first signalling molecule to be included under this class is *cis*-11-methyl-2-dodecenoic acid produced by *Xanthomonas campestris* pv. *campestris*. These signals induce innate immunity and hence act as signalling molecules for inter-kingdom communication between plants and bacteria. Since QS is generally used by the microorganisms to sense and get adapted with the prevailing microenvironment (Williams 2007; Mellbye and Schuster 2011), studies using nonpathogenic *Burkholderia* strains further state the role and importance of QS for inter-kingdom communication between plant and bacteria in the rhizosphere and among different microbial communities (Chan et al. 2011; Suarez-Moreno et al. Suárez-Moreno et al. 2012).

12.4 Quorum Sensing in Gram-Positive Bacteria

Cell-to-cell signalling in Gram-positive bacteria is triggered by signal transduction mechanism which in turn is a two-component system where histidine kinase and a response-regulating protein bring about phosphorylation of the cascade, resulting in the stimulation of signal transduction. In general, post-translationally modified/unmodified peptides which are secreted through an ATP-binding cassette (ABC) exporter protein are used as QS signalling molecules by Gram-positive bacteria (Bassler 2002). Several key processes are found to be under quorum sensing regulation in the case of Gram-positive bacteria. Few to be listed include competence development for DNA uptake by *Bacillus subtilis* and *Streptococcus pneumoniae*, production of microcin by *Lactobacillus sake* and determination of virulence in *Staphylococcus aureus*. *S. aureus* and *S. epidermidis* are often the important species that are found to be responsible for the formation of biofilms on medical devices. Infections characterized due to the biofilm formation by *Staphylococcus* spp. are highly resistant to antibiotic treatment. A group of peptides termed modulins that are phenol soluble have been suggested as the virulence determinants in this bacteria. In *S. aureus*, quorum sensing is observed to occur in two different mechanisms. The first quorum sensing mechanism employs a seven amino acid peptide RAP (RNAIII activating peptide), an autoinducer which regulates TRAP protein (target of RNAIII activating protein) (Balaban and Novick Balaban and Novick 1995). Production of TRAP is stimulated when the RAP reaches its threshold concentration. This phenomenon leads to the enhancement of cell adhesion and stimulation of the second quorum sensing mechanism which is found to be regulated by the Agr

(accessory gene regulator) locus. The Agr system is constituted of RNAII and RNAIII, and their transcription is found to be under the control of P2 and P3 promoters, respectively (Morfeldt et al. 1995).

12.5 Quorum Sensing in Fungi

Quorum sensing, a cell density phenomenon, is usually reported to bring about morphological changes in several microorganisms including bacteria and fungi (unicellular and filamentous) using different QS signalling systems. However, one of the best-studied QS systems is reported in *Candida albicans*, which is a dimorphic and opportunistic human pathogen (Hogan 2006). Morphologically, *C. albicans* is observed to undergo a shift to yeast-like growth from hyphal growth and vice versa and is found to be controlled by a cell density-dependent phenomenon. This phenomenon is governed by farnesol and tyrosol which act as QS signalling molecules (Hornby et al. 2001). However, in the case of *Saccharomyces cerevisiae* (budding yeast), morphological changes and pseudohyphal formation are stimulated by two aromatic alcohols, namely, phenylethanol and tryptophol (Chen et al. 2004). Studies on QS in fungi were initiated long ago and it has been tremendously expanding since past few years. Few examples of filamentous fungi in which QS mechanisms are reported include *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium chrysogenum and Penicillium sclerotiorum*. Oxylipins which are chemically lactone-containing molecules act as QS signalling molecules in these fungi. Morphogenesis, secondary metabolite production including mycotoxins, antibiotics and the phenomenon of sporulation are reported to be under QS regulation in these fungi (Schimmel et al. 1998; Calvo et al. 2002; Tsitsigiannis and Keller 2007; Sorrentino et al. 2010). Apart from this, the high degree of similarities shared by the filamentous bacteria and fungi has driven the researchers towards investigating the presence of other QS signalling molecules which has led to the discovery of different γ-butyrolactone-containing molecules in filamentous fungi. Later, it was reported that several such γ-butyrolactone-containing molecules occurred in fungi and play the role as putative QS molecules. Butyrolactone-I, a secondary metabolite produced by *A. terreus*, acts as a signalling molecule that brings about the stimulation of lovastatin biosynthetic gene machinery (Raina et al. 2012), thereby resulting in the enhanced yield of this secondary metabolite lovastatin. Apart from this, butyrolactone-I is also thought to effect hyphal formation and sporulation (Schimmel et al. 1998). It is attributed with autostimulatory function as well. The transcription of lovastatin biosynthetic genes in *A. terreus* is found to be under the regulation of linoleic acid and its derivatives. This partially explains the key role of oxylipins as QS signalling molecules. Madhani (2011) reported butyrolactone-I and multicolic acid as QS signalling molecules in *A. terreus* and *Penicillium sclerotiorum*, respectively, which in turn brought about 2.9-fold increase in the production rate of lovastatin at fermentor level. γ-Heptalactone is another γ-butyrolactone-containing molecule that was recently identified in *A. nidulans*, where it was reported to act as

QS molecule in the same fungus. It is mainly said to act by decreasing the lag phase thereby affecting the growth of this fungus. Lactonases are reported to be synthesized by different fungi. Uroz and Heinonsalo (2008) are the first to report on *Ascomycota* and *Basidiomycota* lineages (include root-associated fungi) to use AHL lactonase as QS signalling molecule. Hence, fungi belonging to different genera use quorum sensing phenomenon and influence their population-dependent behaviour such as conidia-sclerotia morphological shift and secondary metabolite production (Horowitz Brown et al. 2008; Brown et al. 2009).

12.6 Inter-Kingdom Microbial Signalling in Rhizosphere

12.6.1 Quorum Sensing-Mediated Responses Between Bacteria and Fungi

The synthesis of phenazine by *Pseudomonas chlororaphis* is a cell density-dependent process which is regulated by *phzI* and *phzR*. Here, the quorum sensing molecule is found to be HHL (*N*-hexanoyl-homoserine lactone) (Wood et al. 1997). *P. chlororaphis* strain PCL1391 produces phenazine-1-carboxamide (PCN), and this acts as the antifungal agent against *Fusarium oxysporum* which is responsible for the cause of tomato (*Solanum lycopersicum*) foot and root rot (Chin-A-Woeng et al. 2001). As PCN has been detected only in high cell density of *P. chlororaphis*, it is stated that its production may be stimulated by QS phenomenon. The QS system of *Burkholderia ambifaria* is thought to possess a *CepRI* system, which uses C6-homoserine lactone and C8-homoserine lactone signals. In *Burkholderia cepacia* complex (Bcc) species, production of antifungal molecules, i.e. pyrrolnitrin, burkholdines (occidiofungin-like molecules) and enacyloxins, is reported as QS regulated (Lutter et al. 2001; Zhou et al. 2003; Schmidt et al. 2009; Annelise Chapalain et al. 2013).

12.6.2 Quorum Sensing Response Between Plant and Bacteria

Microorganisms and plants that live in mutual association are found to be able to overcome plant defence mechanisms and thereby bring about the successful colonization of the host (Alqueres et al. 2013). All the beneficial association between plants and microorganisms excluding mycorrhiza or *rhizobia* are generally found to be under systemic regulation or autoregulation. Plants respond to signals generated by microorganisms and other environments by employing signal cascade systems and exhibit induced systemic resistance (ISR) and systemic acquired resistance (SAR) as response against beneficial and harmful rhizosphere bacteria, respectively. Secondary metabolites obtained from the microbial inoculants are found to play a key role in their biocontrol activity against several pathogenic microorganisms, which further contributes to an increase in the systemic resistance of the host plant. Plants use root exudate molecules occurring in the rhizosphere to obtain benefit

from the microorganism by stimulating their root colonization. AHL-secreting bacteria are found to occur in large number in the region surrounding their roots. This states the role of these molecules and plants in deciding the colonizing population (López-Ráez et al. 2012). Plant host species generate a variety of responses to AHL molecules. Hartmann and Schikora (2012) observed a decrease in the concentrations of certain proteins in *Medicago truncatula* when it was treated with AHLs. However, increase in the concentration of the same proteins was observed after the depletion of AHLs. Almost all the plant-associated bacteria have QS systems that are mediated by AHL. Pathogenic microorganisms use QS phenomenon to infect and establish disease in host plant. In this process, plants have developed unique and different mechanisms which use these QS signalling molecules to generate immune response towards its defence against pathogens. The first QS response against bacterial QS molecule (AHL) was reported in legumes *Phaseolus vulgaris*, where it resulted in increased transpiration rate and stomatal conductance (Joseph and Phillip 2003) and in the case of *Medicago truncatula*, it resulted in enhanced expression of genes involved in plant defence, production of plant hormones and regulation of metabolic processes (Mathesius et al. 2003). Acyl homoserine lactones obtained from symbiotic nitrogen fixer (*Sinorhizobium meliloti*) and opportunistic pathogenic bacteria (*Pseudomonas aeruginosa*) generally executes its effects from even at low concentrations of micro molar to nano and bring about huge changes in the production of over 150 proteins. When tomato plant roots were inoculated with *Serratia liquefaciens* MG1, it resulted in induced systemic resistance in plants and was reported by (Hartmann et al. 2004; Schuhegger et al. 2006; Amrutha et al. 2018). This led to high salicylic acid levels as well as increased expression of ethylene-dependent defence genes (i.e. PR1a). Similarly, on inoculating bean and tomato plants with *Serratia plymuthica* HRO which is found to be a producer of C48, producing C4-/C6- and OH C4-/OH C6-acyl homoserine lactones, ISR was induced against the fungal leaf pathogen *Botrytis cinerea* (Liu et al. 2007; Pang et al. 2009). On the other hand, *Arabidopsis thaliana*, when treated with (C4- and C6-) *N*-acyl homoserine lactonic compounds, resulted in the altered expression of certain genes thereby influencing the concentration of plant hormones and plant defence system (Von Rad et al. 2008). Unlike *S. plymuthica*, stimulation of roots of *A. thaliana* with AHLs did not result in any ISR. However, stimulation of *Arabidopsis* plant roots with C10-homoserine lactone led to certain changes in root system (Ortíz-Castro et al. 2008). Furthermore, Liu et al. (2012) and Jin et al. (2012) demonstrated that G-protein-coupled receptors, which are encoded by At-GPA1, mediate the C6- and C8-AHLs triggered root stimulation in *Arabidopsis* plants. Induced systemic resistance was triggered in *A. thaliana* and *Hordeum vulgare* (barley) when the plants encountered with the fungal pathogens *Golovinomyces orontii* and *Blumeria graminis* f. sp. *hordei*. This stimulation is stated to be due to the treatment of the plants with C12- and C14- *N*-acyl homoserine lactones. Presence of C12- or C14-acyl homoserine lactonic compounds brings about certain alterations during the activation of the mitogen-activated protein (MAP) kinases, At-MPK3 and At-MPK6, which results in active expression of *PR1* gene and defence-related transcription factors, WRKY26 and WRKY29. Hence, it can be hereby concluded that

AHLs trigger ISR and root system development in *A. thaliana* (Schenk et al. 2012). Moreover, plant roots are shown to specifically prefer short-chained AHLs for their uproot transfer as they are easily water soluble. However, plants respond differently to unique AHLs in a tissue-specific manner (Mathesius et al. 2003). Plants on encountering with *Agrobacterium tumefaciens*, a bacterium that is responsible for the cause of crown gall tumours in plants, trigger deposition of γ-amino butyric acid (GAbA; also an animal neurotransmitter) near the injury; this in turn leads to destruction of its own QS signal by activation of AttM lactonase in *A. tumefaciens* (Chevrot et al. 2006). On the other hand, QS signalling mechanism in *Sinorhizobium meliloti*, a plant symbiont is reported to be inhibited by L-canavanine, which is produced by alfa-alfa (Keshavan et al. 2005). Hence, occurrence of AHLs in plant vicinity is said to influence plant physiological response. Phenazine synthesis in *Pseudomonas aureofaciens* strain 30–84 is found to be under QS regulation and this pigment synthesis is actively stimulated on inoculation of *P. aureofaciens* onto root surfaces of wheat plant (Chin-A-Woeng et al. 2003). *Erwinia carotovora* causes cell-wall degradation in industrially important plants such as potato by production of various exoenzymes such as cellulases, pectinases and proteases (Kang et al. 2016) which is said to be controlled by quorum sensing mechanisms. *E. carotovora* growth is characterized by two phases. In the first phase, neither exoenzymes nor carbapenem is produced, and during the second phase, both exoenzymes and antibiotic carbapenem are produced as the cells occur at their threshold concentration, which are found to be under the regulation by the *CarI–CarR* QS signalling system, homologous with *V. fischeri LuxI–LuxR* system (Barnard et al. 2007).

12.6.3 Quorum Sensing Response Between Plant and Fungi

Enzymatic reactions mediated by dioxygenase (DOX), lipoxygenase (LOX) and P450 which form three important groups of enzymes collectively result in the formation of oxylipins. Jasmonic acid, traumatin, green leaf volatiles (GLV), divinyl ethers and hydroxy, oxo, or keto fatty acids are few to mention among the 150 oxylipins identified (Mosblech et al. 2009). Reactive oxygen species (ROS) mediated peroxidation and oxidative stress is also found to trigger the formation of phytoprostanes, which include oxylipins. These are said to act as potential signals which govern the plant physiological response on encountering phytopathogens (Mueller et al. 2008).

Oxylipins occurring in fungal species, plant and mammalians are all found to show structural similarities. This explains to certain extent about the oxylipin-mediated interkingdom signalling between *Aspergillus* and its host plant. One of the findings on importance of oxylipin-mediated cross-signalling is when a lipoxygenase ZmLOX3 from maize plant is expressed in *A. nidulans*, it could compensate the loss of *ppoA* and *ppoC* genes, and the LOX null maize mutants could not execute their response against *Aspergillus* and other pathogens. Maize plant deficient of ZmLOX3 was found to be easily susceptible to *Aspergillus flavus* colonization and its mycotoxin (aflatoxin)

production both in vitro and in field experiments (Gao et al. 2009). The strategies employed by *Aspergillus* for its pathogenesis generally include secretion of the enzymes lipase and lipoxygenase within the host plant. These enzymes metabolize lipids, resulting in the release of free fatty acids which are then oxidized to oxylipins. These are termed as host-derived oxylipins. Fungi use oxylipins to establish their invasive growth, formation of spores and mycotoxin.

A variety of QS signalling molecules produced by microorganisms which are responsible for their interaction among themselves and with that of their specific hosts has been discussed so far. Structure of these QS molecules along with their function and producing microorganism is listed in Table 12.1.

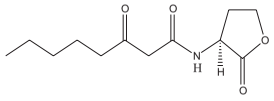
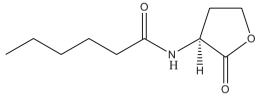
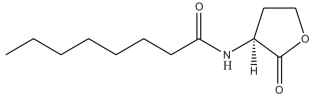
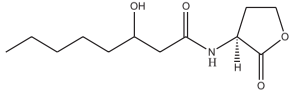
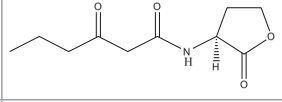
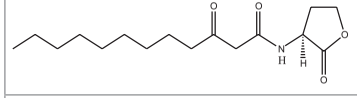
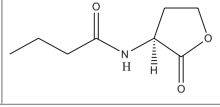
12.7 Bioassays for the Detection of Signal Molecules

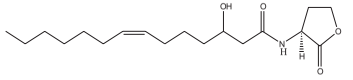
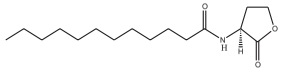
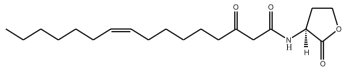
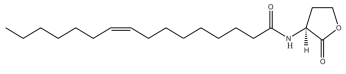
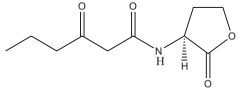
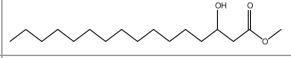
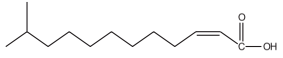
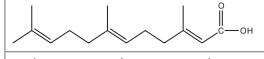
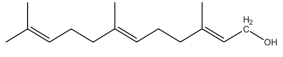
Several procedures and protocols are employed for QS signalling molecules detection. A collection of commonly used protocols is further compiled by Rumbaugh (2011). Among the various techniques used bioassays, thin-layer chromatography (TLC), chromatographic and spectroscopic techniques are regularly used for recognition of signalling molecules.

Microorganisms produce QS molecules or autoinducers in very small quantities and several sensor techniques have been designed and developed for their identification and chemical analysis. Mutant strains are generated which are not capable of synthesizing QS molecules, and studies have shown that addition of QS molecules exogenously results in the expression of the wild-type phenotype in these organisms.

Although a number of bacteria have been reported as biosensor strains, three out of them are routinely used for bioassays. The first one is AHL sensor pDC141E33 from *Agrobacterium tumefaciens*. Here, lacZ and traG are fused, and the produced AHLs can be visualized on TLC plates by incorporating 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) onto TLC plates using agar overlay. The second biosensor strain is *Chromobacterium violaceum*. This biosensor strain is involved in the inhibition or production of violacein (pigment) in *C. violaceum*. Production of violacein is found to be under the regulation of hexanoyl homoserine lactone (HHL). Strain CV026 is miniTn5 mutant of *C. violaceum* that is violacein negative. Incubation of this mutant with AHLs results in induction of violacein production by this mutant. The third biosensor strain is *Serratia liquefaciens* MGI which exhibits active bacterial swarming motility. However, *S. liquefaciens* MG44 strain, which is a SWrI::T45 mutant of MGI, is unable to produce BHL or HHL and hence lacks swarming motility. However, addition of BHL or HHL externally restores serrawetin synthesis and swarming motility in this mutant. Not all QS signalling molecules are detected by single biosensor strain and this could be due to difference in the chemical structure of the signalling molecules. Therefore, more than one biosensor in different combinations is generally preferred to detect the presence of signalling molecules.

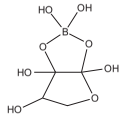
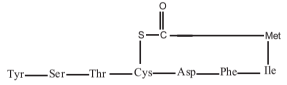
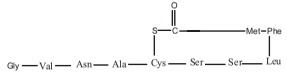
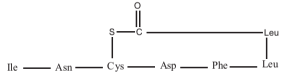
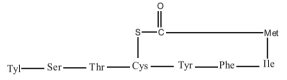
Table 12.1 Structure of quorum sensing molecules and their function

Microorganism	Signal structure	Chemical name	Function	References
Agrobacterium		N-3-oxooctanoyl-L-homoserine lactone (OOHL or 3-oxo-C8)	Synthesis of photosynthetic membrane, conjugal transfer of pTi, root nodulation/ symbiosis	White and Winans (2008)
Burkholderia cenocepacia *Chromobacterium violaceum*		Hexanoyl homoserine lactone (HHL or C6)	Adherence, swarming motility, virulence factor, biofilm formation	Ryan et al. (2015)
Burkholderia pseudomallei		Octanoyl homoserine lactone (OHL or C8)	Siderophore production, biofilm formation, virulence factor	Urlich (2004)
		3-Hydroxy octanoyl-L-homoserine lactone (3-hydroxy-C8)		
Erwinia carotovora		3-Oxo-hexanoyl-L-homoserine lactone (OHHL or 3-oxo-C6)	Antibiotic carbapenem, bioluminescence, virulence factors, exoenzymes	Ferluga et al. (2008)
Pseudomonas aeruginosa		N-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL or 3-oxo-C12)	Biofilm formation, lectin, exoenzymes swarming, protease	Amari et al. (2013)
		N-butanoyl-L-homoserine lactone (BHL or C4)		

Rhizobium leguminosarum		N-(3-hydroxy-7-cis-tetradecenoyl)-L-homoserine lactone (3-hydroxy-7-cis-C14)	Synthesis of photosynthetic membrane, conjugal transfer of pTi, root nodulation/ symbiosis	Gonzalez and Marketon (2003)
Sinorhizobium meliloti		Dodecanoyl-L-homoserine lactone (DDHL or C12)	Exopolysaccharide synthesis, motility, nodulation, plasmid transfer	Gonzalez and Marketon (2003)
		N-3-oxo-(cis-9-octadecenoyl)-L-homoserine lactone (3-oxo-9-cis-C16)		
		9-cis-hexadecenoyl)-L-homoserine lactone (9-cis-C16:1)		
Vibrio fischeri		3-Oxo-hexanoyl-L-homoserine lactone (OHHL or 3-oxo-C6)	Bioluminescence, virulence factors, exoenzymes	Dunlap (1999)
Ralstonia solanacearum		9-Hydroxy palmitic acid methyl ester (PAME)	Not determined/studied	Ferluga et al. (2008)
Xanthomonas campestris		Diffusible signalling factor (DSF)	Exoenzyme production, exopolysaccharide production	O'Connell et al. (2013)
Candida albicans		Farnesoic acid	Inhibits yeast-filamentous morphological transition	Tian et al. (2013) and Hornby et al. (2001)
		Farnesol		

(continued)

Table 12.1 (continued)

Microorganism	Signal structure	Chemical name	Function	References
Vibrio harveyi		AI-2 4S-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate (S-THMF-borate)	Bioluminescence	Henke and Bassler (2004)
Staphylococcus aureus group I strains		Autoinducing peptide-I (AIP-I)	Virulence, antimicrobial peptide synthesis, genetic competence	Sturme et al. (2002)
S. aureus group II strains		Autoinducing peptide-II (AIP-II)		
S. aureus group III strains		Autoinducing peptide-III (AIP-III)		
S. aureus group IV strains		Autoinducing peptide-IV (AIP-IV)		

12.8 Quorum Mimics and Quenching

Several articles reported the role of interfering QS molecules against the action of drugs newly developed against several human pathogenic bacteria. Similarly, mechanisms designed towards the control of phytopathogenic bacteria by interfering with QS signalling system have also been developed. Various types of AHL molecules produced by bacteria results in the bacterial cross-talk. Certain bacteria are found to produce lactonases and acylases capable of disrupting the cell-cell signalling by acting on cyclic ester or amide bond of QS signalling molecule.

However, eukaryotic microorganisms are reported to secrete quorum sensing interfering (QSI) molecules. These molecules have gained huge attention as they are found to act on microbial signalling system influencing it both positively and negatively. Chemical synthesis of structural homologues of certain QS signalling molecules has further increased the available QSI molecules which can be used against pathogenic microorganisms. However, designing and construction of new transgenic plants are till date the effective way to have control over these activities (Hartmann and Schikora 2012). Presence of AHL lactonase in *Bacillus thuringiensis* in part is considered to be responsible for its biocontrol activity. *B. thuringiensis* on mutating for the AHL lactonase showed decrease in its biocontrol efficacy against *E. carotovora*, thereby stating the role of AHL lactonase. Pathogenic bacteria significantly depend on QS regulation system which helps them have coordination leading to the establishment of disease in host plants. Therefore, plants have evolved mechanisms to bring about disruption of the signalling molecules directly or by producing QS mimics to combat against pathogens, thereby helping themselves in prevention or attenuation of the infection. Additionally, plants have developed certain mechanisms through which they sense the QS signalling molecules produced from pathogens and alter their physiological response towards their defence against pathogens. *Bacillus cereus* produces AiiA, which acts by disrupting AHL structure and makes it inactive. The first reported AHL mimic is a halogenated furanone which acts by inhibiting swarming motility in *S. liquefaciens*. It was discovered in the red algae *Delisea pulchra* and was found to show structural similarity to AHLs (Givskov et al. 1996). These furanones bind to the*LuxR*-type proteins in a non-agonist fashion, thereby increasing the *LuxR* disruption (Manefield et al. 2002). QS signalling in bacteria inhabiting the roots of plants including pea, tomato, *M. truncatula* and rice is found to be under strict regulation by the compounds secreted by them (Gao et al., 2003, Teplitski et al. 2000). Another QS signalling molecule which is an α-β unsaturated fatty acid was identified from *X. campesteris*. It was found to exhibit structural and functional similarities with that of farnesoic acid. Farnesoic acid is a potential QS signalling molecule found to exclusively regulate the morphology and virulence of the fungal pathogen *Candida albicans* (Wang et al. 2004).

12.9 Conclusion

Microorganisms are being well documented as unique sources excelling in decomposition of the environmental waste and reconstitution of disturbed ecosystems. Hence, conservation of these microorganisms is very much essential for the establishment of various schemes and strategies including nutrients management with respect to soil physiochemical aspect and plant disease management. Tremendous and active research carried over the past few decades have hypothesized that rhizosphere microorganisms use QS signalling mechanisms to influence their physiological response which in turn establishes communication among themselves and with that of their interaction with host plants. Initially, most of the bacteria are thought to possess just AHL-based *LuxR/LuxI* homologous systems, but now the scenario has become even more complex as new types of QS systems and signalling molecules are being actively reported. Active research is demanded to further understand the role that QS molecules play among the microbial communities as well as during plant-microbe interactions. There are several reports available these days on bacteria that disrupt their own signals. Further detailed studies are required regarding AHLs and other QS molecules production, their disruption and also their effects on other microorganisms and plant during their interaction with host plant.

Acknowledgement The authors acknowledge the financial support from UGC-MJRP 42-481/2013(SR) and UGC-BSR-RFSMS F.4-1/2006(BSR) 7-369/2012(BSR).

References

Ahmad I, Aquil F, Ahmad F, Zahin M, Musarrat J (2008) Quorum sensing in bacteria: potential in plant health protection. In: Ahmad I, Hayat S, Pichtel J (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. Wiley, Weinheim, pp 129–153

Alqueres S, Menses C, Rouws L, Rothballer M, Baldani I, Schmid M, Hartmann A (2013) The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* strain PAL5. Mol Plant Microbe Interact 26:937–945

Amari DT, Marques CNH, Davies DG (2013) The putative enoyl-coenzyme A hydratase DspI is required for production of the *Pseudomonas aeruginosa* biofilm dispersion autoinducer *cis*-2-decenoic acid. J Bacteriol 195:4600–4610

Amrutha RN, Bramhachari PV, Prakasham RS (2018) Quorum-sensing mechanism in rhizobium sp.: revealing complexity in a molecular dialogue. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 249–258

Balaban N, Novick RP (1995) Autocrine regulation of toxin synthesis by *Staphylococcus aureus*. Proc Natl Acad Sci U S A 92:1619–1623

Barnard AM, Bowden SD, Burr T, Coulthurst SJ, Monson RE, Salmond GP (2007) Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. Philos Trans R Soc B Biol Sci 362:1165–1183

Bassler BL (2002) Small talk: cell to cell communication in bacteria. Cell 109:421–424

Brown SH, Scott JB, Bhaheetharan J, Sharpee WC, Milde L, Wilson RA, Keller NP (2009) Oxygenase coordination is required for morphological transition and the host fungus interaction of *Aspergillus flavus*. Mol Plant Microbe Interact 22:882–894

Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459

Chan KG, Puthucheary SD, Chan XY, Yin WF, Wong CS, Too WS (2011) Quorum sensing in *Aeromonas* species isolated from patients in Malaysia. Curr Microbiol 62:167–172

Chapalain A, Vial L, Laprade N, Dekimpe V, Perreault J, Deziel E (2013) Identification of quorum sensing-controlled genes in *Burkholderia ambifaria*. Microbiol Open 2:226–242

Chen H, Fujita M, Feng Q, Clardy J, Fink GR (2004) Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc Natl Acad Sci U S A 101:5048–5052

Chevrot R, Rosen R, Haudecoeur E, Cirou A, Shelp BJ, Ron E, Faure D (2006) GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. Proc Natl Acad Sci U S A 103:7460–7464

Chin-A-Woeng TFC, Van den Broek D, deVoer G, Vander Drift KMGM, Tuinman S, Thomas-Oates JE, Lugtenberg BJJ, Bloemberg GV (2001) Phenazine-1-carboxamide production in the biocontrol strain *Pseudomonas chlororaphis* PCL1391 is regulated by multiple factors secreted into the growth medium. Mol Plant-Microbe Interact 14:969–979

Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ (2003) Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503–523

Dunlap PV (1999) Quorum regulation of luminescence in *Vibrio fischeri*. J Mol Microbiol Biotechnol 1:5–12

Ferluga S, Steindler L, Venturi V (2008) N-acyl homoserine lactone quorum sensing in Gram negative rhizobacteria. In: Karlovsky P (ed) Secondary metabolites in soil ecology. Springer, Berlin, pp 69–90

Galloway WR, Hodgkinson JT, Bowden SD, Welch M, Spring DR (2011) Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and Al-2 quorum sensing pathways. Chem Rev 111:28–67

Gao M, Teplitski M, Robinson JB, Bauer WD (2003) Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Mol Plant-Microbe Interact 16:827–834

Gao X, Brodhagen M, Isakeit T, Brown SH, Gobel C, Betran J, Feussner I, Keller NP, Kolomiets MV (2009) Inactivation of the lipoxygenase ZmLOX3 increases susceptibility of maize to Aspergillus spp. Mol Plant Microbe Interact 22:222–231

Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone mediated prokaryotic signalling. J Bacteriol 178:6618–6622

Gonzalez JE, Marketon MM (2003) Quorum sensing in nitrogen-fixing Rhizobacteria. Microbiol Mol Biol Rev 67:574–592

González O, Ortíz-Castro R, Díaz-Pérez C, Díaz-Pérez AL, Magaña-Dueñas V, López-Bucio J, Campos-García J (2017) Non-ribosomal peptide synthases from *Pseudomonas aeruginosa* play a role in cyclodipeptide biosynthesis, quorum-sensing regulation, and root development in a plant host. Microb Ecol 73:616–629

Hartmann A, Schikora A (2012) Quorum sensing of bacteria and trans-kingdom interactions of *N-acyl* homoserine lactones with eukaryotes. J Chem Ecol 38:704–713

Hartmann A, Gantner S, Schuhegger R, Steidle A, Dürr C, Schmid M (2004) N-acyl-homoserine lactones of rhizosphere bacteria trigger systemic resistance in tomato plants. In: Lugtenberg B, Tikhonovich I, Provorov N (eds) Biology of molecular plant-microbe interactions, vol 4. MPMI-Press, St. Paul, pp 554–556

Henke JM, Bassler BL (2004) Bacterial social engagements. Trends Cell Biol 14:648–656

Hogan DA (2006) Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot Cell 5:613–619

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol 67:2982–2992

Horowitz Brown S, Zarnowski R, Sharpee WC, Keller NP (2008) Morphological transitions governed by density dependence and lipoxygenase activity in *Aspergillus flavus*. Appl Environ Microbiol 74:5674–5685

Jin G, Liu F, Ma H, Hao S, Zhao Q, Bian Z, Jia Z, Song S (2012) Two G-protein-coupled receptor candidates, Cand2 and Cand7, are involved in *Arabidopsis* root growth mediated by the bacterial quorum-sensing signals *N*-acyl-homoserine lactones. Biochem Biophys Res Commun 417:991–995

Joseph CM, Phillip DA (2003) Metabolites from soil bacteria affect plant water relations. Plant Physiol Biochem 41:189–192

Kang JE, Han JW, Jeon BJ, Kim BS (2016) Efficacies of quorum sensing inhibitors Piericidin A and Glucupiericidin A produced by *Streptomyces xanthocidicus* KPP01532 for the control of potato soft rot caused by *Erwinia carotovora* subsp. *atrosptica*. Microbiol Res 184:32–41

Keshavan ND, Chowdhary PK, Haines DC, Gonzalez JE (2005) L-Canavanine made by *Medicago sativa* interferes with quorum sensing in *Sinorhizobium meliloti*. J Bacteriol 187:8427–8436

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

Liu F, Bian Z, Jia Z, Zhao Q, Song S (2012) The GCR1 and GPA1 participate in promotion of Arabidopsis primary root elongation induced by N-acyl-homoserine lactones, the bacterial quorum-sensing system. Mol Plant Microbe Interact 25:677–683

López-Ráez JA, Bouwmeester H, Pozo MJ (2012) Communication in the rhizosphere, a target for pest management. In: Agroecology and strategies for climate change. Springer, Dordrecht, pp 109–133

Lutter E, Lewenza S, Dennis JJ, Visser MB, Sokol PA (2001) Distribution of quorum-sensing genes in the *Burkholderia cepacia* complex. Infect Immun 69:4661–4666

Madhani HD (2011) Quorum sensing in fungi: Q&A. PLOS Pathog 7:e1002301

Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, Kjelleberg S, Givskov M (2002) Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology 148:1119–1127

Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anoliés, G., Rolfe, B. G., Baur, W. D. (2003). Extensive and specific responses of a eukaryote to bacterial quorum sensing signals. *Proceedings of National Academy of Sciences of the United States of America,* 100:1444-1449

Mellbye B, Schuster M (2011) More than just a quorum: integration of stress and other environmental cues in acyl-homoserine lactone signalling. In: Storz G, Hengge R (eds) Bacterial stress responses. ASM Press, Washington, DC, pp 349–363

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663

Morfeldt E, Taylor D, Von Gabain A, Arvidson S (1995) Activation of alpha toxin translation in *staphylococcus aureus* by the transcoded antisense RNA, RNA III. EMBO J 14:4569–4577

Mosblech A, Feussner I, Heilmann I (2009) Oxylipins: structurally diverse metabolites from fatty acid oxidation. Plant Physiol Biochem 47:511–517

Mueller S, Hilbert B, Dueckershoff K, Roitsch T, Krischke M, Mueller MJ, Berger S (2008) General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in Arabidopsis. Plant Cell 20:768–785

O'Connell A, An SQ, McCarthy Y, Schulte F, Niehaus K, He YQ, Ryan RP, Dow JL (2013) Proteomics analysis of the regulatory role of Rpf/DSF cell-to-cell signaling system in the virulence of *Xanthomonas campestris*. Mol Plant Microbe Interact 26:1131–1137

Ortíz-Castro R, Martinez-Trujillo M, López-Bucio J (2008) *N*-acyl homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ 31:1497–1509

Pang Y, Liu X, Ma Y, Chernin L, Berg G, Gao K (2009) Induction of systemic resistance, root colonization and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on *N*-acyl homoserine lactones. Eur J Plant Pathol 124:261–268

Raina S, DeVizio D, Palonen EK, Odell M, Brandt AM, Soini JT, Keshavarz T (2012) Is quorum sensing involved in lovastatin production in the filamentous fungus *Aspergillus terreus*? Process Biochem 47:843–852

Rumbaugh KP (ed) (2011) Quorum sensing: methods and protocols. Methods in molecular biology. Springer, New York

Ryan RP, An SQ, Allan JH (2015) The DSF family of cell–cell signals: an expanding class of bacterial virulence regulators. PLoS Pathog 11:e1004986

Schenk ST, Stein E, Kogel KH, Schikora A (2012) *Arabidopsis* growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal Behaviour 7:178–181

Schimmel TG, Coffman AD, Parsons SJ (1998) Effect of butyrolactone I on the producing fungus *Aspergillus terreus*. Appl Environ Microbiol 64:3707–3712

Schmidt S, Blom JF, Pernthaler J, Berg G, Baldwin A, Mahenthiralingam E (2009) Production of the antifungal compound pyrrolnitrin is quorum sensing regulated in members of the *Burkholderia cepacia* complex. Environ Microbiol 11:1422–1437

Schuhegger R, Ihring A, Gantner S, Bahnweg G, Knappe C, Vogg G, Hutzler P, Schmid M, Breusegem FV, Eberl L, Hartmann A, Langebartels C (2006) Induction of systemic resistance in tomato plants by *N*-acyl-homoserine lactone–producing rhizosphere bacteria. Plant Cell Environ 29:909–918

Sorrentino F, Roy I, Keshavarz T (2010) Impact of linoleic acid supplementation on lovastatin production in *Aspergillus terreus* cultures. Appl Microbiol Biotechnol 88:65–73

Sturme MH, Kleerebezem M, Nakayama J, Akkermans ADL, Vaughan EE, deVos WM (2002) Cell to cell communication by autoinducing peptides in Gram-positive bacteria. Antonie Van Leeuwenhoek 81:233–243

Suárez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonça-Previato L, James EK, Venturi V (2012) Common features of environmental and potentially beneficial plant-associated Burkholderia. Microb Ecol 63:249–266

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviours in associated bacteria. Mol Plant Microbe Interact 13:637–648

Tian J, Weng LX, Zhan YQ, Wang LH (2013) BDSF inhibits *Candida albicans* adherence to urinary catheters. Microb Pathog 64:33–38

Tsitsigiannis DI, Keller NP (2007) Oxylipins as developmental and host–fungal communication signals. Trends Microbiol 15:109–118

Urlich RL (2004) Quorum quenching: enzymatic disruption of *N*-Acylhomoserine lactone-mediated bacterial communication in *Burkholderia thailandensis*. Appl Environ Microbiol 70:6173–6180

Uroz S, Heinonsalo J (2008) Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root associated fungi. FEMS Microbiol Ecol 65:271–278

Von Rad U, Klein I, Dobrev PI, Kottova J, Zazimalova E, Fekete A, Hartmann A, Schmitt-kopplin P, Durner J (2008) The response of *Arabidopsis thaliana* to *N*-hexanoyl- DL-homoserine lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229:73–85

Wang LH, He Y, Gao Y, Wu JE, Dong YH, He C, Wang SX, Weng LX, Xu JL, Tay L (2004) A bacterial cell–cell communication signal with cross-kingdom structural analogues. Mol Microbiol 51:903–912

White CE, Winans SC (2008) The cell-cell communication system of *Agrobacterium tumefaciens*. In: Tzfira T, Citovsky V (eds) *Agrobacterium:* from biology to biotechnology. Springer, New York, pp 593–622

Williams P (2007) Quorum sensing, communication and cross kingdom signalling in the bacterial world. Microbiology 153:3923–3938

Wood DW, Gong F, Daykin MM, Williams P, Pierson LS (1997) N-acyl homoserine lactone mediated regulation of phenazine gene expressions by *Pseudomonas aureofaciens* 30-84 in the wheat rhizosphere. J Bacteriol 174:7663–7670

Zhou H, Yao F, Roberts DP, Lessie TG (2003) AHL-deficient mutants of *Burkholderia ambifaria* BC-F have decreased antifungal activity. Curr Microbiol 47:174–179

13 Role of Medicinal Plants and Endophytic Bacteria of Medicinal Plants in Inhibition of Biofilm Formation: Interference in Quorum Sensing

Chanda Vikrant Berde, Sagar P. Salvi, Prachiti P. Rawool, A. M. V. N. Prathyusha, and Vikrant B. Berde

Abstract

Quorum sensing (QS) plays a major part in the growth of biofilms. QS depends on the presence of bacteria in sufficient numbers which enables achieving threshold level of autoinducer, thus triggering the gene expression responsible for biofilm formation virulence, sporulation, conjugation, etc. Controlling the production of autoinducers can provide an approach for disease control. Studies on quorum sensing inhibition and the quest for QS inhibitors have shown many eukaryotes, particularly plants, and even bacteria to produce anti-QS substances. Several anti-QS methods have been proved including natural products from plant-based secondary metabolites. Many species of medicinal plants yield metabolites that can control the microbial growth and have traditionally been used to treat diseases, particularly microbial infections. Endophytic bacteria of medicinal plants have been researched for their role in disease control. Most of the medicinal properties of the plants have actually been found to be a characteristic of the endophytic community of the plant. Medicinal plants and the endophytes of the medicinal plants with anti-quorum sensing activity thus having potential as biofilm formation inhibitors have been reviewed in this chapter.

Keywords

Quorum sensing · Inhibition · Medicinal plants · Endophytes

C. V. Berde · S. P. Salvi · P. P. Rawool
Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

A. M. V. N. Prathyusha
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

V. B. Berde (✉)
Department of Zoology, Arts, Commerce and Science College, Lanja, Maharashtra, India
e-mail: vikrantzoo@yahoo.co.in

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_13

Virulence gene expression in the pathogenic microorganisms uses a QS system to control. Thus, inhibition of the QS system is considered as an innovative approach for the growth of antipathogenic agents. These agents are used for fighting bacterial infections caused by microbial strains that are resistant to antibiotics. Biofilms can be defined as organized aggregation of microorganisms attached to surfaces, encased in an extracellular matrix. The biofilm life cycle occurs in four stages:

1. Bacterial cells attach to surfaces in the first phase.
2. In the second phase, microcolonies are formed on the surface.
3. Third phase is maturation of the microcolonies to form a biofilm.
4. Last phase involves dispersal of the bacteria.

In the initial stage, the bacteria establish onto the solid living or nonliving substratum by van der Waals forces, steric interactions, and electrostatic (double-layer) interaction. These interactions are collectively known as the DLVO (Derjaguin, Landau, Verwey, and Overbeek) forces (Garrett et al. 2008). Quorum sensing (QS) system is responsible for the bacteria cell density regulation as well as metabolic activity of the biofilm. Thus, QS system is the main regulatory and cell-to-cell communication system. *N*-acylated l-homoserine lactones (AHLs) are produced as autoinducers (AIs) during quorum sensing in Gram-negative bacteria such as *Escherichia coli*.

Biofilms can occur especially on implants in the medical scenario. These implanted devices provide the surface for adherence and formation of a biofilm. Microbial community present in the biofilm is highly complex. It is highly resistant to antibiotics and disinfectants. Thus, they persist and survive removal techniques and methods. Thus, biofilms are a major hazard, and therefore, eliminating the contaminated medical implant is frequently the best strategy for therapy. Persistent infections are also a result of the cells detaching from biofilm and getting in the bloodstream (Ramage et al. 2002, 2005). Biofilms lead to substantial economic and health problems.

There are numerous conventional routes for fighting biofilms such as removal by physical and chemical methods and use of antimicrobials, antiseptics, and disinfectants to eliminate biofilm organisms. However, biofilms are extremely unaffected by these approaches as against the planktonic cells. Thus, novel approaches other than the conventional methods are the need of the day.

Mature biofilms are unaffected by antimicrobial agents due to the altered growth proportion of the organisms in the biofilm (Donlan and Costerton 2002), and subsequently, the subpopulations also gain resistance (Ito et al. 2009). The resistance can be transferred horizontally using plasmid that bears the resistance genes. Thus, many research groups have focused their attention to the development of agents that interfere with the biofilm structure and eventually can regulate biofilm-mediated infections.

According to Pan and Ren (2009), biofilm formation will not take place if the QS process is interrupted as it affects the survival and pathogenesis of the bacteria. Signal production, detection, and gene activation/inactivation are the steps involved in quorum sensing (QS).

QS has been the main target for the development of anti-biofilm strategies that are not based on the use of antibiotics. Therefore, blocking bacterial QS system may prevent QS-controlled phenotypes accountable for food spoilage. Numerous anti-QS methods have been revealed including natural products from plant-based secondary metabolites.

13.1 Anti-Quorum Sensing Activity of Plant Extracts

Natural products have played a significant role as sources of new drugs. The natural products include extracts of different parts of the plants, oils from different plants, and plant metabolites. Many plant species have evolved processes as adaptive methods, to produce metabolites that can influence the growth of microorganisms and these have been used from ages to treat human diseases, particularly infections. In most of the studies, the plants known traditionally for their healing properties were tested. From the different studies carried out, it is evident that biofilms of various pathogenic bacteria can be disrupted or their formation hindered by these plant produces.

A lot of work has been carried out on the biofilms of *Staphylococcus* species. This research data is important keeping in mind the drug resistance increasing tremendously in these genera. Artini et al. (2012) have reported the activity of *Krameria*, *Aesculus hippocastanum*, and *Chelidonium majus* extracts in the inhibition of biofilm formation by *S. aureus*. The major components present in these plants were chelerythrine, sanguinarine, dihydroxybenzofuran, and proanthocyanidin. These compounds were found to be responsible for the inhibition activity. Similar inhibitory activity against *Staphylococcus aureus* was shown by plant extracts containing tannins (Payne et al. 2012). In a study reported by Taganna et al. (2011), the high activity fraction of *Terminalia catappa* extract was found to be rich in tannins. The tannins showed potential to inhibit the phenotypic properties in test strains related to quorum sensing. *Chromobacterium violaceum* produces pigment violacein, and the production is quorum dependent. Tannins have ability to reduce the violacein production by interfering with the QS system. Similarly, biofilm maturation in *Pseudomonas aeruginosa* has also been reported to be affected in the presence of tannins. QS inhibitory potentials of extracts of *Rosa rugosa* have been successfully proved by Zhang et al. (2014). These extracts were found to be rich in polyphenols. The findings strongly propose that polyphenol extract of the plant could be used efficiently as QS inhibitor in food industry, thus increasing the shelf life and food safety.

Anti-biofilm activity has been widely reported for polyphenol containing tea-tree oil, from the *Melaleuca alternifolia* leaves (Kwiecinski et al. 2009). An essential oil extracted from this tree is tea-tree oil. Secondly, bark and leaves of cinnamon trees, containing cinnamaldehyde, also have antibiofilm activity. American cranberry (*Vaccinium macrocarpon*) extracts have been reported to contain active constituent proanthocyanins (PAC), which is attributed for its QS ability. Another example is derivatives of ellagic acid from *Rubus ulmifolius* that have the QS property.

Interestingly, the efficiency, mode of action, and dosage required for biofilm disruption vary from plant to plant. It is pertinet that the Tea-tree eliminates biofilm-forming ability in *S. aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Kwiecinski et al. 2009). Other studies suggest that failure of pathogen to establish biofilm is due to the ability of tea-tree oil to disrupt the adherence factors responsible for the attachment of bacteria to the substratum (Park et al. 2007). Coelho et al. (2012) reported studies of antibacterial activity of tea-tree essential oil (TTO) in combination with ciprofloxacin (CIP) against *P. aeruginosa* biofilms. The results proved that the synergistic effect of TTO with (CIP) resulted in reduction in biofilm biomass more than 70%.

Proanthocyanins have been reported to hinder the growth and biofilm formation of Gram-positive bacteria, such as *Staphylococcus* sp., but not the Gram-negative bacteria (*E. coli*) (LaPlante et al. 2012). Latest studies have shown that cinnamaldehyde, a key active compound present in cinnamon essential oil, can also halt the biofilm formation in *S. aureus* in a dose-dependent manner (Jia et al. 2011).

Coenye et al. (2012) have worked extensively on the anti-biofilm activity of 119 plants, to eliminate the biofilm formed by *Propionibacterium acne*. This study demonstrated that five plant extracts, namely, *Epimedium brevicornum*, *Malus pumila*, *Polygonum cuspidatum*, *Rhodiola crenulata*, and *Dolichos lablab*, among the 119 plants tested, showed a potent antibiofilm activity. They found that *E. brevicornum* and *P. cuspidatum* extracts show a remarkable antibiofilm activity at levels below the minimum inhibitory concentrations. The active ingredients present in these plant extracts were icartin and resveratrol.

Studies on the inhibition of biofilms of *E. coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *P. mirabilis* by using the extracts of *Capparis spinosa* (caper bush) have been carried out by Issac et al. (2011). The inhibition studies showed the extracts showed very good activity at very low concentration showing its high efficiency as an anti-biofilm agent against a wide host range. *Streptococcus mutans* biofilm was inhibited by green tea at 6.2 mg/mL and dandasa extracts at the concentration of 12.5 mg/mL. Faraz et al. (2012) further reported good antibiofilm effects by these extracts at concentrations of 12.5 and 3.1 mg/mL, respectively. Ravichandiran and his team (2012) have reported inhibition of *E. coli* biofilm by bark extracts of *Melia dubia* at the concentration of 30 mg/mL.

Some plant flower extracts are good anti-biofilm inhibitors. *Lagerstroemia speciosa* is a common medicinal plant found in Southeast Asia. Singh et al. (2012) proved that *L. speciosa* fruit extracts significantly blocked biofilm formation at 10 mg/mL concentration. The most effective extracts were isolated from medicinal plants, namely, branches of *Bauhinia acuruana*, fruits of *Chamaecrista desvauxii*, fruits of *B. acuruana*, and leaves of *Pityrocarpa moniliformis*. These plants are from Brazilian xeric shrubland. Biofilm formation was remarkably repressed when these extracts were added (Dda et al. 2011). Forty-five aqueous extracts prepared from 24 Caatinga medicinal plant species were checked for their ability to inhibit biofilm activity of *Staphylococcus epidermidis.*

Biofilm formation of two Gram-positive species of bacteria, namely, *Staphylococcus aureus* and *S. epidermidis*; five Gram-negative bacteria, namely, *Pseudomonas fluorescens*, *P. aeruginosa*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *E. coli*; and three yeast species, i.e. *Candida tropicalis*, *Candida albicans*, and *C. glabrata*, was inhibited by a native plant of Brazil, *Croton nepetaefolius.* This plant contains casbane diterpene which has the anti-quorum activity (Carneiro et al. 2011). *Boesenbergia pandurata* (finger root) also showed inhibition of *Candida* sp. biofilm formation. The biofilms are reduced by 63–98% using the oil of the plant even when sub-MIC concentrations were used (Taweechaisupapong et al. 2010).

P. aeruginosa biofilm formation is strongly inhibited by fresh extract of *Allium sativum* (fresh garlic extract [FGE]). There is drastic reduction in the growth of the biofilm by 6 log units (Harjai et al. 2010). Similarly, eugenol and caffeine have similarly been proved to be effective quorum sensing inhibitors (Zhou et al. 2013; Norizan et al. 2013).

The plants from neotropical rain forest have been widely studied for quorum sensing and biofilm inhibitory activities. Ta et al. (2014) reported the quorum sensing inhibitory activity of 71 plant species belonging to the Meliaceae, Melastomataceae, Lepidobotryaceae, Sapindaceae, and Simaroubaceae families. According to the authors, extracts of these dynamic species may perhaps lead to future development of plant-based treatments for biofilm-associated infections.

The extract of *A. indica* (neem) was most efficient in eradicating *M. smegmatis* biofilms. The neem metabolites are thus promising candidates as tools against mycobacterial infections (Syed et al. 2014). Lee et al. (2013) have worked on varied plant extracts for activity against enterohemorrhagic *E. coli* (EHEC) O157:H7 biofilm. They found 16 plant extracts out of 498 could inhibit biofilm formation up to 85%.

Curcuma longa rhizome contains curcumin or diferuloylmethane [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] as a natural component. The antibacterial activity of curcumin, along with antimicrobial therapy, can be beneficial for dealing with resistant *P. aeruginosa*. The treatment with curcumin was also found to reduce the QS-dependent factors, such as exopolysaccharide production, alginate production, and swimming and swarming motility of uropathogens. In addition to this, curcumin was also found to enhance the vulnerability of a marker strain and uropathogens to conventional antibiotics (Issac et al. 2014).

13.2 Anti-Quorum Sensing Activity of Plant Essential Oils

Quorum sensing mechanism of pathogens has been reported to be interfered by numerous essential oils (EOs). Due to their preservative and antimicrobial effects, EOs are natural constituents used in food industry. Many EOs exert their antimicrobial effect on microbial cell wall which leads to cell death. Moreover, it is also reported that EOs act on the bacteria without evolving antimicrobial resistance (Ohno et al. 2003; Ali et al. 2005).

Cumin oil (*Cuminum cyminum*): The efficacy of cumin seed EO against the biofilm development by *K. pneumoniae* strains, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Serratia marcescens*, has been reported by Safoura et al. (2010) and Abraham et al. (2012). Cumin oil is extracted from medicinal aromatic plants. It is used for flavoring foods and medical formulations (Iacobellis et al. 2005). It contains eugenol and has many medicinal uses.

Cinnamon oil: This oil is effective against pathogenic bacteria capable of forming biofilms such as *S. mutans*, *S. epidermidis*, enteropathogenic *E. coli* (EPEC), *L. monocytogenes*, and *Lactobacillus plantarum* (Filoche et al. 2005; Nuryastuti et al. 2009; Oliveira et al. 2012).

Oregano essential oil: Anti-QS activity of oregano essential oil on biofilm development by *Staphylococci* species, namely, *S. aureus*, *S. lugdunensis*, *S. haemolyticus*, *S. sciuri*, and *E. coli*, was reported by Nebahat et al. (2010).

Vegetable oil: Filogˆonio et al. (2011) and colleagues found vegetable oil to be effective in the dental biofilm control, thus a promising use in the inhibition of dental caries and periodontal diseases.

The effectiveness of three essential oils (thymol, oregano, and cinnamon oils) at sublethal levels on biofilm formation of biofilm-forming bacterial strains, i.e., *Acinetobacter*, *Sphingomonas*, and *Stenotrophomonas*, was researched by Sandra (2014). Thyme oil was found to be most effective and it repressed the formation of a biofilm.

13.3 Anti-Quorum Sensing Activity of Mangrove Plant Extracts

Two mangroves, Solanaceae (Singh et al. 2015) and *Rhizophora* (2013), have been studied for quorum sensing activity. Quorum quenching molecules from Solanaceae family are bioactive proteins that can be utilized to device a healing technique against infectious microorganisms. These bioactive proteins inactivate QS signals from bacteria and inhibit cell-to-cell communication. As a consequence, virulence development in *Pseudomonas aeruginosa* is repressed. The antipathogenic potential of mangrove trees of the genus *Rhizophora* was evaluated against *Pseudomonas aeruginosa* PAO1 and two other clinical isolates. The *Rhizophora apiculata* and *R. mucronata* methanol extracts were found to inhibit QS-dependent virulence factor production, such as LasA protease, LasB elastase, total protease, and pyocyanin pigment production. These extracts also inhibited biofilm formation in all the test strains. This is the first report on the QS inhibitory (QSI) ability of *Rhizophora* spp. against *P. aeruginosa* infections (Annapoorani et al. 2013).

A lot of research on Chinese herbs has been carried out based on the traditional knowledge of Chinese treatment methods. *Centella asiatica* (L.) Urban is the ingredient in Ayurvedic medicine as well as traditional African and Chinese medicine (Vasavi et al. 2016). *C. asiatica* is used in the treatment of skin problems and healing wounds and is an antibacterial and antiviral agent. Triterpene acids and their sugar esters, asiatic acid, madecassic acid, and asiaticosides have anti-biofilm properties. *Centella* extract shows very good anti-biofilm activity. Quorum sensing in

pathogens and its inhibition by plant extracts have been very nicely described by Sadekuzzaman et al. (2015) and Basavaraju et al. (2016).

13.4 Role of Endophytes in Quorum Sensing Inhibition

Endophytic bacteria inhabit inner plant tissues without causing any harm. Favorable properties detected in most of the endophytic microorganisms include tolerance to environmental conditions and resistance to microbial pathogens, and they act as effective biocontrol agent (Parulekar-Berde 2015). In recent years, QS inhibition has become an intense area of research due to its applications in medicine, industry, and biotechnology. In the search for QS inhibitors, research has proved that many eukaryotes, particularly plants, and even bacteria themselves produce anti-QS substances.

Owing to their presence in a specific niche, endophytic microorganisms are able to synthesize diverse types of bioactive molecules. These bioactive compounds are produced in order to get over the challenges faced in these niches. Continuous development of resistance mechanism by pathogens to the currently available health treatments along with pharmaceuticals has led researchers to explore new therapeutic agents. Utilization of quorum sensing (QS) inhibitors in antivirulence approach against pathogenesis is one of the inventive strategies.

Chan et al. (2011) have reported coexistence of QS and quorum quenching (QQ) activities in *Acinetobacter* and *Burkholderia* isolated from ginger (*Zingiber officinale*) rhizosphere. A collective role of bacteria is observed in QS-dependent phenotype of a community that is polymicrobial. Such bacteria can break down a wide range of AHLs.

Endophytes live symbiotically in relation with plant and produce many secondary metabolites including enzymes. *Pterocarpus santalinus* L. (Fabaceae), known commonly as red sandalwood, is a substitute for teakwood. This species is found exclusively in Western Ghats of India. *P. santalinus* has been used as a folk remedy for the treatment of inflammation, mental aberrations, ulcers, and control of diabetes (Krishnaveni and Rao 2000). Shastry and Ravishankar (2013) worked on the endophytic fungi *Ventilago madraspatana* Gaertn. They attributed the anti-QS activity of the endophytes to their hydrolytic enzymes. Endophytic fungi were isolated from *Ventilago madraspatana.*

The endophytic bacteria of *Pterocarpus santalinus* were screened for the existence of N-acyl homoserine lactones (AHLs) degrading ability using biosensor strains and the activity was confirmed by quantifying the violacein production of the biosensor strain. Cell-free lysate of endophytic bacteria, *Bacillus firmus* PT18 and *Enterobacter asburiae* PT39, revealed potent AHL degrading ability by hindering about 80% violacein production in biosensor strain.

Quorum quenching (QQ) activity is related to QS in that the QQ leads to degradation of QS molecules (Barrios et al. 2009); as a result the sufficient buildup of bacterial cell number does not take place, and thus quorum sensing is disrupted (Medina-Martinez et al. 2007). Breakdown of AHL molecules is brought about by

two major enzymes, lactonases and acylases, which act by breaking lactone ring and acyl side chain, respectively (Rashid et al. 2011).

Mookherjee et al. (2018) have presented a summary on the endophytic microorganisms as a treasure trove of secondary metabolites which are mostly bioactive molecules, particularly the screening of endophytic bacteria, purification of QS inhibitors, production of QS inhibitors, and application of QS inhibitors. There is immense prospect for endophytic microorganisms in healthcare and food industry, as long as a comprehensive understanding of the biology of endophyte, its characteristics, pathogenicity, and its ecosystem is developed (Mookherjee et al. 2018).

The work on the endophytic bacteria *C. sativa* offers fundamental understanding of the antivirulence strategies used by endophytes to survive in their ecological niches. The endophytes develop defense mechanisms in order to avert the large number of pathogens attacking associated host plants. These mechanisms ensure that the pathogen does not gain resistance to the plant/endophyte bioactive secondary metabolites. The work of Kusari et al. (2014a) provides evidence for utilizing endophytes as tools for biological control of bacterial phytopathogens.

Radula marginata and *Cannabis sativa* L., two phylogenetically distinct plant species, have been reported by Kusari et al. (2014b) to contain structurally similar secondary metabolites like cannabinoids. Biofilm-forming potential and anti-biofilm ability of endophytic microbial community of the liverwort *R. marginata*, as compared to bacterial endophytic isolates harbored in *C. sativa* plants, were studied. *Radula* and *Cannabis* plants were found to harbor similar endophytic bacterial genera which exhibited similar functional traits like biofilm formation and universal anti-biofilm activities.

Microbacterium testaceum BAC1065 and BAC1093 were isolated from the "Talismã" cultivar. Their cultural extracts strongly inhibited most of the pathogenic bacteria tested. Bean endophytic bacteria were also shown to have the ability to inhibit the quorum sensing of Gram-negative bacteria. The ability to prevent QS has not been reported previously for endophytic microorganisms of *P. vulgaris*. Furthermore, *M. testaceum* with ability to inhibit quorum sensing appears to be widespread in common bean (Lopes et al. 2015).

Quorum sensing influences biofilm maturation and quenching these QS systems may contribute to fighting biofouling. Signal inactivation by enzymatic degradation or modification is one possibility to restrict QS and these enzymes are common in the prokaryotes as well as in eukaryotes. Lactonases and acylases hydrolyze N-acyl homoserine lactone (AHL) molecules which have been most intensively explored. Fetzner (2015) has mentioned the various applications of signal-degrading bacteria as biocontrol agents in the defense of crops against soft-rot disease, the use of signal-degrading bacteria as probiotics in aquaculture, and the entrapment of quorum quenching enzymes or bacteria to control biofouling in membrane bioreactors.

The engineered endophytic strain KJ006 (pKPE-aiiA) is reported to inhibit production of quorum sensing signals by wild-type strain in vitro. It could minimize the occurrence of rice seedling rot caused by *Burkholderia glumae* in situ. Thus, the bacterial endophyte transformed with the aiiA gene can be used as a novel

biological control agent against pathogenic *Burkholderia glumae* that are known to occupy the same ecological niche (Cho et al. 2007).

Therefore, compounds obtained from natural sources and their different formulations could be a novel approach to combating biofilms. Although these agents were effective and showed enormous potential in the treatment of biofilm-associated infections, their mechanisms of action remain unclear. The molecular pathways and animal model studies of these potential agents could provide a clearer view on the pathways affected. Another approach is to look into the synergistic effect of combinations of these agents and antibiotics to eradicate biofilm-associated infections.

Acknowledgements The authors are grateful to their academic institutions for the support extended.

Conflict of Interest The authors declares that they have no competing interests.

References

Abraham NM, Lamlertthon S, Fowler VG, Jefferson KK (2012) Chelating agents exert distinct effects on biofilm formation in Staphylococcus aureus depending on strain background: role for clumping factor B. J Med Microbiol 61:1062–1070

Ali S, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H, Rao LV, Habibullah CM, Sechi LA, Ahmed N (2005) Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. Ann Clin Microbiol Antimicrob 4:20

Annapoorani A, Kalpana B, Musthafa KS, Pandian SK, Ravi AV (2013) Antipathogenic potential of *Rhizophora* spp. against the quorum sensing mediated virulence factors production in drug resistant *Pseudomonas aeruginosa*. Phytomedicine 20:956–963

Artini M, Papa R, Barbato G, Scoarughi GL, Cellini A, Morazzoni P, Bombardelli E, Selan L (2012) Bacterial biofilm formation inhibitory activity revealed for plant-derived natural compounds. Bioorg Med Chem 20:920–926

Barrio AFG, Covo V, Vives M, Medina LM, Achenie L (2009) Quorum quenching analysis in Pseudomonas aeruginosa and *Escherichia coli*: network topology and inhibition mechanism effect on the optimized inhibitor dose. Bioprocess Biosyst Eng 32(4):545–556

Basavaraju H, Sisnity VS, Palaparthy R, Addanki PK (2016) Quorum quenching: signal jamming in dental plaque biofilms. J Dent Sci 11:349–352

Carneiro VA, Santos HS, Arruda FV, Bandeira PN, Albuquerque MR, Pereira MO, Henriques M, Cavada BS, Teixeira EH (2011) *Casbane diterpene* as a promising natural antimicrobial agent against biofilm-associated infections. Molecules 16:190–201

Chan K, Atkinson S, Mathee K, Sam C, Chhabra SR, Camara S et al (2011) Characterization of N-acylhomoserine lactone degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. BMC Microbiol 11:1–13

Cho HS, Park SY, Ryu CM, Kim JF, Kim JG, Park SH (2007) Interference of quorum sensing and virulence of the rice pathogen *Burkholderia glumae* by an engineered endophytic bacterium. FEMS Microbiol Ecol 60:14–23

Coelho FL, Lopes SP, Pereira MO (2012) Effective association of tea tree essential oil with conventional antibiotics to control Pseudomonas aeruginosa biofilm. Biofilm 5 – Proceedings of the international conference, Paris, France, 227:157

Coenye T, Brackman G, Rigole P, DeWitte E, Honraet K, Rossel B, Nelis HJ (2012) Eradication of Propionibacterium acnes biofilms by plant extracts and putative identification of icariin, resveratrol and salidroside as active compounds. Phytomedicine 19:409–412

Donlan RM, Costerton JW (2002) Biofilms: survival mechanism of clinically relevant microorganism. Clin Microbiol Rev 15:167–193

Faraz N, Islam ZU, Rehman R, Sehrish (2012) Antibiofilm forming activity of naturally occurring compound. Biomedica 28:171–175

Fetzner S (2015) Quorum quenching enzymes. J Biotechnol 10:2–14

Filoche SK, Soma K, Sissons and C. H. (2005) Antimicrobial effects of essential oil in combination with chlorhexidine digluconate. Oral Microbiol Immunol 20:221–225

Filogˆonio CF, Soares RV, Horta MC, Penido CV, Cruz RA (2011) Effect of vegetable oil (Brazil nut oil) and mineral oil (liquid petrolatum) on dental biofilm control. Braz Oral Res 25:556–561

Garrett TG, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18:1049–1056

Harjai K, Kumar R, Singh S (2010) Garlic blocks quorum sensing and attenuates the virulence of Pseudomonas aeruginosa. FEMS Immunol Med Microbiol 58:161–168

Iacobellis NS, Cantore PL, Capasso F, Senatore F (2005) Antibacterial activity of Cuminum cyminum *L.* and Carum carvi *L.* essential oils. J Agric Food Chem 53:57–61

Issac ASV, Palani A, Ramaswamy BR, Shunmugiah KP, Arumugam VR (2011) Antiquorum sensing and antibiofilm potential of Capparis spinosa. Arch Med Res 42:658–668

Issac ASVP, Selvam P, Shunmugiah KP, Veera RA (2014) Inhibition of biofilm development of uropathogens by curcumin - an anti-quorum sensing agent from *Curcuma longa*. Food Chem 148C:453–460

Ito A, Taniuchi A, May T, Kawata K, Okabe S (2009) Increased antibiotic resistance of *Escherichia coli* in mature biofilms. Appl Environ Microbiol 75:4093–4100

Jia P, Xue Y, Duan X, Shao S (2011) Effect of cinnamaldehyde on biofilm formation and sarA expression by methicillin-resistant *Staphylococcus aureus*. Lett Appl Microbiol 53:409–416

Krishnaveni KS, Rao SJV (2000) An isoflavone from *Pterocarpus santalinus*. Phytochemistry 53:605–606

Kusari P, Kusari S, Lamshöft M, Sezgin S, Spiteller M, Kayser O (2014a) Quorum quenching is an antivirulence strategy employed by endophytic bacteria. Appl Microbiol Biotechnol 98:7173–7183

Kusari P, Kusari S, Spiteller M, Kayser O (2014b) Biocontrol potential of endophytes harbored in *Radula marginata* (liverwort) from the New Zealand ecosystem. Antonie Van Leeuwenhoek 106:771–788

Kwiecinski J, Eick S, Wojcik K (2009) Effects of tea tree (*Melaleuca alternifolia*) oil on *Staphylococcus aureus* in biofilms and stationary growth phase. Int J Antimicrob Agents 33:343–347

LaPlante KL, Sarkisian SA, Woodmansee S, Rowley DC, Seeram NP (2012) Effects of cranberry extracts on growth and biofilm production of *Escherichia coli* and *Staphylococcus* species. Phytother Res 26:1371–1374

Lee JH, Cho HS, Joo SW, Regmi CS, Kim JA, Ryu SH, Yong RC, Cho MH, Lee J (2013) Diverse plant extracts and trans-resveratrol inhibit biofilm formation and swarming of Escherichia coli O157:H7. Biofouling 29:1189–1203

Lopes RB, Costa LE, Vanetti MC, de Araújo EF, de Queiroz MV (2015) Endophytic bacteria isolated from common bean (*Phaseolus vulgaris*) exhibiting high variability showed antimicrobial activity and quorum sensing inhibition. Curr Microbiol 71:509–516

Mookherjee A, Singh S, Maiti MK (2018) Quorum sensing inhibitors: can endophytes be prospective sources. Arch Microbiol 200:355–369

Medina-Martinez MS, Uyttendaele M, Rajkovic A, Nadal P, Debevere J (2007) Degradation of N-acyl-L-homoserine lactones by *Bacillus cereus* in culture media and pork extract. Appl Environ Microbiol 73:2329–2332

Nebahat B, Leyla V, Berna D, Cigdem S, Abamuslum G, Murat G, HCB K, Mine K (2010) Effect of oregano essential oil on biofilms formed by *Staphylococci* and Escherichia coli. Kafkas Univ Vet Fak Derg 16(suppl A):S23–S29

Norizan SN, Yin WF, Chan KG (2013) Caffeine as a potential quorum sensing inhibitor. Sensors (Basel) 18:5117–5129

Nuryastuti T, van der Mei HC, Busscher HJ, Iravati S, Aman AT, Krom BP (2009) Effect of cinnamon oil on *icaA* expression and biofilm formation by Staphylococcus epidermidis. Appl Environ Microbiol 75:6850–6855

Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, Kodama T, Kashima K, Imanishi (2003) Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter 8:207–215

Oliveira MM, Brugnera DF, Nasciment JD, Batista NN, Piccoli RH (2012) Cinnamon essential oil and cinnamaldehyde in the control of bacterial biofilm formed on stainless steel surfaces. Eur Food Res Technol 234:821–832

Pan J, Ren D (2009) Quorum sensing inhibitors: a patent review. Expert Opin Ther Pat 19:1581–1601

Park H, Jang C, Cho Y, Choi C (2007) Antibacterial effect of tea-tree oil on methicillin-resistant *Staphylococcus aureus* biofilm formation of the tympanostomy tube: an *in vitro* study. In Vivo 21:1027–1030

Parulekar-Berde C (2015) Bioprospecting of endophytes of medicinal plants. J Pharm Bio Sci 3:210–211

Payne DE, Martin NR, Parzych KR, Rickard AH, Underwood A, Boles BR (2012) Tannic acid inhibits *Staphylococcus aureus* surface colonization in an IsaA-dependent manner. Infect Immunol 81:496–504

Ramage G, Savile SP, Wickes BL, Lopez-Ribot JL (2002) Inhibition of *Candida albicans* biofilm formation by Farnesol, a quorum-sensing molecule. Appl Environ Microbiol 68:5459–5463

Rashid R, Moroshoshi T, Someya N, Ikeda T (2011) Degradation of N-acylhomoserine lactone quorum sensing signaling molecules by potato root surface-associated Chryseobacterium strains. Microbes Environ 26:144–148

Shastry RP, Ravishankar Rai V (2013) Hydrolytic enzymes and quorum sensing inhibitors from endophytic fungi of *Ventilago madraspatana* Gaertn. Biocatal Agric Biotechnol 2:120–124

Ramage G, Saville SP, Thomas DP, Lopez-Ribot JL (2005) *Candida* biofilms: an update. Eukaryot Cell 4:633–638

Syed HA, Khalid A, Sikander K, Sherwani NB, Shana UK (2014) Detection of *Mycobacterium smegmatis* biofilm and its control by natural agents. Intl J Curr Microbiol Appl Sci 3:801–812

Sadekuzzaman M, Yang S, Mizan MFR, Ha SD (2015) Current and recent advanced strategies for combating biofilms. Compr Rev Food Sci Food Saf 14:491–509

Safoura D, Morteza S, Mohsen B (2010) Effect of cumin (Cuminum cyminum) seed essential oil biofilm formation and plasmid integrity of Klebsiella pneumoniae. Pharmacogn Mag 6:57–61

Sandra SL (2014) Essential oil show specific inhibitory effects on bacterial biofilm formation. Food Control 36:224–229

Singh BN, Singh HB, Singh A, Singh BR, Mishra A, Nautiyal CS (2012) Lagerstroemia speciosa fruit extract modulates quorum sensing-controlled virulence factor production and biofilm formation in Pseudomonas aeruginosa. Microbiology 158:529–538

Singh G, Tamboli E, Acharya A, Kumaraswamy C, Mala K, Raman P (2015) Bioactive proteins from Solanaceae as quorum sensing inhibitors against virulence in *Pseudomonas aeruginosa*. Med Hypotheses:84: 539–84: 542

Ta CA, Freundorfer M, Mah TF, Otárola-Rojas M, Garcia M, Sanchez-Vindas P, Poveda L, Maschek JA, Baker BJ, Adonizio AL, Downum K, Durst T, Arnason JT (2014) Inhibition of bacterial quorum sensing and biofilm formation by extracts of neotropical rainforest plants. Planta Med 80:343–350

Taganna JC, Quanica JP, Perono RMG, Amor EC, Rivera WL (2011) Tannin-rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in *Chromobacterium violaceum* and the

QS-controlled biofilm maturation and LasA staphylolytic activity in *Pseudomonas aeruginosa*. J Ethnopharmacol 134:3865–3871

Taweechaisupapong S, Singhara S, Lertsatitthanakorn P, Khunkitti W (2010) Antimicrobial effects of *Boesenbergia pandurata* and *Piper sarmentosum* leaf extracts on planktonic cells and biofilm of oral pathogens. Pak J Pharm Sci 23:224–231

Vasavi HS, Arun AB, Rekha PD (2016) Anti-quorum sensing activity of flavonoid rich fraction from Centella asiatica L. against Pseudomonas aeruginosa PAO1. J Microbiol Immunol Infect 49:8–15

Zhang J, Rui X, Wang L, Guan Y, Sun X, Dong M (2014) Polyphenolic extract from *Rosa rugosa* tea inhibits bacterial quorum sensing and biofilm formation. Food Control 42:125–131

Zhou L, Zheng H, Tang Y, Yu W, Gong Q (2013) Eugenol inhibits quorum sensing at sub-inhibitory concentrations. Biotechnol Lett 35:631–637

Antimicrobial and Anti-quorum Sensing Activities of Medicinal Plants

14

Angela E. Peter, Pola Sudhakar, B. V. Sandeep, and B. Ganga Rao

Abstract

Medicinal plants have been used for several centuries for prevention, treatment, and cure of diseases. The study of many of these traditional medicinal plants has led to the isolation and characterization of bioactive compounds. Some of these compounds have been chemically modified and used as drugs to mitigate ailments. Several commercially available antibiotics are derived from plants. The rise of multidrug-resistant strains of pathogens has led to an intensive search for compounds that can curb this alarming trend. The phenomenon of quorum sensing sheds a whole new light on the process of mitigation of microbial infection, where the plant-derived compounds block essential pathways (like virulence factor expression and biofilm formation) controlled by quorum sensing. Since the discovery that halogenated furanones produced by red marine algae *Delisea pulchra* can interfere with the process of quorum sensing in several Gram-negative bacteria, several medicinal plants (*Mentha piperita*, *Syzygium aromaticum*, *Rosmarinus officinalis*, *Jasminum sambac*, *Lilium brownii*, *Ocimum sanctum*, etc.) have been assessed for anti-quorum sensing potential. Some of them possess only anti-quorum sensing activity, and a few others possess both antimicrobial and anti-quorum sensing activity. Some studies have also identified the compounds responsible for inhibition of quorum sensing pathways. This chapter discusses the study of medicinal plants for their anti-quorum sensing activity.

A. E. Peter · P. Sudhakar (✉) · B. V. Sandeep
Department of Biotechnology, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India
e-mail: sudhakarpola@gmail.com

B. G. Rao
College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_14

Keywords
Medicinal plants · Quorum sensing · Quorum quenching · Anti-QS activity

14.1 Introduction

There are numerous medicinal plants in the world, some of which are found to be widely distributed, while others are endemic. The geographic and demographic conditions of that area determine the kind of phytochemicals and secondary metabolites produced by plants inhabiting the area. These various phytochemicals confer the plant with specific medicinal properties. Sometimes, the entire plant is medicinally important, while at other times, certain parts of the plant are used for the treatment of some medical conditions. Numerous traditionally acclaimed medicinal plants have been scientifically evaluated, and the specific bioactive compounds have been identified. In some cases, when the bioactive compounds are highly potent, they have even been chemically synthesized and marketed as drugs for the treatment and cure of specific medical conditions.

Antibiotics vary in their mode of action. Some inhibit protein or nucleic acid synthesis, while others disrupt membrane structures, and some interfere with the synthesis of peptidoglycan, a necessary constituent of microbial cell walls. One major concern with the use of antibiotics is the development of antibiotic-resistant strains. Antibiotic-resistant strains can develop naturally or by the improper use of antibiotics. These are mutants of the susceptible strains that have acquired the capacity to escape destruction by the antibiotic. As a result, the focus has shifted from developing antimicrobial agents to a more promising strategy in recent times.

14.2 Quorum Sensing

Quorum sensing is a kind of cell-to-cell communication in bacteria. In this process, bacteria communicate with each other by the secretion of some chemical signaling molecules known as autoinducers (AIs). These autoinducers are produced by the bacteria and released, and subsequently the amount of the autoinducer is assessed. The level of the autoinducer increases with the population of the autoinducer-producing bacteria. Once a sufficient number of cells, called a "quorum," are present, the extracellular autoinducer reaches a threshold level. This results in a widespread alteration in the gene expression, in all the autoinducer-producing bacterial cells. This communication may occur within a species, between two species, or between bacteria and their host. This process enables groups of bacteria to coordinate their functions and act as multicellular organisms. Thus, quorum sensing enables bacteria to regulate the gene expression depending on its population

density. Most of the quorum sensing-controlled mechanisms do not account for much when undertaken by an individual bacterium but can have exceptional results when a group of bacteria is involved. Virulence factor expression, biofilm formation, bioluminescence, sporulation, and conjugation are some quorum sensing-controlled mechanisms.

This phenomenon opens up a whole new revolutionary field for the treatment of microbial infections. While antimicrobial activity infers inhibition of growth or killing of the microorganism, anti-quorum sensing activity disturbs the bacterial communication system, thus attenuating microbial pathogenicity. Anti-quorum sensing agents were first characterized in red marine algae (*Delisea pulchra*) (Zahin et al. 2010a). Since this, several studies have been undertaken on terrestrial plants to ascertain if they also possessed such properties. Traditional medicinal plants from different regions of China (Siew-Mian and Tham 2012), India (Singh et al. 2009a), South Florida (Adonizio et al. 2008), and Egypt (Zaki et al. 2012), different spices (cloves, cinnamon), fruits (mango, pomegranate), and essential oils (lavender, sandalwood) have been tested for anti-quorum sensing activity. Some of these studies yielded a positive result, with the particular plant exhibiting anti-quorum sensing activity. This is, however, still a developing field in the nascent stages, which holds much promise for the future.

14.3 Mechanisms of Quorum Sensing

There are primarily three mechanisms of quorum sensing in bacteria, based on the type of autoinducer molecule involved and the mode of gene expression. One mechanism is seen in most Gram-negative bacteria and another in most of the Gram-positive bacteria. A third mechanism is also observed, having few similarities to both Gram-negative and Gram-positive bacteria-mediated quorum sensing, and some features different from both.

14.3.1 Model I: Quorum Sensing in Gram-Negative Bacteria(Waters and Bassler 2005)

The model organism for quorum sensing in Gram-negative bacteria is *Vibrio fischeri*, which lives as a symbiont of a squid. This bacterium resides in the luminous organ of the squid and stimulates the production of luminescence, helping the squid, while it is benefited as the light-producing organ is rich in nutrients.

This method of quorum sensing, which controls the expression of the luciferase gene, which is responsible for luminescence of the squid, was the first quorum sensing mechanism discovered, which was later found to exist in the majority of the Gram-negative bacteria.

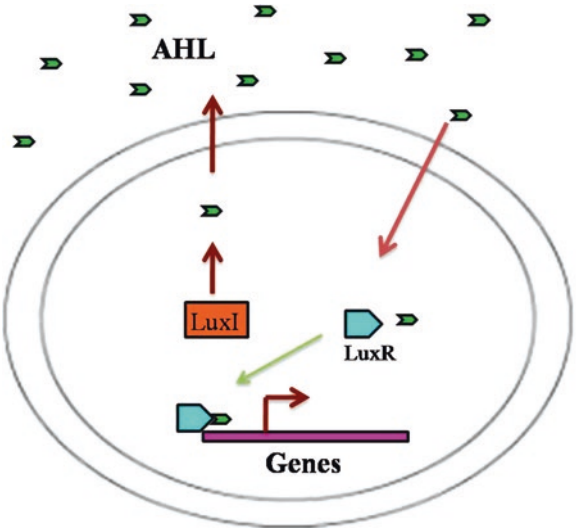


Fig. 14.1 Mechanism of quorum sensing in Gram-negative bacteria

Protein LuxI, which is a synthase, produces AHL (acyl-homoserine lactone autoinducer 3OC6-homoserine lactone). This AHL molecule can freely pass through the membrane; and when the amount of AHL reaches a threshold limit, the AHL binds to LuxR, a receptor and a DNA-binding transcriptional activator. This AHL-bound LuxR complex then activates the lux*I*CDABE operon which produces luciferase, responsible for light emission, and induces the expression of *lux*I, responsible for creating a positive feedback loop amplifying the entire process, causing the entire population to produce light.

Though other Gram-negative bacteria have similar mechanisms of activation of gene expression, they differ in the structure of AHLs produced. It was also found that the LuxR of each bacterium is highly specific for the AHL produced by that particular bacterial strain. Figure 14.1 explains the mechanism of quorum sensing in Gram-negative bacteria.

This hence helps in intraspecies communication.

14.3.2 Model II: Quorum Sensing in Gram-Positive Bacteria (Waters and Bassler 2005; Winzer and Williams 2001)

The autoinducer molecules of Gram-positive bacteria are small inducer peptides (oligopeptides, <10 amino acids). Quorum sensing in Gram-positive bacteria is well studied in *S. aureus.* The general mechanism is described in Fig. 14.2.

*agr*D codes for the AIP (autoinducing peptide) in *S. aureus*. These are impermeable to the bacterial membrane and require the assistance of ArgB protein to be exported from the bacterial cell. Once the level of the modified AIP reaches the

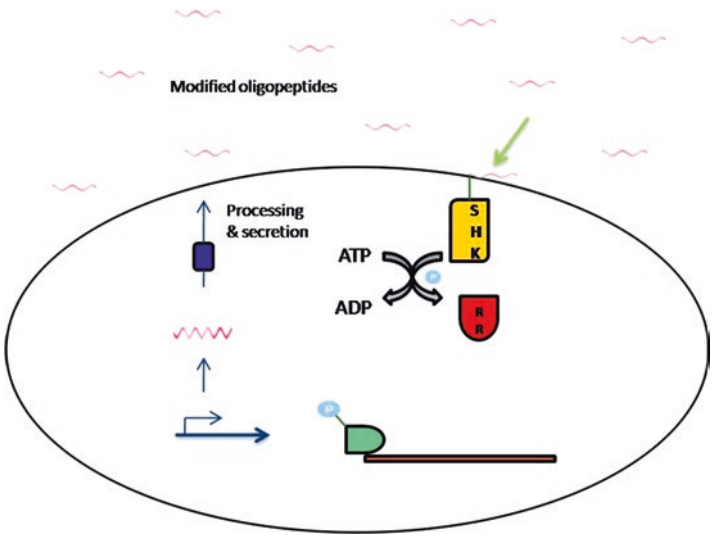


Fig. 14.2 Mechanism of quorum sensing in Gram-positive bacteria. *RR* response regulator, *SHK* sensor histidine kinase

threshold levels, they bind to AgrC receptor, which causes the phosphorylation of AgrA. This phosphorylated AgrA induces the expression of a regulatory RNA, RNA III, responsible for the production of various cell adhesion factors. This phosphorylated AgrA also activates the *agr*BDCA which stimulates the production of more AIP, hence shifting the equilibrium from cell adhesion to production of virulence factors, when there are an increased number of bacterial cells.

The signaling cassette of *S. aureus* is similar to peptide signaling cassettes of other Gram-positive bacteria.

14.3.3 Model III: Quorum Sensing in Other Bacteria (Bassler 2002)

This method was observed in *Vibrio harveyi.*

In this method, two kinds of autoinducer molecules are produced by the bacteria: one is HAI-I, which is similar to the autoinducer produced by other Gram-negative bacteria. The second autoinducer is AI-2, which is entirely different from AI of either Gram-negative or Gram-positive bacteria. It is a furanosyl borate diester. Once the threshold limits of AI are achieved, the expression of genes is induced by a mechanism similar to Gram-positive bacterial quorum sensing, by phosphorylation, causing the transcription of genes coding for quorum sensing-controlled mechanisms (Fig. 14.3).

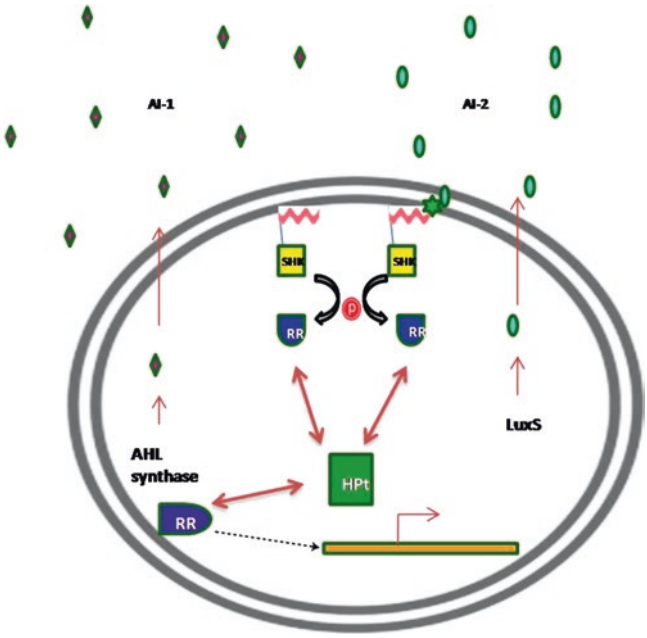


Fig. 14.3 Mechanism of quorum sensing in some bacteria. *RR* response regulator, *SHK* sensor histidine kinase, *HPt* histidine phosphotransfer protein

14.3.4 Model IV: Quorum Sensing in Fungi (Albuquerque and Casadevall 2012)

Quorum sensing was first discovered in the pathogenic fungus, *Candida albicans.* Since then, however, there has been a steady increase in the knowledge of quorum sensing systems in fungi. The autoinducers responsible for mediating the process in fungi are farnesol, tyrosol, phenylethanol, and tryptophol, the latter three being alcohols derived from aromatic amino acids, tyrosine, phenylalanine, and tryptophan. The signaling cascades that are operating in response to the autoinducer molecules that cause the expression of specific genes in fungi are poorly understood, due to their complexity. Farnesol from *C.* albicans, however, regulates filamentation and biofilm formation, reduces oxidative stress, specifically modulates drug efflux, and has detrimental effects on other microbes including bacteria (*S. aureus*) and other fungi (*Aspergillus* sps., *S. cerevisiae*). In other fungi, quorum sensing mediates differentiated expression of genes; however, the autoinducer molecules are not yet purified or identified.

14.4 Importance of Quorum Sensing

The field of quorum sensing holds impetus and relevance, in various fields like medicine, agriculture, industry, and aquaculture, as quorum sensing-mediated mechanisms render the bacteria susceptible to its consequent inhibition. In the case of human pathogens, biofilm formation and virulence expression are critical for the growth, multiplication and ability to cause disease. Since quorum sensing controls these critical steps for rendering them pathogenic, it is envisioned that the inhibition of quorum sensing can help in attenuation of a pathogen and render it noninfectious.

14.5 Quorum Quenching

The process of quenching or extinguishing the cell-to-cell communication mediated by small signaling molecules is known as quorum quenching. Following are some of the methods of quorum quenching.

14.5.1 Quorum Quenching in Gram-Negative Bacteria(Hentrez and Givskov 2003)

I. *Blocking the Signal Generation*

A critical step in the production of acylated homoserine lactones (autoinducer molecules) is the generation of homoserine lactone ring moiety, for which SAM (S-adenosyl methionine) acts as the amino group donor. It was observed that the presence of various analogs for SAM, such as S-adenosyl homocysteine, S-adenosyl cysteine, and sinefungin, led to the ability to block the production of the AHL signaling molecule.

II. *Inhibition of Signal Propagation*

Since the concentration of AHL is critical for the propagation of the signal, both enzymatic (AiiA from *Bacillus* sps. Catalyze the hydrolysis of AHL molecules) and nonenzymatic (alkaline hydrolysis at high pH) methods for degradation were studied. This line of analysis is of substantial clinical interest.

III. *Thwarting Receptor Binding*

Another method that is of interest in the blocking of quorum sensing signaling is the possibility of blocking of the binding of the AHL molecule to its receptor. This can be done either by competitive inhibition, where analogs structurally similar to AHLs are used, or by noncompetitive inhibition, where analogs that bind to other binding sites on the receptor are employed (Fig. 14.4).

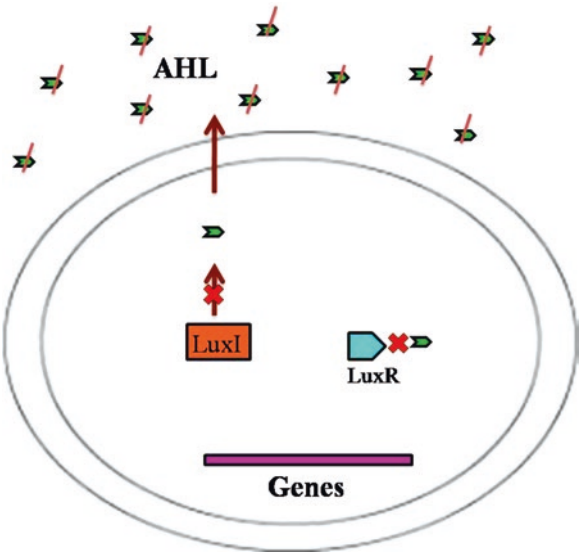


Fig. 14.4 Mechanism of quorum quenching in Gram-negative bacteria

14.5.2 Quorum Quenching in Gram-Positive Bacteria (Kalia 2013)

Different peptide blocking agents have been identified that inhibit quorum sensing-mediated gene expression in Gram-positive bacteria. Siomycin I is a peptide antibiotic that selectively inhibits the growth of Gram-positive bacteria by disruption of biofilm of *Enterococcus faecalis*. RNA III inhibiting peptide (RIP) affected the adhesion of *S. aureus* and *S. epidermis*. Truncated AIP II affected the cognate receptor AgrC-II and also inhibited virulence in four different strains of *S. aureus* (Fig. 14.5).

14.5.3 Quorum Quenching in Fungi (Hogan 2015)

The use of some chemical molecules achieves inhibition of quorum sensing in fungi. The mechanisms of inhibition are not well defined, as the process of quorum sensing in fungi is not so well understood. Nevertheless, few chemical molecules have been identified to quell the quorum signal in certain specific fungi.

14.6 Anti-quorum Sensing Activity

The molecules that act as antagonists for the inhibition of quorum sensing are of great interest, as they represent highly attractive targets for the development of novel therapeutics. It is speculated that this method of controlling microbial infections will circumvent the existing problem of antibiotic resistance. It was contemplated that some phytochemicals produced by plants might inhibit quorum sensing.

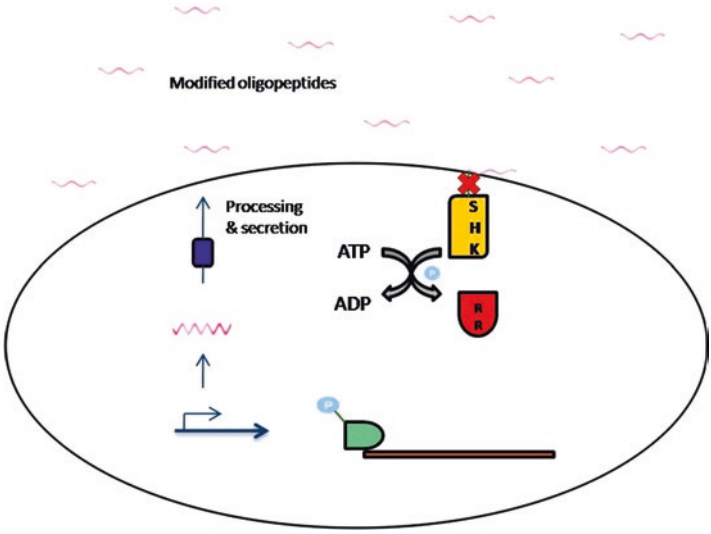


Fig. 14.5 Mechanism of quorum quenching in Gram-positive bacteria

Anti-quorum sensing compounds (halogenated furanones) were first characterized from Australian macro (red) algae *Delissea pulchra* (Zahin et al. 2010b). Spurred on by this discovery, there was a new interest in the discovery of more such quorum sensing inhibitors from other algae and plants in the scientific community. This led to the screening of several seaweeds, marine sponges, edible fruits and vegetables, essential oils (Packiavathy et al. 2012), and in particular plants already known to possess medicinal properties. Another factor that led to this accelerated screening of different terrestrial and marine sources for anti-quorum sensing activity was the development of fast and straightforward assay systems using biosensor strains that enabled the easy and efficient detection of anti-quorum sensing activity. This led to the unearthing of anti-quorum sensing ability of several algae, sponges, and plants.

This chapter focuses on the anti-quorum sensing properties of medicinal plants in particular.

14.7 Methods for Assessment of Anti-quorum Sensing Activity (McLean et al. 2004; Bacha et al. 2016; Khan et al. 2008)

A simple and rapid screening method was developed by McLean and his coworkers, to assess anti-quorum sensing activity of bacterial or plant samples. This method employs either one of the indicator cultures: *Pseudomonas aureofaciens* 30–84 or *Chromobacterium violaceum* ATCC 12472. This technique is a soft agar overlay protocol, based on the inhibition of pigment formation. In the case of plant samples, the test sample (leaf/flower/stem) is directly placed on LB agar plates. Subsequently,

the plates are overlaid with LB soft agar, which contains an inoculum of either one of the reporter bacterial strains. The plates are incubated overnight. Detection is facilitated as the reporter bacterial strains regulate pigment production by quorum sensing and are readily inhibited by AHL analogs and other antagonists. Inhibition of quorum sensing is indicated by a lack of pigmentation of the indicator culture in the vicinity of the tested sample. If there is lack of pigmentation coupled with an inhibition of the growth, the plant sample under study exhibits both anti-quorum sensing and antimicrobial properties.

This method of assay is rapid and accurate and can be quickly followed and mastered. As a result, this is commonly used, with slight modifications, wherever necessary by several researchers to ascertain anti-quorum sensing properties of different plant samples. Among the two reporter bacterial strains mentioned, *Chromobacterium violaceum* ATCC 12472 is more frequently used. This synthesizes a violet-colored pigment called violacein. The quorum signaling molecule involved is N-hexanoyl-L-homoserine lactone (HHL) produced by the autoinducer synthase CviR. The binding of this HHL to its receptor CviR triggers the expression of genes for the production of the violet pigment violacein.

Instead of placing whole plant samples, plant extracts can be pipetted onto sterile paper disks which are put on the agar plates (disk-diffusion method). Alternatively, wells can be made in the agar plates to hold the sample extracts (cup-plate method). The colorless, opaque but viable halo around the disks/well indicates the inhibition of quorum sensing (Figs. 14.6 and 14.7).

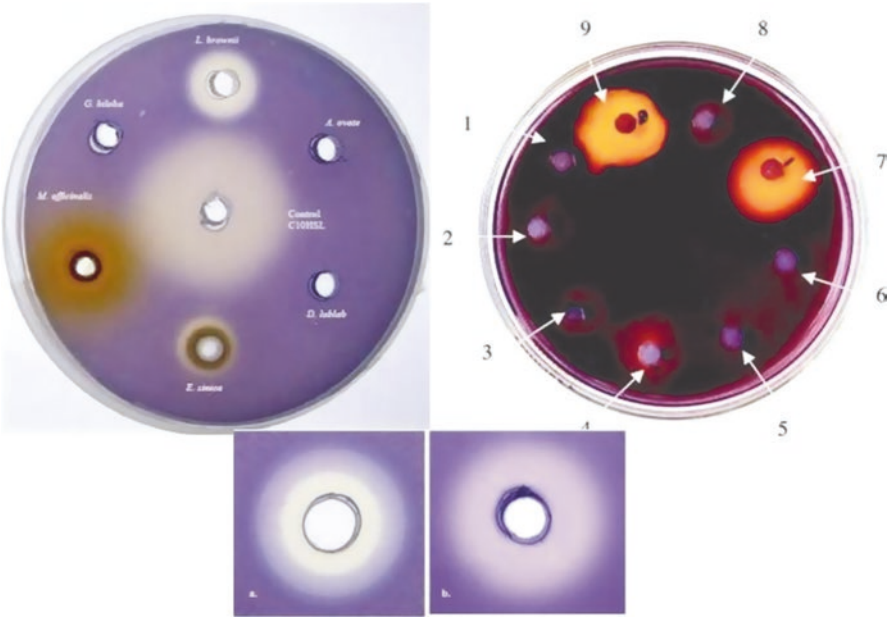


Fig. 14.6 Anti-QS activity indicated by clear zone/halo due to inhibition of violacein formation (Siew-Mian and Tham 2012; Singh et al. 2009c)

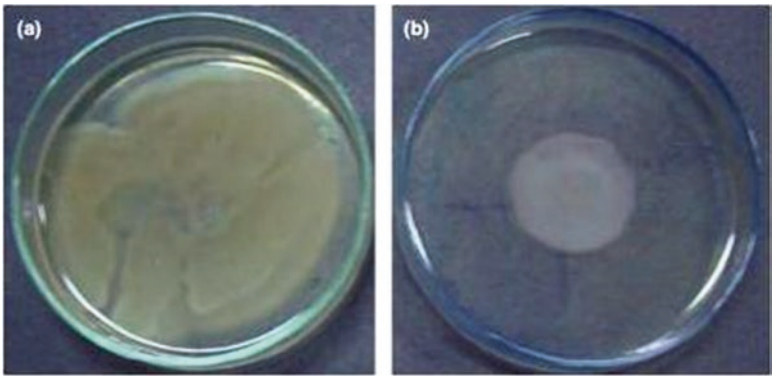


Fig. 14.7 Inhibition of swarming motility in the plate (**b**) when compared to control plate (**a**) using reporter strain PAO1 (Khan et al. 2008)

Another reporter strain of *E. coli*, AI1-QQ.1, is also used to assess anti-QS activity. This reporter consists of a gene encoding a lethal protein fused to a promoter induced in the presence of quorum sensing signaling molecule AHL. Consequently, the strain is unable to grow in the presence of AHL signaling molecules, unless a nontoxic QS-inhibiting compound is present.

Another method to strengthen the assumption of anti-QS activity of a plant uses a bioreporter strain *P.* aeruginosa, PAO1. The phenomenon of swarming in bacteria is considered to be a virulence factor as it is involved in the process of biofilm formation due to mass translocation of cells and this relies on the expression of biosurfactant molecules, the expression of which is under quorum sensing control in PAO1. Hence, any compound inhibiting the swarming motility in PAO1 is expected to interfere with quorum sensing and its regulated traits.

14.8 Anti-quorum Sensing Activities of Medicinal Plants

Several medicinal plants of different regions have been screened for their anti-quorum sensing activity. Some studies are a preliminary screening of medicinal plants; others extend to the detection of the compounds responsible. Some medicinal plants exhibiting anti-quorum sensing activity are discussed below (Fig. 14.8).

Cinnamomum verum is widely distributed in the tropical regions of the world. The bark is an important spice and a flavoring agent. It is known to exhibit antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties. It is also used for neurological disorders, cardiovascular diseases, and the improvement of lipid profile (Rao and Gan 2014).

The anti-QS activity of essential oil of cinnamon against reporter strains CV12472 and CV026 in the presence of natural C_6-AHL was assayed. A slightly

Fig. 14.8 Some plants possessing anti-QS activity

higher anti-QS activity was observed against CV12472 (zone of inhibition 12 mm) when compared to CV026 (zone of inhibition 11 mm) (Waters and Bassler 2005).

Mentha piperita is an important aromatic and medicinal crop. It is initially native to Europe, Canada, and the USA but is now cultivated in different parts of the world. The oil obtained is strongly scented and has medicinal and high commercial value as it is also used as a flavoring agent. The plant is used as an antiemetic, antispasmodic, and nasal decongestant. It is known for its antimicrobial and anti-inflammatory activity. It is also used in different gastrointestinal and hepatic disorders.(Shah and D'Mello 2004)

The commercially available essential oil of *M. piperita* was subjected to anti-QS activity against reporter strains CV12472 and CV026 in the presence of natural C_6-AHL. A higher zone of inhibition (11 mm) was obtained in the case of CV12472, when compared to that of CV026 (10 mm), showing that the essential oil of *M. piperita* possesses anti-QS activity.(Waters and Bassler 2005)

Syzygium aromaticum is native to East Indonesia but is produced in several regions like India, Malaysia, Sri Lanka, Indonesia, Madagascar, and Tanzania. It is an important spice. It is rich in several phenolic compounds and essential oils. It is well known for numerous pharmacological properties, which render it invaluable in a broad spectrum of medical conditions. It is known to possess antimicrobial, antioxidant, antiviral, antinociceptive, and larvicidal activity (Cortes-Rojas et al. 2014).

The ability of clove essential oil to inhibit quorum sensing was assayed using bioreporter strains CV12472 and CV026 in the presence of natural C_6-AHL. The essential oil of clove showed greater quorum sensing inhibition ability for CV12472 (zone of inhibition 19 mm) when compared to CV026 in the presence of natural C_6-AHL (zone of inhibition 17 mm). At higher concentration, clove oil showed inhibition of pigment formation of CV12472, indicating that the inhibition of quorum sensing is a function of the concentration of sample used. Inhibition of swarming motility by clove oil was also studied with reporter strain PAO1. It was observed that swarming motility was inhibited by clove oil, thus strengthening its anti-QS behavior. GC-MS analysis of clove oil was further studied. Major ingredient eugenol was individually tested for anti-QS activity and inhibition of swarming activity. Pure eugenol showed no anti-QS activity or inhibition of swarming motility. This implies that the anti-QS activity and inhibition of swarming motility exhibited by clove oil are due to the other compounds, like α-caryophyllene and β-caryophyllene of clove oil, either in isolation or combination with the various other compounds (Waters and Bassler 2005).

Moringa oleifera, commonly known as drumstick tree, is native to India but found in other tropical and subtropical regions of the world. It is known to possess antidiabetic and anticancer properties. The leaves, fruits, roots, and seeds are used for the treatment of abdominal tumors, scurvy, paralytic attacks, helminthic, bladder, prostate troubles, sores, and skin infections (Singh et al. 2009b).

The anti-quorum sensing potential of aqueous extract of leaf, fruit, and seed was studied using biomonitor strain *C. violaceum* (ATCC 12472) by disk diffusion assay. Strong anti-QS activity was observed for both leaf and fruit extracts. No activity was observed for seed extract (Singh et al. 2009a).

Tecoma capensis is commonly referred to as Cape honeysuckle. It is found in warm and cold regions of the world. It is known to possess antiplasmodial activity, relieve pain, and induce sleep (Saini and Singhal 2012).

Anti-QS activity was determined using *C. violaceum.* The ethanolic extract of both flower and leaf exhibited anti-QS activity with a zone of inhibition 11 ± 1.0 mm and 13 ± 0.5 mm, respectively (Al-Hussaini and Mahasneh 2009).

Lavandula angustifolia is commonly called as lavender; it is known for its carminative, antiflatulence, and anticolic properties. It is a sedative and possesses spasmolytic activity (Lis-Balchin and Hart 1999).

C. violaceum was used to determine the anti-QS potential of flowers (ethanolic extract) of *L. angustifolia.* An opaque zone of 9.5 ± 0.5 mm was observed, indicating that the flowers possess anti-QS activity (Al-Hussaini and Mahasneh 2009).

Rosmarinus officinalis (rosemary) is a popular perennial culinary herb cultivated all over the world. It is known for its antimicrobial, anticancer, antidiabetic, anti-inflammatory, antioxidant, antidiuretic, antiulcerogenic, and antithrombotic activity (Habtemariam 2016).

The ethanolic extracts of both flowers and leaves of *R. officinalis* exhibited anti-QS activity (zone of inhibition 9.0 ± 0.5 mm and 13 ± 0.5 mm, respectively) when assayed using *C.* violaceum (Al-Hussaini and Mahasneh 2009).

Jasminum sambac, commonly called Jasmine, is widely cultivated in several tropical regions like India and Malaysia. Traditionally, the oil extracted is used to treat cancer and heart disease, as an antidepressant, to soothe pain and anxiety, and to make skin smooth (Sabharwal et al. 2013).

The ethanolic extract of leaves and flowers was tested for their anti-QS activity using *C. violaceum*. Both extracts exhibited anti-QS activity with zone of inhibition 10.5 ± 0.9 mm and 9.0 ± 0.5 mm, respectively (Al-Hussaini and Mahasneh 2009).

Populus nigra is known for its antioxidant and anti-inflammatory properties (Dudonné et al. 2011).

The ethanolic extract of the leaves of *P. nigra* exhibited anti-QS activity, with a zone of inhibition of 8.5 ± 0.5 mm, when tested using *C. violaceum* (Al-Hussaini and Mahasneh 2009).

Populus alba, the white poplar tree, is widely distributed in Europe, Asia as well as North Africa. It is known to possess antifungal, antioxidant, antitumor, antiseptic, and antiviral activity (Haouat et al. 2013).

The ethanolic extract of the leaves of *P. alba* exhibited anti-QS activity, with a zone of inhibition of 10 ± 1.0 mm, when tested using *C. violaceum* (Al-Hussaini and Mahasneh 2009).

Sonchus oleraceus, commonly called sow thistle, is native to Asia and Europe. It possesses high antioxidant activity and is commonly used for alleviation of pain (Vilelathor et al. 2009a).

The ethanolic extract of the aerial parts of *S. oleraceus* was assayed for anti-QS activity (using *C. violaceum*). A zone of inhibition of 18 ± 0.5 mm indicated that it possessed an excellent anti-QS activity (Al-Hussaini and Mahasneh 2009).

Laurus nobilis is commonly referred to as the sweet bay tree and is a native to southern Europe. It is known to possess neuroprotective, antioxidant, antiulcerogenic, anticonvulsant, analgesic, anti-inflammatory, antimicrobial, insect-repellant, immunostimulant, and antimutagenic activity (Patrakar et al. 2012).

Ethanolic extract of the leaves, flowers, fruits, and bark was tested for anti-QS activity using *C. violaceum.* The highest activity was exhibited by the flowers (zone of inhibition 24 ± 0.9 mm), followed by the leaves (zone of inhibition 17.5 ± 0.5 mm) and bark (zone of inhibition 19 ± 0.5 mm), and the least activity was exhibited by the fruits (zone of inhibition 15 ± 0.9 mm) (Al-Hussaini and Mahasneh 2009).

Adhatoda vasica Nees is found in many parts of India and several regions of the world. It is a traditional medicinal plant of India, used in Ayurveda for treating various ailments. Pharmacognostic activities include antiulcerogenic, anti-allergy, antitubercular, abortifacient, antimicrobial, insecticidal, uterotonic, anti-asthmatic, bronchodilatory, and wound-healing activity (Gangwar and Ghosh 2014).

The anti-QS activity of hydroalcoholic leaf extract of *A. vasica* was checked using reporter strain *C. violaceum* CV12472. Anti-QS zone of 12 ± 0.3 mm was observed (Zaki et al. 2012).

Bauhinia purpurea L. is not only native to Asia but also found in some areas of America. It possesses antipyretic, anti-inflammatory, antinociceptive, antimicrobial, analgesic, antidiabetic, antioxidant, and antidiarrheal activity. It is also known for its wound-healing, nephroprotective, and hormone regulation capacity (Kumar and Chandrashekar 2011).

The hydroalcoholic extract of *B. purpurea* leaves was evaluated for inhibition of quorum sensing. A zone of 10 ± 0.1 mm indicated that the leaf extract possessed the ability to inhibit quorum sensing (Zaki et al. 2012).

Lantana camara L. is commonly known as Lantana or Wild Sage. It is an ornamental plant that is initially from America; now found in several parts of the world, including Africa and New Zealand. The plant possesses antimicrobial, antiulcerogenic, hemolytic, hypoglycemic, anti-inflammatory, antifilarial, anticancer, antifertility, and wound-healing properties (Kalita et al. 2012).

The leaf extract (hydroalcoholic) of *L. camara* was subjected to anti-QS assay using bioreporter strain CV12472. Anti-QS zone of 9 ± 0.6 mm was obtained, suggesting that the leaf of *L.* camara possesses mild quorum sensing-inhibitory properties (Zaki et al. 2012).

Myoporum laetum G. Forst. is found in South America, California, New Zealand. It is a shrub or small tree found in open grasslands or coastal regions. It is used traditionally as a medicinal plant in different parts of the world, like Egypt (Preston 2012).

The hydroalcoholic leaf extract of *M. laetum* when tested for anti-QS gave an inhibitory zone of 15 ± 0.4 mm, indicating that it may possess good ability to inhibit the expression of genes controlled by quorum sensing (Zaki et al. 2012).

Piper longum L. (long pepper) is native to Indo-Malaysian region. Though it can be commonly found growing wild in the tropical rainforests of India, Nepal, Indonesia, Malaysia, Sri Lanka, and the Philippines, it is also widely cultivated as it is a commercial crop of great value. It is used as a spice and as a traditional medicine. Pharmacognostic activities include stimulant effects, immunomodulatory, hepatoprotective, anti-inflammatory, antiamoebic, hypocholesterolemic, and antimicrobial activity (Khushbu et al. 2011).

The fruit extract of *P. longum* was subjected to quorum sensing-inhibitory assay, using CV12472 bioreporter strain. A zone of inhibition of 6 ± 0.1 mm indicated that the fruits possess mild ability to inhibit quorum sensing (Zaki et al. 2012).

Taraxacum officinale F. H. Wigg, commonly called dandelion, is distributed in several regions across the globe. It has been widely used in traditional medicinal systems. The root is considered as a gastrointestinal remedy, aiding in the liver and digestive function, while the leaf is used as a diuretic. Pharmacological activities include anti-inflammation and hypoglycemic activity (Yarnell and Abascal 2009).

A study of the anti-QS ability of the hydroalcoholic extract of the aerial parts of *T. officinale* exhibited its ability to quench the quorum sensing process for the production of violacein in reporter bacterial strain CV12472 (zone of inhibition observed 7 ± 0.4 mm) (Zaki et al. 2012).

Hemidesmus indicus (L.) Schult is a climbing plant that grows in different parts of India and is used by native healers for nephric complaints, syphilis, and sore mouth. It has antibacterial, anticancer, antidiabetic, antidiarrheal, anti-inflammatory, antioxidant, antiulcerogenic, antivenom, renoprotective, cardioprotective, and hepatoprotective effects (Weissner 2014).

The hydroalcoholic extract of the root of *H. indicus* was assayed for anti-QS activity at three different concentrations, 400 µg, 800 µg, and 1200 µg, using two bioreporter strains, CV 12472 and CV026. The highest activity was observed against CV 12472 at 800 µg, followed by the other two concentrations (zone of inhibition 800 µg, 16.0 ± 0.4 mm; 400 µg, 14.0 ± 0.4 mm; 1200 µg, 11.0 ± 0.3 mm). A similar trend was observed for using CV026 strain (zone of inhibition 800 µg, 14.0 ± 0.4 mm; 400 µg, 12.0 ± 0.4 mm; 1200 µg, 6.0 ± 0.3 mm). The extract also exhibited the ability to reduce swarming motility of PAO1 reporter strain (Zahin et al. 2010b).

Holarrhena antidysenterica is found in tropical and subtropical regions of Asia and Africa. It is used in Indian Ayurvedic medicine to treat diarrhea and dysentery. It is known for its antidiabetic, antidiarrheal, diuretic, antihelminthic, antimalarial, antimicrobial, antimutagenic, and antihypertensive activity (Sinha et al. 2013).

The bark of *H. antidystenterica* was assessed for anti-QS activity at three different concentrations, with two reporter strains, CV 12472 and CV026. More significant inhibition was observed with CV 12472 than CV026. In the case of CV 12472, a very high zone of inhibition was observed at the highest concentration, which consequently reduced with a decrease in concentration (zone of inhibition 1200 μg, 21.0 ± 0.5 mm; 800 μg, 15.0 ± 0.4 mm; 400 μg, 12.0 ± 0.3 mm). Relatively lesser anti-QS activity was exhibited against CV026 strain (zone of inhibition 800 μg, 11.0 ± 0.4 mm; 400 μg, 10.0 ± 0.4 mm; 1200 μg, 5.0 ± 0.2 mm). The extract also reduced the swarming motility of PAO1 reporter strain (Zahin et al. 2010b).

Mangifera indica, mango, is grown in many parts of the world. The plant is known for its antidiabetic, antitetanus, analgesic, antipyretic, antimicrobial, antiulcerogenic, antimalarial, cardioprotective, and bronchodilatory properties (Parvez 2016).

The ability to inhibit quorum sensing was assessed using hydroalcoholic leaf extract. The extract more efficiently inhibited quorum sensing against CV 12472 than CV 026 reporter strain (zone of inhibition CV 12472 1200 μg, 14.0 ± 0.4 mm; 800 μg, 12.0 ± 0.3 mm; 400 μg, 8.0 ± 0.2 mm; CV026 1200 μg, 11.0 ± 0.4 mm; 800 μg, 9.0 ± 0.4 mm; 400 μg, NIL). The inhibition of swarming motility was also good (65.9% reduction at a concentration of 800 μg/ml, determined using PAO1) (Zahin et al. 2010b).

Psoralea corylifolia is an important Indian and Chinese medicinal plant, widely distributed in the Asian subcontinent. It is known for its immunomodulatory, anti-inflammatory, antitumor, and antibacterial activity. The seed extract is used for a variety of diseases like leukoderma and impotence (Mounika 2016).

The anti-QS activity of hydroalcoholic seed extract was studied against reporter strains CV 12472 and CV026. The extract was more potent against CV 12472 than CV026. In the case of both extracts, the inhibitory capacity increased with increase in the concentration of extract (zone of inhibition CV 12472 1200 μg, 17.0 ± 0.5 mm; 800 μg, 11.0 ± 0.4 mm; 400 μg, 9.0 ± 0.2 mm; CV026 1200 μg, 14.0 ± 0.6 mm; 800 μg, 11.0 ± 0.5 mm; 400 μg, 8.0 ± 0.3 mm). The extract also exhibited an excellent ability (69.5% reduction, at a concentration of 1000 μg/ml) to reduce the swarming motility of PAO1 bioreporter strain. This signifies that the extract is potent and can control quorum sensing-mediated expression of genes (Zahin et al. 2010b).

Punica granatum is found in India and more arid regions of Southeast Asia, the East Indies, and tropical parts of Africa. It is known to possess high antioxidant activity and anticarcinogenic and anti-inflammatory activity. It is also used for dental conditions, in diabetes, and in male infertility (Jurenka 2008).

The hydroalcoholic extract of pomegranate rind was assayed for anti-QS properties with bioreporter strains CV 12472 and CV026. The extract was more potent on CV026 than CV 12472. The highest activity was observed at the highest concentration employed, 1200 µg, and no inhibition was observed at the minimum concentration in the case of both the reporter strains (zone of inhibition CV026 1200 µg, 12.0 ± 0.4 mm; 800 µg, 10.0 ± 0.4 mm; 400 µg, NIL; CV 12472 1200 µg, 7.0 ± 0.1 mm; 800 µg, 9.0 ± 0.2 mm; 400 µg, NIL). The extract was also tested for its capacity to inhibit swarming motility of PAO1 strain. It was found that the extract was potent and there was a 65.9% reduction in the swarming motility at an extract concentration of 500 µg/ml. This proves that the extract of pomegranate rind has a good anti-QS activity (Zahin et al. 2010b).

Aloe barbadensis is a well-known important medicinal plant. It improves the digestive system, protects the immune system, and helps fight stress. It is known for its wound-healing, anticancer, anti-arthritic, antidiabetic, and antimicrobial properties (Nandal and Bhardwaj 2012).

The anti-QS activity of hydro-acetone of aloe leaf extract was assessed using CVO26 bioreporter strain. There was no inhibition of violacein pigment production. Inhibition of swarming activity of reporter strain PAO1 was observed, the percent of reduction being 79.9%. This indicated that aloe could have anti-QS activity, as it showed inhibition of swarming activity. It is recommended that another assay be performed using other reporter strains like CV 12472 to establish the anti-QS activity (Siew-Mian and Tham 2012).

Angelica sinensis is a herb found in China, Japan, and Korea. The dried root is used traditionally to strengthen heart, liver as well as lubricate the bowel. It is considered as a blood tonic and is used for regulating the menstrual cycle. It is known for its anticoagulant, antispasmodic, and antifibrotic activity. It is also used for dysmenorrheal, cardiovascular disease, immune support, and hematopoiesis (Head 2004).

The root extract was studied for anti-QS activity. There was an inhibition of pigmentation; zone of inhibition of 13.5 ± 0.3 mm was observed for 50 µl of extract used. There was no inhibition of swarming when checked with PAO1 reporter strain; the extract promoted swarming when compared to the control (Siew-Mian and Tham 2012).

Astragalus membranaceus is a herbal immunomodulator and an antidiabetic drug. The roots have been used in many herbal formulations in China for the treatment of diabetes (Agyemang et al. 2013).

The root extract exhibited a very high inhibition of violacein production (zone of inhibition 34.0 ± 0.0 mm for 50 µl of extract used) when assayed for anti-QS

activity with CV026 strain.). No inhibition of swarming motility was detected when assayed with PAO1 reporter strain (Siew-Mian and Tham 2012).

Cnidium monnieri is a plant commonly used in the traditional Chinese system of medicine. The plant is a source of osthole (7-methoxy-8[3-methyl-2-butenyl]-2H-1-benzopyran-2-one), which is known for its anticancer, anti-inflammatory, antioxidant, immunomodulatory, antimicrobial, and antiparasitic properties. It is also well known for its neuroprotective, hepatoprotective, and cardiovascular benefits (Zhang et al. 2015).

50 µl of a hydro-acetone extract of the seed was subjected to anti-QS assay using reporter strain CV026. No inhibition of pigmentation was observed. The extract was however found to inhibit swarming of reporter strain PAO1 to a great extent (percent of reduction- 78.8%). This indicated that *C. monnieri* could have anti-QS activity, as it showed inhibition of swarming activity. It is recommended that another assay be performed using other reporter strains like CV 12472 to establish the anti-QS activity (Siew-Mian and Tham 2012).

Crataegus cuneata is widely distributed throughout the Northern temperate regions of the world. It is known for its activity on the reproductive system (Kumar et al. 2012).

A study of the anti-QS activity of the fruit extract using CV026 revealed that the extract was able to inhibit pigmentation (zone of inhibition 14.2 ± 0.4 mm). No inhibition of swarming motility was detected when assayed with PAO1 reporter strain (Siew-Mian and Tham 2012).

Dioscorea nipponica is used for rheumatoid arthritis, asthma, and bronchitis. It is also known for its anticancer activity (Ho et al. 2011).

The tuber extract of *D. nipponica* was assayed for anti-QS activity using CV026. A considerable zone of inhibition of pigment formation of 13.8 ± 0.2 mm indicates that it could possess anti-QS activity. There was no inhibition of swarming when checked with PAO1 reporter strain; the extract promoted swarming when compared to the control (Siew-Mian and Tham 2012)

Ephedra sinica is found in Asia, Europe, and some areas of America. It is used in Chinese and Indian traditional medicinal systems. It is known to have anti-inflammatory activity. It is a source of the neurotransmitter epinephrine (Abourashed et al. 2003).

A 50 µl hydro-acetone branch extract of *E. sinica* was subjected to anti-QS assay with reporter strain CV026. There was inhibition of violacein production (zone of inhibition 12.0 ± 0.0 mm). The extract promoted swarming, as there was an increase

in the colony of PAO1 (compared to control) which was used as a reporter strain (Siew-Mian and Tham 2012).

Lilium brownii is a traditional medicinal plant native to China and India, but has been cultivated in different countries of Europe also. It is used in the treatment of backaches, dizziness, impotence, urinary disorders, and fever. It is known for its antipyretic and antidiabetic properties (Okubo et al. 2012).

The bulb extract of *L. brownii* was checked for anti-QS activity. A zone of inhibition of 17.3 ± 0.3 mm indicated that there was an inhibition of pigment formation. The extract was also able to inhibit swarming greatly; a reduction percent of 64.8% was observed on reporter strain PAO1. This suggests that the plant has potent anti-QS activity (Siew-Mian and Tham 2012).

Magnoila officinalis is a common Chinese medicinal plant. It possesses antioxidant, anti-inflammatory, antitumor, and antimicrobial activity (Shen et al. 2010). The bark extract of *M. officinalis* was screened for anti-QS activity.

There was a high inhibition of violacein production; a creamy-white halo (diameter 23.7 ± 0.3 mm) was observed. The extract promoted swarming, as there was an increase in the colony of PAO1 (compared to control) which was used as a reporter strain (Siew-Mian and Tham 2012).

Panax pseudoginseng is commonly called false Ginseng or Indian Ginseng. It is found in India, China, Tibet, Nepal, Bhutan, and Myanmar. It is a conventional folk medicine. It is known for its anticancer, anti-asthma, and anticonvulsive effects. Traditionally, it is used for the treatment of headaches, hemorrhagic disease, dyspepsia, and palpitations (Nayar and Sastry 1990; Selvam 2012).

Screening of the root extract for anti-QS activity using CV026 revealed the ability to inhibit pigmentation (zone of inhibition 12.7 ± 0.3 mm). The extract was also able to inhibit swarming greatly; a reduction percent of 60.2% was observed on reporter strain PAO1. This suggests that the plant has potent anti-QS activity (Siew-Mian and Tham 2012).

Albiza schimperiana is traditionally used as a medicine for the treatment of bacterial infections like pneumonia and other parasitic infections such as malaria (Kokila et al. 2013).

The methanol extract of the root of *A. schimperiana* displayed quorum sensing activity, when screened using *E. coli* reporter strain AI1-QQ.1, suggesting the presence of AHL interfering molecules in the extract (Bacha et al. 2016).

Justicia schimperiana is known to be used in the traditional system of medicine in Ethiopia. The leaves are popularly used for the treatment of liver disease, diarrhea, dysentery, and other stomach disorders (Correa and Alcantara 2011).

Petroleum ether extract of the seed of *J. schimperiana* revealed the ability to quench quorum signaling, hinting that the extract possibly possesses AHL interfering molecules (Bacha et al. 2016).

Prunus armeniaca is commonly known as apricot. It is a deciduous tree that is native to the continental regions of the globe but is also widely cultivated in other areas. The antimicrobial, anticancer, antioxidant, and hepatoprotective activity of *P. armeniaca* have also been reported. It is consumed as a fruit and is used in the traditional system of medicine for asthma, constipation, and cough and to soothe irritated skin (Raj et al. 2012).

No zone of inhibition was observed when the plant extract (seed kernel) was tested with reporter strain CV026 for quorum sensing activity. However, inhibition of swarming was identified with PAO1 reporter strain, as exhibited by a 29% reduction in colony area (Koh and Tham 2011).

Prunella vulgaris belongs to the mint family and is very popularly used in European, Chinese, and Indian traditional medicinal system. It is used to treat fever and throat infections and for wound healing. Its antimicrobial, anti-inflammation, antidiabetic, and antistress activity has also been scientifically evaluated (Rasool and Ganai 2013).

Anti-quorum sensing activity was assessed using reporter strain CV026 and a zone of inhibition of 15.5 mm was observed indicating the ability of *P. vulgaris* to inhibit quorum sensing. No inhibition in swarming motility was identified; on the contrary, the whole plant extract was observed to promote swarming motility when tested with PAO1 (Koh and Tham 2011).

Nelumbo nucifera, commonly known as the lotus, is an aquatic plant found in Asia, known for its medicinal properties. Traditionally, it is used in the treatment of tissue inflammation, cancer, diarrhea, skin diseases, nervous disorders, and leprosy. Several bioactive compounds like β-sitosterol, quercetin, ginnol, and nuciferine have been reported and characterized from lotus plant (Paudel and Panth 2015).

Leaf extract of lotus possesses the ability to inhibit quorum sensing, as a zone of inhibition of 16 mm was observed when CV026 reporter strain was used. Promotion of swarming motility was observed compared to the control when PAO1 reporter strain was used (Koh and Tham 2011).

Panax notoginseng is a Chinese medicinal herb widely cultivated in different parts of China. It is used to promote blood circulation, for the treatment of fractures, injuries, vertigo and reduces swelling and pain. Its anti-inflammatory, antioxidant, antitumor, antimicrobial, antidiabetic, renal protective, and hepatoprotective activity has been scientifically evaluated (Ng 2006).

The hydro-acetone flower and root extract of *P. notoginseng* exhibited anti-QS activity against both CV026 and PAO1 reporter strains. A zone of inhibition of pigmentation of 20 mm was observed for CV026 strain, and 32% inhibition of swarming motility was observed in the case of flower extract. The root extract exhibited higher activity, a zone of inhibition of 24 mm was observed for CV026, and 50% inhibition of swarming motility was observed with PAO1 (Koh and Tham 2011).

Areca catechu is a variety of palm plant grown extensively in Asian countries as a seed crop. The alkaloids present in the seed are intoxicating and addictive when chewed. It is an antidepressant, a sedative, and a narcotic-analgesic. It is proved to possess antioxidant, antivenom, anticancer, antihelminthic, and molluscicidal activity. Several alkaloids, like guvacine, guvacoline, isoguvacine, arecaidine, arecolidine, norarecaidine, and norarecoline, have been reported to form seeds of *A. catechu (*Jaiswal et al. 2011*)*.

The seed extract of *A. catechu* showed the ability to inhibit QS when tested with two reporter strains: CV026 and PAO1. A clear zone of 18 mm (absence of pigmentation) was observed in the case of CV026. Efficient inhibition of swarming motility was observed, with a high percentage of 79% against reporter strain PAO1 (Koh and Tham 2011).

Imperata cylindrica is commonly known as Cogon grass and is a perennial monocot plant. It is traditionally used in the treatment of diabetes, gout, common cold and cough, anemia, and urinary calculi. It possesses antihelminthic, antibacterial, anticancer, antidiuretic, antidiarrheal, and anti-arthritic activity (Parvathy et al. 2012).

The hydro-acetone extract of the underground stem exhibited the ability to inhibit QS as illustrated by the study on reporter strain CV026, where pigmentation inhibition was depicted by a zone of inhibition of 20 mm. When the reporter strain PAO1 was used for analysis, no inhibition of swarming motility was observed; in fact, the plant extract was found to promote swarming (Koh and Tham 2011).

Myristica cinnamomea is a folk medicine in many regions of Asia like Malaysia, Singapore, and Sumatra. It is known for its antimicrobial, nematocidal, and anti-ulcerogenic activity.

QS inhibition was observed with reporter strain CV026. In particular, malabaricone C from the methanolic bark extract was found to inhibit violacein production

by CV026. Similarly, anti-QS activity was observed with reporter strain PAO1; malabaricone C was found to decrease the production of pyocyanin by PAO1. However, no adverse effects on the viability of PAO1 were observed (Chong et al. 2011).

Acacia nilotica L. is widely distributed in the tropical and subtropical regions. It is used traditionally for the treatment of cancer, diarrhea, tuberculosis, leprosy, bleeding piles, cold, cough, and fever. Antioxidant activity, inhibition of lipid peroxidation, and prevention of DNA damage of *A. nilotica* have also been scientifically evaluated.

The hydrolyzed crude extract and the hydrolyzed ethyl acetate fraction of the pods of *A. nilotica* exhibited a dose-dependent inhibition of violacein production by the biomonitor strain CV 12472. Among the two extracts, the hydrolyzed ethyl acetate fraction showed higher QS inhibition. The inhibitory effect of hydrolyzed ethyl acetate fraction ranged from 15.24 ± 0.82 to 100 ± 4.04%, while the inhibitory effect of hydrolyzed crude extract ranged from 11.11 ± 0.73 to 91.26 ± 4.62% (Singh et al. 2009c).

Allium sativum, commonly called garlic, is a small underground bulb which is native to Central Asia but is widely cultivated in several parts of the world. It is famous for its medicinal properties and as a spice used in culinary preparations. It has been used traditionally for the management of blood pressure, high cholesterol, heart attack, and coronary disease. It was found to be useful for the treatment of different cancers, diabetes, gout, microbial infections, diarrhea, and arthritis (Neeraj et al. 2014).

It possesses anti-QS activity as observed with reporter strains *C. violaceum*, *P.* aeruginosa, and *A. tumefaciens* strain NLT4 (Kalia 2013).

Ocimum sanctum, commonly referred to as holy basil or tulsi, is a native to India but cultivated in other regions. It is a folklore treatment for various conditions like cancer, diabetes, pain cough, liver conditions, and hypotension. Its antimicrobial, wound-healing, antidiabetic, antioxidant, anticancer, immunogenic, antihelminthic, antiulcerogenic, cardioprotective, and larvicidal activity has been established (Rahman et al. 2011).

Quorum quenching ability of *O. sanctum* was assessed using *C. violaceum* and PAO1 reporter strains. Inhibition of violacein production and pyocyanin pigment formation and biofilm production were observed, respectively (Kalia 2013).

Pisum sativum, commonly known as a garden pea, is used extensively as a pulse for consumption. Pharmacognostic evaluation has revealed that it possesses antidiabetic, anticancer, antioxidant, anti-inflammatory, and antimicrobial activity. Pea plant is rich in apigenin, hydroxybenzoic, hydroxycinnamic, luteolin, and

quercetin, all of which have been reported to contribute to its therapeutic properties including anticarcinogenic property (Rungruangmaitree and Jiraungkoorskul 2017).

The seed, root, and leaf extracts of *P. sativum* inhibited violacein production in the reporter strain CV0blu, indicating that it possessed anti-QS activity. In addition, the seed extract exhibited the ability to inhibit the swarming motility of *S. liquefaciens* MG1 reporter strain and C4HSL-inducible protease and N-acetylglucosaminidase in CV026 reporter strain (Kalia 2013).

Medicago sativa is commonly called alfalfa and is used as an Ayurvedic and homeopathic medicine to treat central nervous system disorders. It is reported to have antioxidant, anti-inflammatory, antidiabetic, and neuroprotective activity (Bora and Sharma 2011).

The anti-QS activity of *M. sativa* exhibited the ability of the seed extract to inhibit the production of violacein pigment by the biomonitor strain *C. violaceum (*Kalia 2013*)*.

Alyssum maritimum is commonly referred to sweet alyssum. The flower and aerial parts of the plant are used as an infusion for kidney stones. It is also known to possess hepatoprotective and antiulcerogenic activity (Parada et al. 2009).

Slight inhibition of pigment formation was observed with reporter strain *C.* violaceum CV0blu indicates the moderate ability to inhibit QS (Kalia 2013).

Ananas comosus, commonly referred to as pineapple, is one of the most important commercial fruit crops in the world. It is known to possess anti-inflammatory, antioxidant, antimicrobial, analgesic, and hypoglycemic effects (Hossain et al. 2015).

The quorum quenching ability of *A. comosus* was studied using *C. violaceum* and PAO1 biomonitor strains. With both biomonitor strains, anti-QS activity was observed. Inhibition of the production of violacein by the *C. violaceum* and inhibition in pyocyanin pigment, staphylolytic protease, elastase production, and biofilm production by PAO1 indicate the ability of the plant to act as a QS inhibitor (Kalia 2013).

Manilkara zapota, commonly known as sapota or chickoo, is a tropical widely cultivated fruit. It is known to possess antimicrobial, antitumor, anti-inflammation, antipyretic, analgesic, hepatoprotective, hypoglycemic, antidiarrheal, and hypocholesterolemic effects. Several of its phytoconstituents have been isolated and characterized like erythrodiol, lupeol acetate, D-quercitol, myricitrin, quercitrin, and manilkoraside (P. Milind and Preeti 2015).

Inhibition of pigment production by reporter strain *C. violaceum* demonstrates the anti-QS ability of the plant. With PAO1 reporter strain, the plant extract was able to obstruct pyocyanin pigment, staphylolytic protease, elastase production, and biofilm production, suggesting its ability to act as a suitable quorum quenching agent (Kalia 2013).

In addition to the plants mentioned above, there are many more medicinal plants screened for inhibition of quorum sensing. A few more plants screened for anti-QS activity are listed in the table below (Table 14.1).

Table 14.1 Plant-based quorum sensing inhibition (Kalia 2013; Koh et al. 2013)

S. no.	Plant name	Organism employed	Anti-QS activity observed
1.	*Vanilla planifolia* extract	*C. violaceum* CV026	Inhibition of violacein production
2.	Rasberry extracts	*C. violaceum*	Inhibition of violacein production
3.	Blueberry extracts	*C. violaceum*	Inhibition of violacein production
4.	Grape extracts	*C. violaceum*	Inhibition of violacein production
	Grape fruit juice	*E. coli*	Inhibition of biofilm formation
		P. aeruginosa	Inhibition of biofilm formation
		S. Typhimurium	Inhibition of biofilm formation
5.	Squash exudate: γ-hydroxybutyrate (GHB)	*A.* tume*faciens*	Inhibition of AHL signaling
6.	Tomato seedlings exudate: γ-hydroxybutyrate (GHB)	*A. tumefaciens*	Inhibition of AHL signaling
7.	*Musa paradisiaca*	*C. violaceum*	Inhibition of pigment formation
		P. aeruginosa PAO1	
8.	Tea tree	*C. violaceum* CV026	Inhibition of pigment formation
9.	*Cinnamomum zeylanicum*	*P. aeruginosa*	Inhibition of biofilm formation
	Cinnamon oil component-cinnamaldehyde	*E. coli*	Inhibition of biofilm formation
		V. harveyi	Inhibits AHL- and AI-2 mediated QS
	Cinnamaldehyde and its derivative: 4-NO2-cinnamaldehyde	*Vibrio* sps.	Inhibition of AI-2 mediated QS-bioluminescence, protease activity, pigment formation
10.	Thyme	*C. violaceum*	Inhibition of violacein production
11.	Turmeric	*C. violaceum*	Inhibition of violacein production
12.	Ginger	*C. violaceum*	Inhibition of pigment formation
13.	*Ocimum basilicum* (sweet basil): Rosmarinic acid	*P. aeruginosa*	Inhibition of protease and elastase production, biofilm formation, and virulence factors
14.	*Passiflora incarnata* leaf extract	*C. violaceum* CV0blu	Inhibition of violacein production
15.	*Ruta graveolens* leaf extract	*C. violaceum* CV0blu	Inhibition of violacein production

14.9 Conclusion

The discovery of penicillin in the early nineteenth century by Fleming fueled the drive to discover more such agents that could eradicate disease-causing microorganisms. Many plants yielded bioactive compounds which were characterized, synthetically produced on a large scale, and used as antibiotics. However, microorganisms are opportunistic and devised mechanisms to evade destruction by antibiotics. This, coupled with the indiscriminate use of these antibiotics, led to the development of multidrug-resistant pathogens. A quest to unearth new molecules that could combat these multidrug-resistant strains began.

As quorum sensing mediates gene expression of several important events that are critical to causing infections, it was envisioned that inhibition could be exploited to contain the growth and expression of virulence of pathogens. Different plants were screened for their ability to inhibit quorum sensing. This chapter deals with some of the plants that exhibited anti-quorum sensing activity on testing. It was observed that many medicinal plants have the potential to act as inhibitors for quorum sensing. Further study is required though, to identify the phytoconstituents that are responsible for inhibition.

A novel approach would be to use plants that have both antimicrobial and anti-quorum sensing activity. Many plants exhibit antimicrobial activity, but only few exhibit both antimicrobial and anti-quorum sensing activity. Identification of such plants and a detailed study of the individual compounds responsible for the activity and the mechanism of action will lead to the development of new lead molecules that can be used to give sustainability to combat microbial infections.

References

Abourashed EA, El-Alfy AT, Khan IA, Walker L (2003) *Ephedra* in perspective- a current review. Phytother Res 17:703–712

Adonizio A, Kong K, Mathee K (2008) Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. Antimicrob Agents Chemother 52(1):198–203

Agyemang K, Han L, Liu E, Zhang Y, Wang T, Gao X (2013) Recent advances in *Astragalus membranaceus* anti-diabetic research: pharmacological effects of its phytochemical constituents. Evid Based Complement Alternat Med 2013:654643

Albuquerque P, Casadevall A (2012) Quorum sensing in fungi- a review. Med Mycol 50(4):337–345

Al-Hussaini R, Mahasneh AM (2009) Microbial growth and quorum sensing antagonist activities of herbal plant extracts. Molecules 14:3425–3435

Bacha K, Tariku Y, Gebreyesus F, Zerihun S, Mohammed A, Weiland-Brauer N, Schmitz RA, Mulat M (2016) Antimicrobial and anti-quorum sensing activities of selected medicinal plants of Ethiopia: implication for development of potent antimicrobial agents. BMC Microbiol 16(139)

Bassler BL (2002) Small talk: cell-to-cell communication in bacteria. Cell 109:421–424

Bora KB, Sharma A (2011) Evaluation of antioxidant and Cerebroprotective effect of *Medicago sativa* Linn. against ischemia and reperfusion insult. Evid Based Complement Alternat Med 2011, 9 pages. Article ID 792167. https://doi.org/10.1093/ecam/neq019. Hindawi Publishing Corporation

Chong YM, Yin WF, Ho CY, Mustafa MR, Hamid A, Hadi A, Awang K, Narrima P, Koh C, Appleton DR, Chan K (2011) Malabaricone C from *Myristica cinnamomea* exhibits anti-quorum sensing activity. J Nat Prod 74:2261–2264

Correa GM, Alcantara AF (2011) Chemical constituents and biological activities of species of *Justicia*- a review. Braz J Pharmacog 22(1):220–238

Cortes-Rojas DF, Fernandes de Souza CR, Oliveria WP (2014) Clove (*Syzygium aromaticum*): a precious spice. Asian Pac J Trop Biomed 4(2):90–96

Dudonné S, Poupard P, Coutière P, Woillez M, Richard T, Mérillon J, Vitrac X (2011) Phenolic composition and antioxidant properties of poplar bud (*Populus nigra*) extract: individual antioxidant contribution of phenolics and transcriptional effect on skin aging. J Agric Food Chem 59(9):4527–4536

Gangwar AK, Ghosh AK (2014) Medicinal uses and pharmacological activity of *Adhatoda vasica*. Int J Herb Med 2(1):88–91

Habtemariam S (2016) The therapeutic potential of rosemary (*Rosmarinus officinalis*) Diterpenes for Alzheimer's disease. Evid Based Complement Alternat Med 2016:1

Haouat AC, El Guendouzi S, Haggoud A, David S, Sqalli H, Ibnsouda S, Iraqui M (2013) Antimycobacterial activity of *Populus alba* leaf extracts. J Med Plants Res 7(16):1015–1021

Head K (2004) *Angelica sinensis* (Dong quai). Altern Med Rev 9(4):429–433

Hentrez M, Givskov M (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 112(9):1300–1307

Ho M, Hsieh Y, Chen J, Chen K, Chen J, Kuo W, Lin S, Chen P (2011) Antimetastatic potentials of *Dioscorea nipponica* on melanoma *in vitro* and *in vivo*. Evid Based Complement Alternat Med 2011:1

Hogan DA (2015) Talking to themselves: autoregulation and quorum sensing in fungi. Eukaryot Cell 5(4):613–619

Hossain MF, Akhtar S, Anwar M (2015) Nutritional value and medicinal benefits of pineapple. Int J Nutr Food Sci 4(1):84–88

Jaiswal P, Kumar P, Singh VK, Singh DK (2011) *Areca catechu* L.: a valuable herbal medicine against different health problems. Res J Med Plant 5920:145–152

Jurenka J (2008) Therapeutic application of pomegranate (*Punica granatum* L.): a review. Altern Med Rev 13(2):128–144

Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245

Kalita S, Kumar G, Karthik L, Rao KVB (2012) A review on medicinal properties of *Lantana camara* Linn. Res J Pharm Technol 5(6):711–715

Khan MSA, Zahin M, Hasan S, Husain FM, Ahmad I (2008) Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol 49:354–360

Khushbu C, Roshni S, Anar P, Carol M, Mayuree P (2011) Phytochemical and therapeutic potential of *Piper longum* Linn a review. Int J Res Ayurveda Pharm 2(1):157–161

Koh KH, Tham F (2011) Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. J Microbiol Immunol Infect 44:144–148

Koh C, Sam C, Yin W, Tan L, Krishnan T, Chong Y, Chan K (2013) Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors 13:6217–6228

Kokila K, Priyadharshini SD, Sujatha V (2013) Phytopharmacological properties of *Albizia* species: a review. Int J Pharm Pharm Sci 5(3):70–73

Kumar T, Chandrashekar KS (2011) *Bauhinia purpurea* Linn.: a review of its ethnobotany, phytochemical and pharmacological profile. Res J Med Plant 5(4):420–431

Kumar D, Arya V, Bhat ZA, Khan NA, Prasad DN (2012) The genus *Crataegus*: chemical and pharmacological perspectives. Braz J Pharmacol 22:1187

Lis-Balchin M, Hart S (1999) Studies on the mode of action of the essential oil of lavender *Lavandula angustifolia* P. Miller. Phytother Res 13(6):540–542

McLean RJC, Pierson III LS, Fuqua C (2004) A simple screening protocol for the identification of quorum signal antagonists. J Microbiol Methods 58:351–360

Mounika SC (2016) A review on medicinal importance of Babchi (*Psoralea corylifolia*). Int J Recent Sci Res 7(6):11504–11512
Nandal U, Bhardwaj RL (2012) *Aloe vera:* a valuable wonder plant for food, medicine and cosmetic use- a review. Int J Pharm Sci Rev Res 13(1):59–67
Nayar MP, Sastry ARK (1990) Red data book on Indian plants, vol 3. Botanical Survey of India, Calcutta, pp 28–29
Neeraj S, Sushila K, Dheeraj D, Millind P, Minakshi P (2014) Garlic: a pungent wonder from nature. Int Res J Pharm 5(7):523–529
Ng TB (2006) Pharmacological activity of Sanchi ginseng (*Panax notoginseng*). J Pharm Pharmacol 58(8):107–119
Okubo H, Hiramatsu M, Masuda J, Sakazono S (2012) New insight into *Lilium brownie* var. *volchesteri*. Floricult Ornament Biol 6(2):44–52
Milind P, Preeti (2015) Chickoo: a wonderful gift from nature. Int J Res Ayurveda Pharm 6(4):544–550
Packiavathy IASV, Agilandeswari P, Musthafa KS, Pandian SK, Ravi AV (2012) Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against gram negative bacterial pathogens. Food Res Int 45:85–92
Parada M, Carrio E, Bonet MA, Valles J (2009) Ethanobotany of the Alt Emporda region (Catalonia, Iberian Peninsula). Plants Used Hum Trad Med 124:609–618
Parvathy NG, Padma AR, Renjith V, Rahate KP, Saranya TS (2012) Phytochemical screening and anti-helmenthic activity of methanolic extract of *Imperata cylindrica*. Int J Pharm Pharm Sci 4(1):232–234
Parvez GMM (2016) Pharmacological activities of mango (*Mangifera indica*): a review. J Pharmacogn Phytochem 5(3):01–07
Patrakar R, Mansuriya M, Patil P (2012) Phytochemical and pharmacological review on *Laurus nobilis*. J Med Plants Res 1(2):597–602
Paudel KR, Panth N (2015) Phytochemical profile and biological activity of *Nelumbo nucifera*. Evid Based Complement Alternat Med 2015:1–16
Preston RE (2012) Flora of North America. Taxon eds, Freeman & Rabeler, p 17
Rahman S, Islam I, Kamruzzaman M, Alam K, Jamal AHM (2011) *Ocimum sanctum* L.: a review of phytochemical and pharmacological profile. Am J Drug Discov Devel. https://doi.org/10.3923/ajdd.2011. ISSN 2150-427x, © 2011 Academic Journals Inc
Raj V, Jain A, Chaudhary J (2012) *Prunus armeniaca* (Apricot): an overview. J Pharm Res 5(8):3964–3966
Rao PV, Gan SH (2014) Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med 2014:1
Rasool R, Ganai BA (2013) *Prunella vulgaris* L.*:* a literature review on its therapeutic potentials. Pharmacologia 4(6):441–448
Rungruangmaitree R, Jiraungkoorskul W (2017) Pea, *Pisum sativum* and its anticancer activity. Pharmacog Rev 11(21):39–42
Sabharwal S, Sudan S, Ranjan V (2013) *Jasminum sambac* Linn (Motia): a review. Int J Pharm Res Bio Sci 2(5):108–130
Saini NK, Singhal M (2012) Evaluation of anti-nociceptive activity of Methanolic extract of *Tecoma capensis* Thunb. (Bignoniaceae) leaves in rats. Asian Pac J Trop Med 5:781–784
Selvam ABD (2012) Pharmacognosy of negative listed plants. Botanical Survey of India, Ministry of Environment and Forests, Kolkata, pp 138–148
Shah PP, D'Mello PM (2004) A review of medicinal uses and pharmacological effects of *Mentha piperita*. Nat Prod Rad 3(4):214–221
Shen J, Man K, Huang P, Chen W, Chen D, Chen Y, Liu P, Chou M, Chen Y (2010) Honokiol and Magnolol as multifunctional antioxidative molecules for dermatologic disorders. Molecules 15:6452–6465
Siew-Mian S, Tham F (2012) Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia. Malays J Microbiol 8(1):11–20

Singh BN, Singh BR, Singh RL, Prakash D, Dhajarey R, Upadhyay G, Singh HB (2009a) Oxidative DNA damage protective activity, antioxidant activity and anti-quorum sensing potentials of *Moringa oleifera*. Food Chem Toxicol 47:1109–1116

Singh BN, Singh BR, Singh RL, Prakash D, Dhajarey R, Upadhyay G, Singh HB (2009b) Oxidative DNA damage protective activity, antioxidant activity and anti-quorum sensing potentials of *Moringa oleifera*. Food Chem Toxicol 47:1109–1116

Singh BN, Singh BR, Singh RL, Prakash D, Sharma BK, Singh HB (2009c) Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L. Food Chem Toxicol 47:778–786

Sinha S, Sharma A, Reddy PH, Rathi B, Prasad NVSRK, Vashistha A (2013) Evaluation of phytochemical and pharmacological aspects of *Holarrhena antidysenterica* (Wall.): a comprehensive review. J Pharm Res 6:488–492

Vilelathor FC, Padilha MM, Santos-e-Silva L, Alves-da-Silva G, Giusti-Paiva A (2009a) Evaluation of the antinociceptive activity of extracts of *Sonchus oleraceus* L. in mice. J Ethnopharmacol 124(2):306–310

Zahin M, Hasan S, Aqil F, Khan MSA, Husain FM, Ahmad I (2010a) Screening of certain medicinal plants for their anti-quorum sensing activity. Indian J Exp Biol 48:1219–1224

Zaki AA, Shaaban MI, Hashish NE, Amer MA, Lahloub M (2012) Assessment of anti-quorum sensing activity of some ornamental and medicinal plants native to Egypt. Sci Pharm 81:251–258

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in Bacteria. Annu Rev Cell Dev Biol 21:319–346

Winzer K, Williams P (2001) Quorum sensing and the regulation of virulence gene expression in pathogenic bacteria. Int J Med Micobiol 291:131–143

Zahin M, Hasan S, Aqil F, Khan MSA, Husain FM, Ahmad I (2010b) Screening of certain medicinal plants for their anti-quorum sensing activity. Indian J Exp Biol 48:1219–1224

Vilelathor FC, Padilha MM, Santos-e-Silva L, Alves-da-Silva G, Giusti-Paiva A (2009b) Evaluation of the antinociceptive activity of extracts of *Sonchus oleraceus* L. in mice. J Ethnopharmacol 124(2):306–310

Yarnell E, Abascal K (2009) Dandelion (*Taraxacum officinale* and *T mongolicum*). Integrative Med 8(2):35–38

Weissner W (2014) Anantamul (*Hemidesmus indicus*): a review of biomedical studies and U.S. products. Ayurveda J Health XII(4):40–52

Zhang Z, Leung W, Cheung H, Chan C (2015) Osthole: a review on its bioactivities, pharmacological properties and potential as alternative medicine. Evid Based Complement Alternat Med 2015:916916

Part IV

Role of Quorum Sensing in Food Industry

Bacterial Quorum Sensing: Challenges and Prospects in Food Microbiology

15

L. Veeranjaneya Reddy, Y. J. Wee, L. Prasanna Anjaneya Reddy, and Pallaval Veera Bramhachari

Abstract

Communication has long been considered as a unique activity of humans. But now it is found that communicative behaviour is present in all living systems including non-multicellular organisms and food fermentations. QS is a microbial communication method which depends on cell number that can control many activities in bacteria such as virulence, biofilm formation, competence and bioluminescence. For QS continuous secretion and observation of hormone-like molecules called auto-inducers or QS molecules is required. QS was first noticed in Gram-negative bacteria *V. fischeri* and termed as autoinduction (AI). After that it came to know that QS is present in many ecological niches. Unlike other environments in food matrix, QS molecules are produced but do not have regular distribution. External environment exerts its importance in varying sensing signals. In fact, physical factors, viz. pH, temperature, water and oxygen availability, are now known to influence the sensing processes. Spoilage of food may be defined as a process that makes food unattractive or deplorable for eating and outcome of microbial activity that ultimately dominates according to widespread ecological determinants. The present chapter will provide an outlook on (i) role of QS in food fermentation and

L. V. Reddy (✉)
Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India
e-mail: lvereddy@gmail.com

Y. J. Wee
Department of Food Science & Technology, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea

L. P. A. Reddy
Department of Lipid Science, Central Food Technological Research Institute (CFTRI), Mysuru, India

P. V. Bramhachari
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_15

food spoilage, (ii) factors that affect the activity of QS in various types of foods and inhibitors that can be used as biopreservatives, (iii) future perspectives that necessitated to understand how microbial behaviour in foods affects QS and (iv) utilization of QS in food preservation and food security.

Keywords
QS · Food microbiology · Farnesol · Tyrosol · Aromatic alcohols · Wine production

15.1 Introduction

Over the last two decades on microorganisms, we have understood that simple single-cell organisms have a surprising mechanism for its cooperative social behaviour. It comes to knew with the observation of growth kinetics of marine bioluminescent bacteria that commencement of log phase occurs without a lag and increase of bioluminescence after mid logarithmic phase only. This phenomenon is probably due to an inhibitor in the medium. Later, Nealson et al. (1970) proposed the phenomenon of 'cell density-dependent AI' after observing luminescence in marine symbiont bacterium *V. fischeri* that inhabits light organs of *E. scolopes*, produced at high cell densities. The term 'QS' particularly refers to the bacterial biomass linked, coordinated gene expression in populations that experience threshold signal concentrations to provoke a coordinated response cellular population (Fuqua et al. 2001). QS is essential for microbes to compete in the race to colonize new niches, utilize available substrates and battle host defences. This behaviour depends on the secretion of its own sensing molecules and detection of diffusible signal molecules from outside-related organisms through 'QS'.

Bacteria use QS to synchronize gene expression systems that trigger communal performance. Bacterial language is chemical in nature using certain signalling molecules. QS depends on secretion, recognition and collective reaction to extracellular signalling molecules described as auto-inducers or QS molecules. They possess specific receptors which can detect these AIs. Upon binding of inducer to receptor, it triggers transcription of specific genes, including AIs. On the other hand, rise in the population increases the inducer concentration, and at a certain point it reaches the threshold level, and the analogous receptor gets activated. The activated receptor induces the upregulation of corresponding genes in all responded organisms; this allows them to start transcription at the same time. QS regulates interactions both in signal producing organisms and between different species present in surrounding medium that is between disease-causing and useful microbes, higher organisms (symbiosis, growth promotion, pathogenicity). It has importance in agriculture (in the areas of plant–microbe interactions apropos to pathogenicity(plant growth promotion, biocontrol) ecology (behaviour of microbes), medicine (colonization and disease causing of animal hosts), food industry (food production, spoilage), aquatic, industrial plant biofouling (Smith et al. 2004).

The major confronting issues in food industry with microbes are contamination, survival and possible foodborne disease. Nevertheless, for microbes, any food resource either natural or processed is just one more ecological system for their survival. But in human point of view, microbial attack causes tremendous economic losses and poses serious public health consequences through spoilage of food by food-related pathogenic bacteria (Dhama et al. 2013). Generally, food spoilage will occur by two consequences: one is ageing and another one is microbial colonization. Spoilage of food by microbes is an amalgamated process and causes a variety of biochemical and textural changes by microbial metabolites. Microbial contamination is a largely universal cause of spoilage of microbes that occurs during food preparation, packing and delivery (Gram et al. 2002). This will decrease the shelf life of food. Food is not adequately utilized owing to microbial spoilage which results in rejection and liberation of toxic metabolites. *Salmonella* spp., *Campylobacter* spp., *E. coli*, *L. monocytogenes*, *Yersinia intermedia* and *P. putida* are few common foodborne pathogens and cause serious problems in the food industry.

Antibiotics are used to destroy or slow down the development of foodborne pathogens and enhance the shelf life of food. Continuous usage of antibiotics will generate drug-resistant strains capable of resisting most commonly used antibiotics, and this requires new strategies to prevent pathogen attack. QS will lend a hand as one of the influential targets for investigation of novel agents, viz. natural compounds, antibodies and enzymes, that can regulate microbial behaviour by disrupting their cellular communication system. It was also reported presence of mechanisms for sensing, mimicking, or destroying QS signals by some of plants, animals and microbes. *P. aeruginosa*, *A. hydrophila*, *V.anguillarum*, *Streptococcus* sp. and *B. cepacia* are well-known food-related pathogenic organisms, form their biofilms on both natural and artificial surfaces using cell-to-cell communication. Better understanding of QS mechanism by signal molecules detection, study mechanism of activities in foods and food ingredients may facilitate in developing novel, advanced tools to prevent biofilm formation and microbial persistence. The current review provides knowledge on the role of QS and signalling molecules in pathogenicity, food spoilage of food-related bacteria. It will also touch upon possibilities to utilize potent QS inhibitors in food preservation and food safety.

15.2 An Ode to the Quorum Sensing

QS is one of the most significant discoveries in the field of microbiology for the past 30 years. Fuqua and Winans (1994) had coined the term QS for a microbial cell-to-cell communication system. It is a well-known occurrence in bacteria and regulates interactions not only signal producing organism but also between various genus and species of pathogenic-beneficial microbes, growth and pathogenicity perspective. QS is a process by which microorganisms are known to interact by different types of small/signal molecules and this communication based on the synthesis, secretion and reaction with petite, diffusible signal molecules called AIs. During the initial

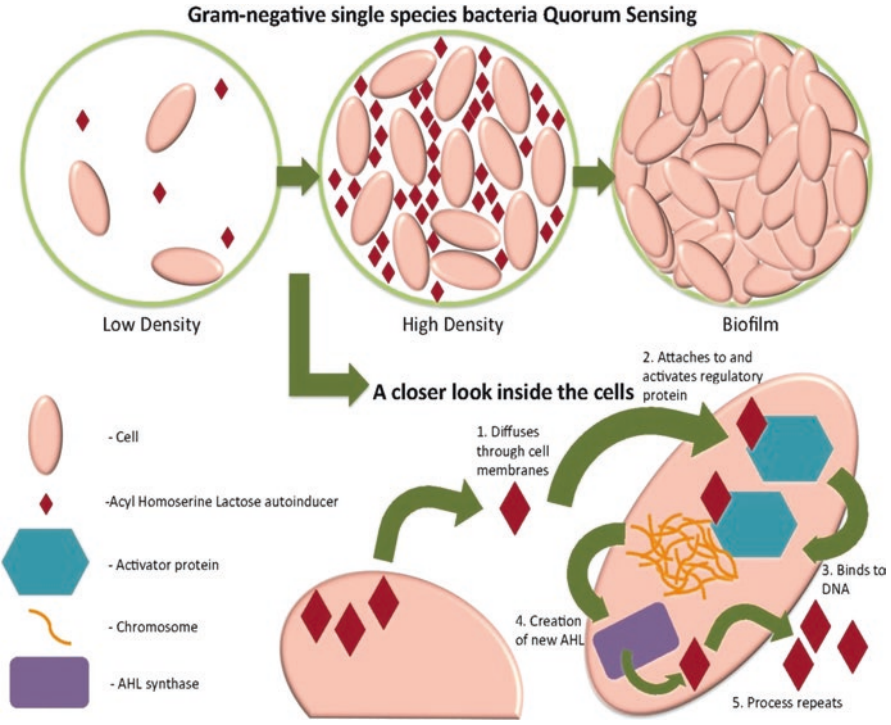


Fig. 15.1 Generalized quorum-sensing mechanism. (Source: https://en.wikipedia.org/wiki/File:Quorum_sensing_of_Gram_Negative_cell.pdf)

stage of bacterial growth, the production and secretion of signal molecules/AIs are at a low concentration, and it increases along with an increase in bacterial density in environment/food matrix in latter phases. Bacteria observe alterations in the concentration of AIs; when it attains a threshold level, it brings about phenotypic effects by QS regulating gene expression (Czajkowski and Jafra 2009) (Fig. 15.1). AI occurs devoid of any external interference. The QS-regulated processes usually damage other bacteria and self-survival under adverse conditions.

QS mechanism is present in Gram-positive and Gram-negative bacteria. Peptide and fatty acid derivatives are two groups of signal molecules that are implicated in bacterial QS. In Gram-positive bacteria oligopeptides that naturally consist of membrane-bound sensor kinase receptors and cytoplasmic transcription factors involved in direct alterations in gene expression. However, fatty acid derivative signalling molecules are involved in QS-regulated gene expression of Gram-negative bacteria. QS is a ubiquitous mechanism that presents in several bacterial species, viz. *Brucella*, *Agrobacterium*, *Enterobacter*, *Burkholderia*, *Pseudomonas*, *Erwinia*, *Serratia*, *Ralstonia*, *Vibrio* and *Yersinia*, which are well-known Gram-negative human and plant pathogenic bacteria that use QS for production and regulation of virulence factors (Williams 2007). *Bacillus*, *Staphylococcus*, *Enterococcus*,

Streptomyces and *Streptococcus* utilize QS to obtain antimicrobial peptides secretion, genetic competence, and also for biofilms (Podbielski and Kreikemeyer 2004). The *Rhizobium* utilizes it for soil nitrogen fixation. In this process symbiotic bacteria and symbiosome development is regulated by QS (Hoang et al. 2004). Extremophiles, haloalkaliphilic archeon *N. occultus Halomonas*, hyperthermophilic bacterium *T. maritima* and acidophilic bacteria *A. ferrooxidans* (Johnson et al. 2005; Rivas et al. 2007) possesses QS mechanism (Llamas et al. 2005; Paggi et al. 2003). Processes that regulated by QS mechanism (bioluminescence, secretion of virulence factors and biofilms) are not much productive and expensive in single bacterial cell yet becomes uncomplicated and more productive within group (Bassler and Losick 2006).

15.3 Mechanisms of Quorum Sensing

Bacterial language is chemical in nature using certain signalling molecules. QS relies on the synthesis, secretion, recognition and group-level response to QS molecules. They possess specific receptors which can detect these AIs. After binding of AI to receptor, it induces transcription of respective genes, together with those for AI synthesis. Molecules implicated in QS (QS) possess the ability to induce their own production; hence they are known as 'AIs'. The total QS process will run in four steps:

1. Production of signal molecules by the microbial cell.
2. Secretion of signal molecules into the surrounding environment (either actively or passively).
3. Detection of threshold level signal molecules by specific receptors.
4. Alterations in gene regulation.

AHL-mediated QS is well studied bacterial communication mechanisms (Table 15.1). The most important agricultural bacterial species are *A. tumefaciens*,

Table 15.1 AHL-mediated quorum sensing systems

Quorum-sensing process	Outcome	References
At low population density, AHL synthase uses S-adenosylmethionine (SAM) and acyl chains to synthesize AHL	Signal generation	Schaefer et al.
Short-chain AHLs diffuse passively	Signal accumulation	Dong et al. (2012)
Long-chain signals require active efflux	Signal accumulation	Dong et al. (2012)
At high population density, LuxR-type (R) transcription factor recognizes signal	Signal reception	Schuster et al.
R proteins and AHLs bind to target DNA leading to increased AHL signal production and activation of quorum-sensing regulation	Autoinduction	Zhu and Winans (1999)

E. carotovora, medically significant *Burkholderia* and *P. aeruginosa* species also employ this mechanism (Dong et al. 2000; Williams 2007). Additionally, this process undergoes 2-component system in this system, the AI, transported to intercellular space by an ABC transporter. The accumulated signals are detected by a 2-component sensor that transports the sensory information to trigger a similar response regulator for changing the expression of QS regulon through regulatory RNAs and intracellular transcription factors (Novick 2003; Waters and Bassler 2005).

15.4 Signal Molecules of Quorum Sensing

The bacterial cell signalling systems are largely categorized into four main categories: (AI-1), (AI-2), (AI-3) and (AIP). In Gram-negative bacteria both (AI-1) and (AI-3) are present, Gram-positive bacteria utilize (AIP) system, and (AI-2) is present in both types. The AI-2 is considered as a universal signalling molecule due to its ability to alter gene expression in different bacterial species and genera. These four categories of signalling systems can group simply based on the mechanism: one is AHL-dependent and another is AIP-dependent QS systems. The QS signal is detected by cytosolic transcription factor in AHL-dependent systems and in second one signal is detected by 2-component membrane-associated response regulatory system (Dong et al. 2012; Sifri 2008).

15.4.1 Auto-Inducer (AI-1)

AI-1 is the main group of signalling molecules in Gram-negative bacteria, and it contains N-acylhomoserine lactones (N-AHL). They contain a conserved homoserine lactone (HSL) ring with a variable acyl side chain. The variation and specificity is present in a mixed bacterial population QS communication because of length and saturation level of acyl chains attached to the existence or lack of oxo or hydroxyl substitutions at C-3 position of acyl chain (Shaw et al. 1997). It is known that N-AHL system is synthesized by LuxI/LuxR system and this becomes the model system for other QS systems. It consists of two components: LuxI (AI-1) and LuxR (transcription factor). Gene expression is under the control of LuxR in the presence of AI (Fig. 15.2). At higher population density, local AI-1 levels reach their threshold levels (required concentration to diffuse back into cell), bind to LuxR and initiate transcription of *luxCDABEGH* operon by binding Lux boxes present in promoter (Devine et al. 1989). The product of this operon, luciferase, catalyses the process responsible for luminescence. Homologous LuxI/LuxR systems capable in producing specific AHLs were identified in numerous Gram-negative bacteria. Virulence factors expression in *P. aeruginosa* and *S. marcescens* were regulated by these signalling mechanisms. *P aeruginosa* contains two systems homologous to LuxI/LuxR. LasI/LasR is responsible for biofilm formation, synthesis of extracellular enzymes and transcription of another QS system.

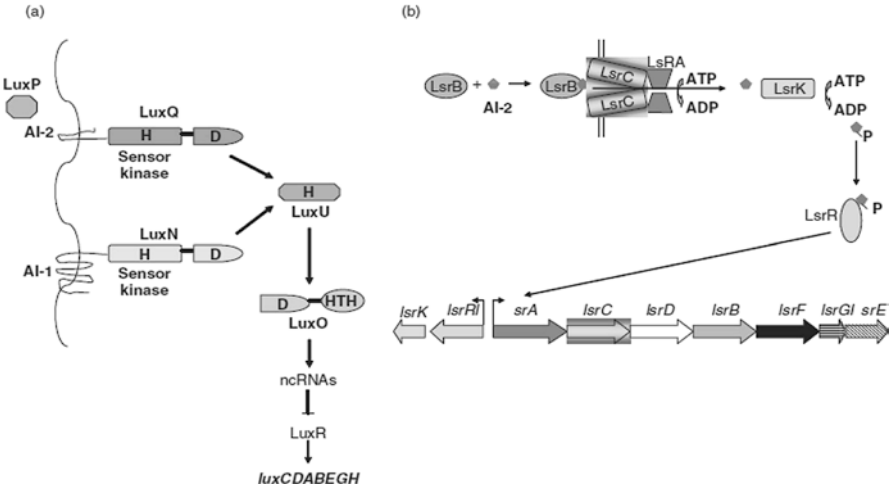


Fig. 15.2 The LuxS/AI-2 quorum-sensing system. (**a**) *Vibrio harveyi* (**b**) in *Salmonella*, *E. coli*

15.4.2 Auto-Inducer (AI-2)

AI-2 was also first detected in Gram-negative bacteria. It is the most common bacterial AI identified up to now as it is present in 500 bacterial species. AI-2 contains a set of interconverting AI molecules derived from 4,5-dihydroxy-2,3-pentanedione (DPD). DPD is synthesized from a by-product of SAM metabolism. AI-2 synthesizes by LuxS in multiple steps by the exchange of ribose homocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), and then it cyclises into quite a lot of furanones in presence of water (Schauder et al. 2001). Different bacterial species recognize different forms of DPD as their active AI-2 signals. For instance, in *V. harveyi*, AI-2 has boron41; *E. coli* and *Salmonella* spp. have a non-borated cyclized DPD moiety42 (Fig. 15.1e). AI-2 has interesting interconversion property; this attribute helps bacteria for inter-species communication. Some bacteria, such as *P. aeruginosa*, do not have LuxS enzyme and thus do not make AI-2. Nevertheless, they can detect AI-2 produced by other bacterial species and modify their gene expression pattern.

Detection of AI-2 accumulated in cell's environment can be performed by two different mechanisms. First one is present in *V. harveyi*, it detects BAI-2 form of AI-2. This mechanism senses periplasmic AI-2 first by binding signal with LuxP, an AI-specific binding protein then after AI-2/LuxP complex acts together with a sensor kinase, LuxQ. This interaction initiates a phosphotransfer cascade that ends in synthesis of luminescence. Till date, the LuxP/LuxQ cascade was detected in *Vibrio* spp. only (Fig. 15.3a). Second one is present in *E. coli* and *S. typhimurium* (Fig. 15.3b). In this mechanism, LuxS-regulated Lsr system is present in place of LuxP/LuxQ system. Detected and diffused AI-2 initiates a cellular reaction by reaching into cytoplasm. In this process periplasmic protein, LsrB helps in recognition of signal by binding to R-THMF form of AI-2. After binding, LsrK

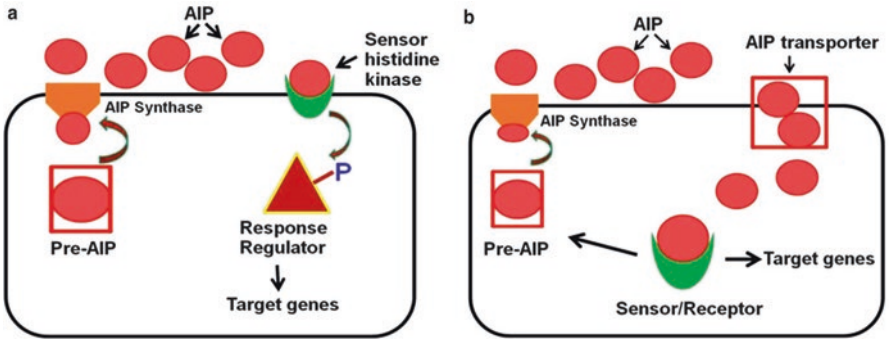


Fig. 15.3 Autoinducing peptide (AIP)-mediated quorum sensing in Gram-positive bacteria by (**a**) two-component signaling and (**b**) an AIP-binding transcription factor

phosphorylates AI-2 together with LsrR (transcriptional repressor) and releases repression of *lsr* operon that may upregulate additional operons (Taga et al. 2001). AI-2 signals structure of two has been determined by co-crystallization with two different AI-2-binding proteins: a furanosyl borate diester (BAI-2) used by *V. harveyi* to regulate luminescence and a furanone [2R,4SL]-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran [R-THMF]) used by *S. typhimurium* (Miller et al. 2004).

15.4.3 Auto-Inducer (AI-3)

AI-3 was first detected in enterohemorrhagic *E. coli* (EHEC) O157:H7 spent media as a compound found that activates the expression of genes concerned with attachment and actin rearrangement in eukaryotic cells (Sperandio et al. 2003). AI-3 signalling helps microbe to survive in their hosts, pathogenesis and flagella motility. These three signals activate expression of a pathogenicity island named locus of enterocyte effacement (LEE), Shiga toxin and flagella regulon (Moreira et al. 2010). There are no reports on presence of AI-3 in Gram-positive bacteria. As AI-3 production is not present in *luxS* mutants, it was thought that LuxS was involved in AI-3 production. Further studies demonstrated that need for AI-3 production in these mutants was not attributable to SAM. AI-3 production was initiated by addition of L-aspartate in media and has not shown any effect on AI-2 production (Walters et al. 2006). AI-3 is recognized by a 2-component system that contains sensor kinase (QseC) and response regulator (QseB). When sufficient concentration of AI-3 present, QseC endures autophosphorylation and transfers phosphate to QseB. This mechanism induces genes conscientious for flagella biosynthesis and motility by upregulating master flagellar regulator gene *flhDC* (Clarke et al. 2006). The complete cascade responsible for regulation of these genes remains ambiguous, but probably known as it involves QseA, a LysR family regulator that is predisposed by cell-to-cell signalling, directly upregulates LEE genes (Sperandio et al. 2002).

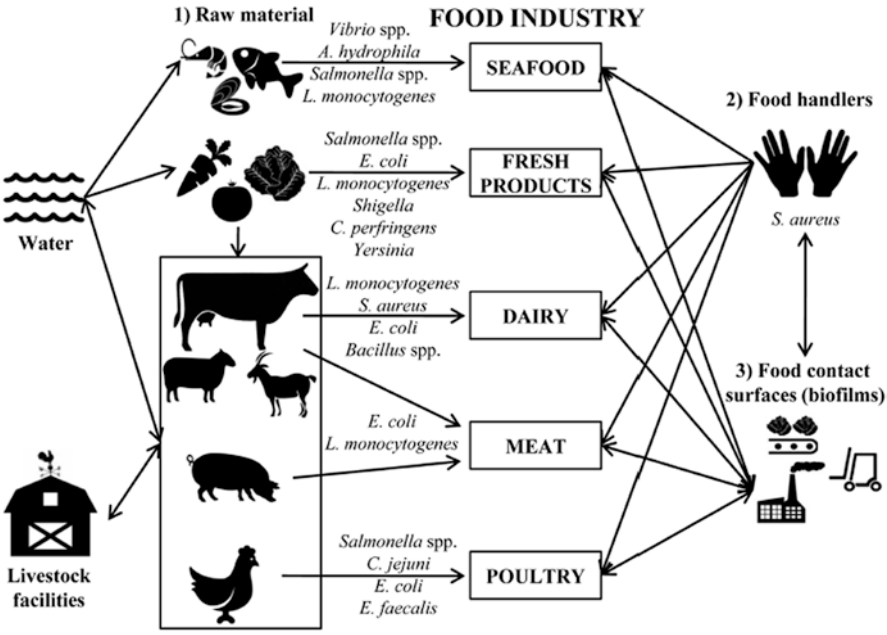


Fig. 15.4 Schematic representation of contamination sources in different food processing industries

15.4.4 Auto-Inducing Peptide (AIP)

This type of cell signalling is largely present in Gram-positive species and first detected in *S. aureus*. In this process bacteria utilize small post-translationally modified peptides for signalling. These small peptides are generally synthesized from oligopeptides by a variety of modifications and secreted out into the surrounding environment through transporters. When reaching threshold levels, the sensor kinases recognized then after initiate phosphorylation of response regulator. The peptides involved in QS use to have their specific cognate receptors (Fig. 15.4). For example, QS peptide signalling is present in opportunistic foodborne pathogen *C. perfringens* which controls sporulation, toxin production, virulence (Ma et al. 2015) and biofilm formation (Vidal et al. 2015). In *L. monocytogenes* it regulates virulence, invasion and biofilm formation (Riedel et al. 2009; Abee et al. 2011) (Fig. 15.5).

15.5 QS and Biofilm Formation in Food Industry

The term 'biofilm' was defined as an 'Aggregate of microorganisms in which cells that are repeatedly entrenched within a self-produced matrix of extracellular polymeric substances (EPS) adhere to each other and/or to a surface' by IUPAC, Polymer

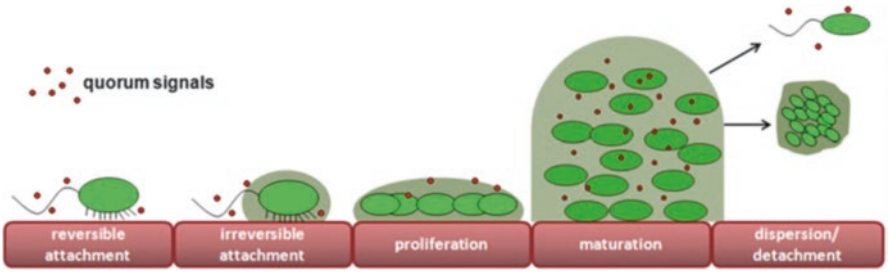


Fig. 15.5 QS signals and biofilm formation by *Pseudomonas aeruginosa*

Division (Vert et al. 2012). The microorganism present in a biofilm displays primitive homeostasis and circulatory systems, genetic material exchange and metabolic cooperation (Costerton et al. 1995). Bacteria can form biofilms on all kinds of surfaces of food processing units from plastic to wood which is very complex to eliminate them completely. The development of biofilms/attached cells on foods and food processing areas usually affects food adversely, especially in raw and inadequate processed foods (Franck 2001). Biofilms have become a big problem in food industry and moreover are thought to cause contamination by a variety of foodborne pathogens, and this can adversely affect the product safety, stability, quality and value (Fig. 15.6). It is very difficult to remove the attached pathogenic microorganisms on minimally processed products due to the difference in surface morphologies, hydrophobic cutin and abrasions in the epidermis of fruits and vegetables (Burnett and Beuchat 2001).

In food processing units, there are two categories of biofilms, one forms films on surfaces that have direct contact with flowing product and known as process biofilms; and second one forms film in the processing area, viz. in places where cleaning, sanitation is poor along with drains and known as environmental biofilms (Table 15.2). Process biofilms are rapid growers when compared to environmental biofilms. The food processing units produce a large amount of perishable, semi-perishable foods and food ingredients; for instance, the dairy industry products such as milk and cream come under perishable products; cheese, butter and yoghurt come under semi-perishable and milk powders; and whey protein concentrates and caseinates come under food ingredients. Contamination of dairy products by pathogenic organisms is minor worry for the dairy industry. Maintaining strict microbiological guidelines is very much required to obtain quality, functionality and safety for their product which leads the company to competitiveness in international markets.

It is well demonstrated that QS participates in all phases of biofilm formation in various bacterial species (Stoodley et al. 2002; Otto 2013). But it was showed that monoculture conditions for biofilm formation in *Salmonella* (Thompson sp.) on stainless steel (72 h at 25 °C) are the same for both AI-2-positive and AI-2-negative strains. Microbes on improperly cleaned and disinfected food processing surfaces are an intricate heterogeneous community, nothing like the pure species biofilms

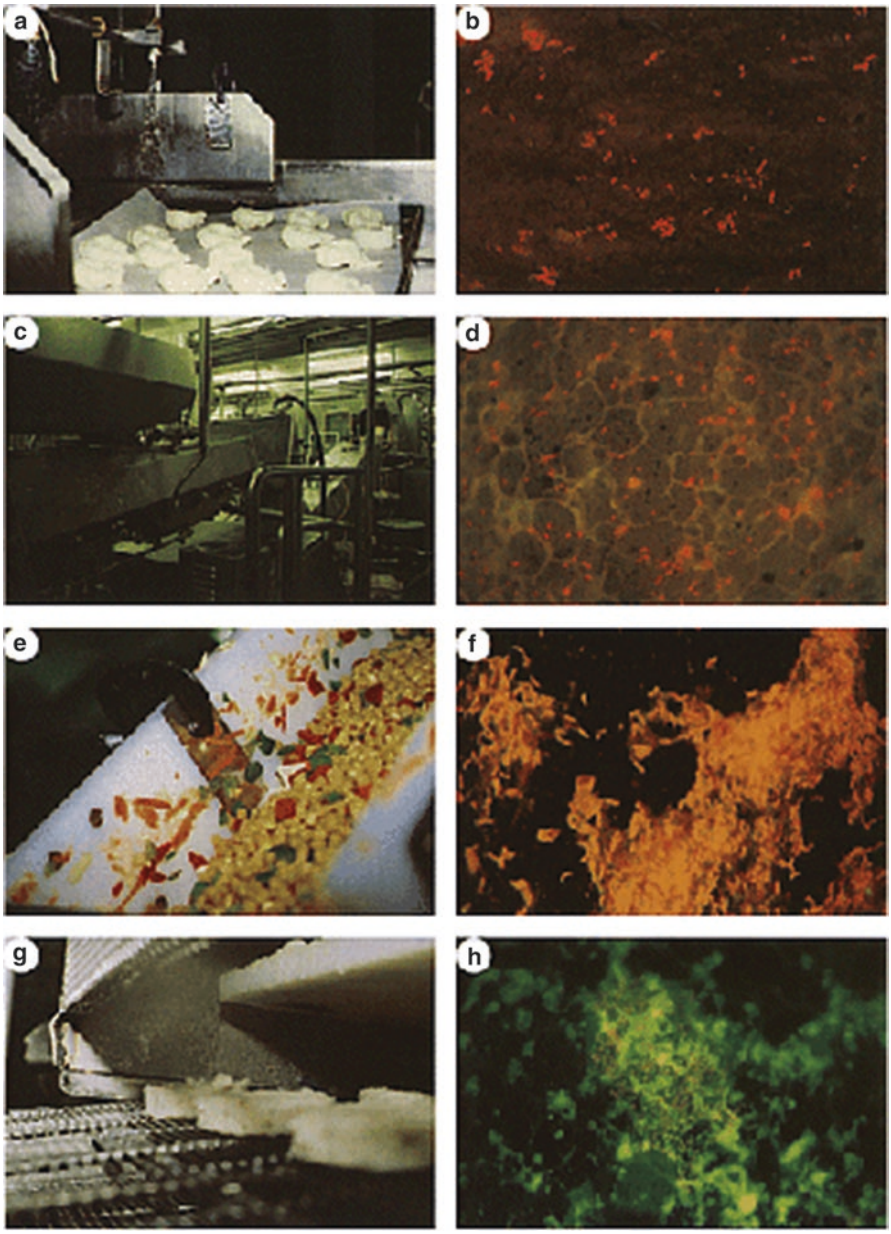


Fig. 15.6 Individual cell and microcolony development on various food processing surfaces **a**, **b** = individual cells developing on an egg glaze bath after 8 h; **c**, **d** = individual cells of *S. aureus* developing within a buttermilk line after 8 h; **e**, **f** = microcolony development after 6 h on a prepared salad line; **g**, **h** = development of microcolonies within 90 min on a fishcake forming machine

Table 15.2 List of bacterial biofilms which appear in food processing surfaces

S.no.	Factory type	Area exposure	Time (h)	Swab count ($cfucm^{-2}$)	% area coverage
1	Canned products	Waste can area	24	>3.5 × 107	15.0
2	Canned products	Blancher extractor	24	>4.7 × 107	26.5
3	Meat substitute	Mixer—undersurface of ledge	20	>5.9 × 107	56.1
4	Potato	Ceiling	24	5.5 × 105	3.6
5	Cod cakes	Conveyor	1.5	4.6 × 106	11.3
6	Biscuits	Steam clean room	16	>1.4 × 107	20.2
7	Poultry products	Wall in rack washing area	6		
8	Peas	Inspection belt guard	48		

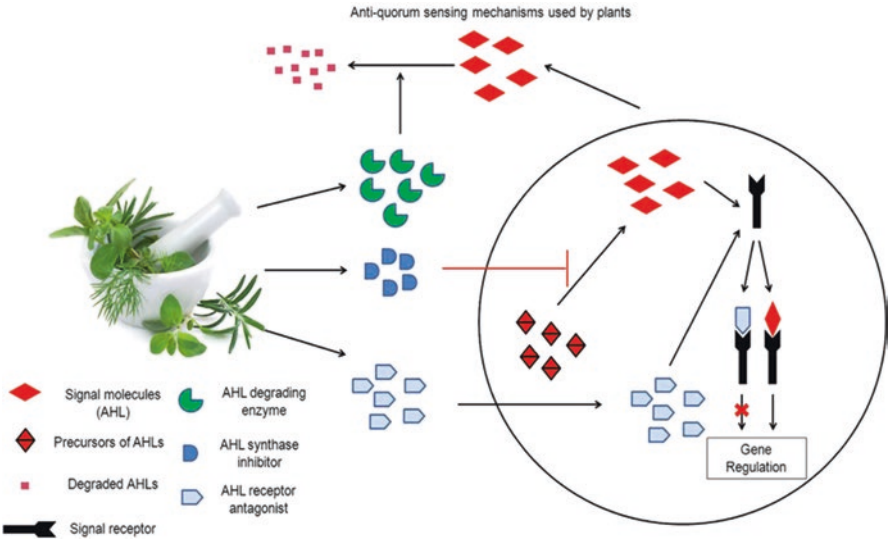


Fig. 15.7 Anti-quorum-sensing mechanism of natural plant compounds

investigated in the laboratory (Stoodley et al. 2002). The biofilm-forming capacity may influence by interactions between the different species of individual strains, and this may be a QS-mediated process (Parsek and Greenberg 2005). Hence, to confirm the specific effect of AHLs on biofilm formation by a single organism in multispecies environments requires a supplementary study.

Biofilm formation in foodborne bacteria in relation to QS has been studied well. It was first reported in *P. aeruginosa* growing in a flow-through reactor and observed that $3OC_{12}$– homoserine lactone (C_{12}) (QS signal molecule) was required during the differentiation of biofilm (Fig. 15.7) (Davies et al. 1998). This function of QS molecule in biofilm received significant attention. *Hafnia alvei* products are common

bacterial food contaminants basically isolated from meat, dairy and fish. *Hafnia alvei* strain 071 is known to form biofilms, but its mutant *H. alvei* 071 *halI* has failed to form the biofilm. From this, it can be suggested that lack of QS in mutant cells of *H. alvei* 071 inhibited biofilm formation process by inhibiting the differentiation process which is obligatory for transforming individual cells into complex multicellular structures (Vivas et al. 2008). The production of EPS in *V. cholerae* and *S. liquefaciens* is under the control of AI-2 and essential for cell aggregation in biofilm formation (LV et al. 2014). This was not observed in bacteria isolated from food processing surroundings. QS is not much important in biofilm structural development. But for some species it is essential for surface attachments, structural development, maturation and even for the control of dispersion of cells from biofilms (Davies et al. 1998; Boles and Horswill 2008a, 2008b; Lv et al. 2014). It is quite interesting that the utilization of *Hafnia alvei* metabolites for the biofilm development by *S. enterica* serovar Enteritidis PT4 growing stainless steel (SS). Biofilms pose significant problems in food-processing environments. Significant contribution of QS in biofilm formation by foodborne pathogens on food processing surfaces could provide novel research dimensions in removing these surface-attached microbial communities. However, QS inhibition may eliminate biofilm formation capacity of pathogenic organism and thus reduce food spoilage which helps in quality food production and food safety (Karatan and Watnick 2009).

In bacteria production of enzymes is an intricate process and affected by various physical and bacterial population factors, such as QS and phase variation (Liu et al. 2007; Khiyami et al. 2006). QS signal molecules were shown to control enzymes, proteases production in biofilms (Swift et al. 1999; Liu et al. 2007; Khajanchi et al. 2009). Relationship between the presence of QS signal molecules AHL, biofilm formation and enzyme production is not yet clear (Khajanchi et al. 2009). For example, in *staphylococcus* activation of *agr* system (QS system involving in dispersal of cells from biofilms) increased its enterotoxin D (SED) expression (Boles and Horswill 2008a, b; Marta et al. 2011). Recently it is observed that QS and cell-to-cell signalling amid bacteria in biofilms can also negate the use of cleaning chemicals. Signalling system in bacteria helps observe neighbouring area and alter their gene expression accordingly to get more resistance. By inhibiting the QS contribution in the production of EPS can be used in reverse direction to control biofilm growth. Following the application of quenchers that avert QS, biofilms were found more readily removed with bactericidal chemicals (Anand et al. 2014).

15.6 Quorum Sensing in Food Processing Spoilage

15.6.1 Food Spoilage

Food processing locations grant a variety of favourable conditions for microbial contamination as they contain moisture, nutrients and microorganisms from their raw materials. Inadequate disinfection of food processing instruments and surfaces causes the survival of foodborne pathogens. This surviving pathogenic microbe in

due course results in biofilms formation. Dairy, brewing, poultry and meat processing are main places of known problematic sectors with contamination (Frank et al. 2003; Jessen and Lammert 2003; Somers and Wong 2004; Chen et al. 2007). Food processing industries are generally base for common foodborne pathogens, viz. *Yersinia enterocolitica*, *Campylobacter*, *Listeria*, *Salmonella* and *Staphylococcus aureus* worldwide and spread diseases to people who consume inappropriately cooked/contaminated products (Fig. 15.5) (Farber and Peterkin 1991; Dewanti and Wong 1995; Kim et al. 2008). Food spoilage is a complex process and known to occur with the help of several chitinolytic, lipolytic, proteolytic and pectinolytic biochemical reactions. The identification of QS signals in spoiled food has provided a new facet in understanding the food spoilage process. Later it was clearly evident that QS regulated also food deteriorations thereof. Many types of signalling molecules were detected in several types of spoiled food products. Ragaert et al. (2007) showed the controlling mechanism of human infecting and food spoilage microbial gene expression by disrupting the QS circuit. Variety of signalling compounds (AI-1, AI-2) were detected in milk, meat and vegetables (Rahman et al. 2017; Liu et al. 2006; Pinto et al. 2007). Another serious typhoid fever causing pathogen *Salmonella enterica* serovar Typhi (*S. Typhi*) is responsible for 217,000 deaths annually by infecting 21.7 million humans (Crump and Mintz 2010). It is known to form biofilms (Kalai Chelvam et al. 2014) and stick on food industry materials, viz. stainless steel, rubber and plastics (Steenackers et al. 2012). Maintaining hygiene conditions in manufacturing plant is highly required to remove microbes and to prevent colonization or persistence of microbes on processing lines. Different methods of mechanical, chemical and thermal processes are used to avert biofilm formation as professionally as possible.

15.6.2 Milk and Dairy Products

Milk and dairy products are very easily liable to bacterial spoilage. A broad range of bacteria resistant to high and low temperatures are present along the different milk processing stages. The processing locations and materials persisted with some type of bacterial species like *B. cereus* spores which stick on processing surfaces, help rapid adhesion of other bacterial cells on the processing line (Marchand et al. 2012). *Geobacillus*, well-known thermophilic bacilli, can grow at 65 °C, and their thermo-resistant spores pose problems for the milk powder production (Palmer et al. 2010). *Pseudomonas* are well-known food pathogen that survive and form biofilms at low temperatures on surfaces of milk cooling tanks and pipelines preceding to heat processing and often secrete thermo-resistant glycolytic, lipolytic, proteolytic and lecithinolytic enzymes that participate in milk spoilage (Marchand et al. 2009). Milk and other dairy products are experiencing many problems with Psychrotrophic bacteria as they can survive at low temperatures. Besides this, *Pseudomonas* biofilms have lived compatibly in multi-species biofilms with other pathogenic bacteria (e.g., *Listeria monocytogenes*) (Marchand et al. 2009). *L. monocytogenes* is a common contaminant in many dairy and other foods which can

survive and grow under a wide range of harsh conditions. *L. monocytogenes* was known to produce AI-2 and small peptides as signalling molecules by two QS systems, luxS and accessory gene regulator (agr). Spoilage of milk by microbes is because of the degradation of lipids and proteins present in milk by lipolytic and proteolytic enzymes, later on it was found that in *Serratia* spp., the secretion of extracellular enzymes is regulated by AHL-based QS system. Christensen et al. (2003) reported that inoculation of pasteurized milk with wild-type *S. proteamaculans* caused spoilage after 18 h of storage at 37 °C, whilst inoculation with a mutant containing an inactivated *sprI* gene did not cause spoilage. Nevertheless, adding up of 3-oxo-C6-HSL to milk inoculated with *sprI* mutant caused its spoilage, entailing the role of AI molecules in spoilage. Correspondingly, QS play an important role in spoilage of milk and dairy products by psychrotrophic bacteria *Pseudomonas* spp., *Serratia* spp., *Enterobacter* spp. and *H. alvei* (Whitfield et al. 2000; Pinto et al. 2007). Moreover, the detection of furanosyl BAI-2 signals in significant amounts in regular milk containing a low bacterial population (10^2 CFU/mL) implies potential association of interspecies communication in milk spoilage (Lu et al. 2004). It was recently found that *B. subtilis* utilized *luxS*-mediated QS for spoilage of dairy products (Gopal et al. 2015; Duanis-Assaf et al. 2015).

15.6.3 Meat Processing

Meat processing is one of the important segments. India has immense potential for production of processed meat and exports. Being highly nutritious, it is easily susceptible to microbial pathogen attacks. Food pathogens such as *C. jejuni* and *Pseudomonas* spp. are common in meat and meat products spoilages even at low temperature conditions (3–8 °C). Additionally, other *Salmonella* serovar sable and *S. typhimurium* are present in chicken and meat products spoilage (Jackson et al. 2013). There are reports on QS role in fresh meat products spoilage stored under refrigerated conditions and slime contaminated meat surfaces (Jay et al. in 2003). Spoilage of frozen ground beef and chicken by *Pseudomonadaceae* and *Enterobacteriaceae* bacteria was already reported. In the spoiled samples AHL signals, such as C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL and C12-HSL, have been noticed (Liu et al. 2006). Lu et al. (2004) investigated the effect of food preservatives such as sodium propionate, sodium benzoate, sodium acetate and sodium nitrate on QS and its signal molecules production. Nychas et al. (2003) found the presence of QS signals at low temperature (5 °C) stored cell-free spoiled minced pork meat. Supplementation of spoiled cell-free meat extract with QS signal molecules to *P. fluorescens* and *S. marcescens* augmented the duration of lag phase in *P. fluorescens* but not in *S. marcescens*. In the same experiment when compared to control samples it was also found that supplementation increased the metabolic activity of both strains. It was found that enhance in metabolic activity is linked to the presence of functional compounds in cell-free meat extract, including QS signal molecules (Nychas et al. 2003).

15.6.4 Fish and Seafood Products

Though the concentration of easy digesting compounds (glucose, lactate, free amino acids) in fish is less than that found in meat, the spoilage process is similar (Nychas et al. 2007). As other food products spoilage of fish is linked with the presence of single/multiple specific spoilage organisms (SSOs) (Gram et al. 2002). *S. putrefaciens* and *Pseudomonas* spp. were detected in iced marine and freshwater fish, respectively, and at the same time *Pseudomonas* spp. and *Shewanella* spp. were detected in fish obtained from the Mediterranean Sea (Gram and Huss 1996; Koutsoumanis and Nychas 1999). Vacuum-packaged cold smoked salmon is known to spoil by *Enterobacteriaceae* and LAB, *Carnobacterium* sp., and/or *Lactobacillus* sp. AHLs were detected in a various spoiled commercial fish products, such as cold-smoked salmon, fish fillets, and minced fish (Gram et al. 1999, 2002). However, researchers have not been able to understand QS systems role in spoilage of cold-smoked salmon.

The research team led by Gram and colleagues recently reported the production of AHLs (mainly 3-hydroxy-C8-HSL) by nonbioluminescent *P. phosphoreum* and *Aeromonas* spp. strains isolated from cultivable spoilage flora of packed cod fillets. The results of the study suggest a possible role of an AHL-based system in the regulation of chitinase activity, which enhances degradation of crustaceans (Flodgaard et al. 2005). In addition, a broad range of AHLs (3-oxo-C6-HSL, C6-HSL, C8-HSL and C12-HSL) have been detected in *Enterobacteriaceae* (mainly *H. alvei* and *S. liquefaciens*) and *Pseudomonadaceae* (mainly *P. fluorescens* and *P. putida*) constituting spoilage flora of rainbow trout fillets and appeared concomitantly with significant proteolytic activity. The activities of several exoenzymes (chitinase, lipase, protease) in *S. proteamaculans* B5a are affected by 3-oxo-C6-HSL and also suggested a QS-based regulation of food spoilage by this strain.

15.6.5 Fruits and Vegetables

Fruits and vegetables were commonly spoiled by *Erwinia* and *Pseudomonas* with the help of their pectinolytic enzymes (pectin lyases, pectate lyase, polygalacturonase, pectin methyl esterases). In these organisms it is illustrious that production of enzymes is inhibited by a broad range of AHLs (mainly 3-oxo-C6-HSL, C6-HSL). Furthermore, it is observed that inoculation of bean sprouts with AHL producing pectinolytic *P. carotovorum* improved spoilage rates (Rasch et al. 2005). The pectinolytic activity of *Pseudomonadaceae* and *Enterobacteriaceae* (mostly *Erwinia* spp.) high cell densities (10^8 to 10^9 CFU g/L) cause enzymatic browning, off-tastes, off-odours and/or texture breakdown. It is suggested that the rot disease is regulated by a QS mechanism because in *E. carotovora* pectolytic activity is under control of AHLs. *S. marcescens* and *S. liquefaciens* secretion of numerous unconnected and potentially food quality-relevant proteins such as lipase LipA, metalloprotease PrtA and surface-layer protein (S-layer) SlaA (Riedel et al. 2006) is guided by QS. A LuxI homolog containing *S. plymouthica* RVH1 was isolated from a raw vegetable

processing line. It was found that production of 3 AHLs, C4-HSL, C6-HSL and 3-oxo-C6-HSL regulated by SplI and absolute loss of 3-oxo-C6-HSL production and decrease in C4-HSL and C6-HSL production was observed by inactivation of AHLs. SplI-dependent QS is participated in synthesis of extracellular nuclease, chitinase, protease and an antibacterial compound 2,3-butanediol (van Houdt et al. 2006). By specific mutations in *splI*, production of exoenzymes in RVH1 strain was reduced once again rectified by addition of C6-HSL or 3-oxo-C6-HSL (van Houdt et al. 2007).

15.7 Quorum Sensing in Probiotics

Probiotics are viable bacteria, when administered in sufficient quantity they provide health benefit to the host. QS is known to attribute the beneficial effects of probiotics. It inhibits the onset of pathogenic bacteria's virulence by interfering with the signalling system. Gut microbiota release a variety of soluble small molecules of diverse chemical nature (surface and exogenous proteins, nucleases, lectins, peptides, amines, bacteriocins, fatty and amino acids, lactones, furanones, etc.). With the help of sensing molecules microbes' recognize surroundings and interact with equivalent cell surface, membrane, cytoplasm and nucleic acid receptors, to respond quickly and coordinatingly by stimulation of particular group of genes for the sustainability of host genome and microbiome. Universal method of intestinal bacterium and eukaryotic cells 'cross-talk' can be summarized as follows:

1. Bacteria release quorum-sensing molecule into intestinal lumen and are intact on the intestinal epithelium.
2. Bacteria and the eukaryotic cell interactions occur through direct contact. Eukaryotic cell biochemical pathways are specifically altered by modulins produced by bacteria.
3. Glycosylation modifications of functional molecules show an effect on the gut microbiota and help to fight against pathogens or viral infections (Freitas et al. 2003).

It also confirmed the reliance of several gut properties on the cross-talk between multiple species (Lopez Boado et al. 2001). Probiotic *L. acidophilus* La-5 produces lactacin B when it senses live bacteria and bacteriocin expression is inhibited by an auto-induction mechanism involving the secreted peptide IP_1800 (Tabasco et al. 2009a). Moslehi-Jenabian et al. (2009) observed luxS gene's role in protecting the probiotic lactobacilli from acidic stress response and their survival in gut. A recent report describes a bacterium isolated from gut intestine of fish based on QS which has probiotic characteristics by effectively reducing the quantity of AHLs and proteases activity of pathogen *A. hydrophila* (Chu et al. 2011).

From the above investigations, it is understandable that AHL-mediated QS systems in Gram-negative bacteria are comprehensively linked with food contamination. Food spoilage microbes influenced by QS along with their QS molecules are

Table 15.3 Food contaminating bacteria influenced by quorum-sensing-regulated phenotypes

Organism	Food product	Signal dependent phenotype	Signalling molecules
Pseudomonas fluorescens	Milk	Proteolytic milk spoilage	395 C4-HSL and 3OC8-HSL
Serratia proteamaculans strainB5a	Milk	Lipolytic and proteolytic milk spoilage	3-Oxo-C6- HSL
Pseudomonas fluorescens	Milk	Proteolytic milk spoilage	L-HSL α-amino-γ - butyrolactones
Pseudomonas phosphoreum and *Aeromonas* spp.	Cod fillets	Chitinolytic activity	3-Hydroxy-C8-HSL
Erwinia carotovora	Vegetables	Cellulolytic and proteolytic spoilage	3-Oxo-C6- HSL
Pectobacterium sp. A2JM	Bean sprouts	Pectinolytic and proteolytic spoilage	3-Oxo-C6- HSL
Serratia plymuthica RVH1	Vegetables	Chitinase and protease activity	3-Oxo-C6- HSL and C6-HSL
Hafnia alvei and *Serratia* spp.	Vacuum packed meat	Proteolytic spoilage	N-3-oxohexanoyl HSL
Pseudomonas spp.	Meat	Biofilm formation and proteolytic spoilage	AHLs
Photobacterium phosphoreum and *Aeromonas* spp.	Cod fillets	Chitinolytic spoilage	3-Hydroxy-C8-HSL

listed in Table 15.3. However, information is scanty on AI-2 and no information on AIPs produced by Gram-positive bacteria in food spoilage. In addition, occurrence of QS signalling compounds in pasteurized/refrigerated milk, meat and fish products, vacuum-packaged and under modified atmosphere tells us that the present-day preservation methods are inadequate. Understanding the types of spoilage organisms and their QS systems in different kinds of food products shall provide attractive ways to develop QSIs that can be utilized as advanced food preservatives.

In 2010, 5262 foodborne outbreaks were reported in the European Union and caused 25 deaths along with a huge number of human infections and hospitalizations (European Food Safety Authority EFSA 2012). *Salmonella*, *Campylobacter* and viral toxins were detected in the majority of epidemic infections that occurred in 2010. Further, it also noticed that *L. monocytogenes* and *E. coli* are in the list of major foodborne pathogens responsible for severe human infections and financial losses. Bacteria mentioned above are all capable to form biofilms on food processing equipment and food surfaces.

15.8 Quorum-Sensing Inhibition in Food Preservation

Contribution of QS signalling systems in spoilage of a variety of foods suggests that one can utilize the inhibition and/or control of cell-to-cell communication as the potential methods of preventing or delaying food spoilage. Such type of inhibition is usually known as quorum quenching. In contrast to present-day antibiotics, QS inhibiting (QSI) molecules exclusively affect QS but not bacterial growth. But the antibiotics inhibit bacterial growth and finally kill them. Hence bacteria will not develop resistance to QSIs (Kalia and Purohit 2011; Kalia 2013). Different quorum-quenching strategies are the following: (i) inhibition of QS signal synthesis, (ii) QS signal degradation in extracellular environment, (iii) inhibition of QS signal detection by receptor blockage and (iv) disruption of efflux pumps; targeting these QS components are highlighted. All these approaches were extensively investigated and successful at the clinical level, but reports on their usage in food preservation are scarce. Among all QS systems inhibition, Gram-negative signals *N*-acyl-homoserine lactones (AHLs) have been mainly targeted. The blockage of AHL synthesis could be the best effective communication interception system among all AHL-based QSI systems. But only a few numbers of investigations were carried out in this area (Parsek et al. 1999; Pechere 2001). Perhaps, in various potential ways available, degradation of QS signal in the extracellular environment by enzymes was the most profoundly investigated strategy to date.

Many compounds that inhibit QS without disturbing growth of bacteria have been reported. Among them, *Delisea pulchra* (the red alga) halogenated natural furanones were first characterized molecules that have AHLs structural resemblance and inhibit the gene expression mediated by binding to LuxR homologue instead of AI (Manefield et al. 1999; Rice et al. 1999). These QSIs silence several AHL-regulated phenotypes such as luminescence and toxin production by *V. harveyi* (Manefield et al. 2000) and *C. violaceum* (Martinelli et al. 2004), bacterial resistance of *P. aeruginosa* biofilms to tobramycin and SDS (Hentzer et al. 2003), swarming of *S. liquefaciens* (Givskov et al. 1996), expression of virulence factors and antibiotic production by *E. carotovorum* (Manefield et al. 2001). The furnones are also effective in vivo models, for example, virulence of *P. aeruginosa* was decreased in a pulmonary mouse model of infection (Wu et al. 2004), and mortality with *V. anguillarum* in rainbow trout was also decreased (Rasch et al. 2004). In addition, this approach in combination with antibiotics may be an unconventional method in management of infectious diseases by multidrug-resistant pathogens such as *P. aeruginosa*, in which QS-regulated virulence mechanisms have already reported (Jimenez et al. 2012).

Since the discovery of anti-QS effect of red alga *D. pulchra*, many plant extracts have also been investigated for their QS inhibition (Manefield et al. 1999; Alvarez et al. 2012) (Table 15.4). In view of consumers demand in avoiding artificial food preservatives, supplementation of natural preservatives that inhibit QS and do not

Table 15.4 List of the various plant-based anti-quorum-sensing compounds

Plant name	Part/active principle	Assay organisms	Test characters	Reference
QSI activity by dietary plants				
Medicago truncatula Gaertn.	Seedlings	*Escherichia coli* JM109 [p(SB536)], *Chromobacterium violaceum* CV026, *Escherichia coli* JM109 [p(SB401)]		Kalia (2013)
Lotus corniculatus L.	Seedlings	*C. violaceum* CV026, *Agrobacterium tumefaciens* NTL4	Violacein pigment, “-galactosidase activity	Kalia (2013)
Pisum sativum L. (pea)	Seedlings	*C. violaceum, Pseudomonas aeruginosa PA01*	Violacein pigment, swarming motility	Fatima et al.
Moringa oleifera Lam.	Leaf and fruit (aqueous extract)	*C. violaceum* 12,472	Violacein pigment	Koh et al. (2013)
Phaseolus vulgaris L. (bean)	AHL-mimic QS molecules	*Sinorhizobium fredii* SMH12, *Pantoea ananatis* AMG501	Biofilm formation	Montano et al. (2015)
Allium cepa L. (onion)	Pantolactone and myristic acid	*P. aeruginosa*	Virulence factors	Abd-Alla and Bashandy (2012)
QSI activity by fruits				
Rubus idaeus L. (raspberry)		*C. violaceum* CV026	Violacein production	Kalia (2013)
Vitis sp. (grape)		Furocoumarins *E. coli* O157:H7, *Salmonella typhimurium, C. violaceum*	Biofilm formation, violacein production	Kalia (2013)
Vaccinium angustifolium Aiton (blueberry)		*Vibrio harveyi*	Autoinducer bioassay	Kalia (2013)
QSI activity by plants used as spices and medicinal plants				
Curcuma longa L. (turmeric)	Curcumin	*P. aeruginosa* PA01	Virulence factor expression	Rudrappa et al. (2008), Packiavathy et al. (2013)
Allium sativum L. (garlic)	Ajoene	*P. aeruginosa*	QS-controlled virulence factors	Jakobsen et al. (2012)
Vanilla planifolia Jacks. ex Andrews (vanilla)	Aqueous methanolic extract of beans	*C. violaceum*	Violacein	Kalia (2013)

(continued)

Table 15.4 (continued)

Plant name	Part/active principle	Assay organisms	Test characters	Reference
Terminalia catappa L.	Tannin-rich fraction	*P. aeruginosa* PA01 *and C. violaceum*	Violacein and carbapenem antibiotic production	Jakobsen et al. (2012)
Syzygium cumini (L.) Skeels. *Pimenta dioica* (L.) Merr.	Ethyl acetate fractions	*C. violaceum*	Violacein production	Vasavi et al.

have negative influence over the taste and odour of foods is one of the new strategies. Patulin and penicillic acid are *Penicillium* secondary metabolites and can enhance inhibition of biofilm formation of *P. aeruginosa.* Tobramycin treatment also reduces virulence of *P. aeruginosa* in a pulmonary mouse model of infection (Ramussen et al. 2005). Essential oils (Eos) have reported as effective anti-QS agents and EOs of tea tree, rosemary, ginger, rose, chamomile, eucalyptus, marjoram, clary sage, juniper and many others demonstrated for their intensive anti-QS effect (Szabo et al. 2010; Kerekes et al. 2013). EOs also known to have antibiofilm properties that formed by pathogens like *Salmonella*, *Listeria*, *Pseudomonas* and *Staphylococcus* (Morten et al. 2012).

Koh et al. (2013) studied a large number of plant metabolites for QS signal synthase activity and reported that it is very rare property for plants to have anti-signal synthase activity. Competitive and non-competitive metabolites can block binding of signal to its cognate receptor and inhibits signal processing. However, plants possess the ability to degrade the bacterial signalling molecules and this will block the bacteria virulence factors by disrupting their cell-to-cell communication systems (Koh et al. 2013). *Piper caucasanum* Bredemeyer, *P. brachypodon* Benth, *P. bogotense* essential oils and clove solvent extracts (*Syzygium aromaticum* (L.) Merrill & Perry) are largely used in food and flavour industries which have reported as anti-QS metabolites. Besides these, many other medicinal plant extracts/natural compounds which interfere with bacterial QS can possibly utilise them as food preservatives instead of present-day artificial preservatives.

Many microorganisms with enzymatic QSI activity have been identified. The broad range of bacterial enzymatic QSI activity advocated that disrupting of bacterial communication is one of significant strategies to confer a competitive advantage among populations. So far 3 main sets of AHL inactivating enzymes were recognized and classified according to AHL cleavage/modification mechanism:

1. AHL lactonases: Belongs to metalloprotein enzyme group and hydrolyse the ester bond of homoserine lactone ring (HSL) and produces respective acyl-homoserines (Dong et al. 2000, 2012) (Fig. 15.1).

2. Acylases: These enzymes cleave the AHL amide bond generating equivalent free fatty acid and homoserine lactone ring (Leadbetter and Greenberg 2000; Lin et al. 2003) (Fig. 15.1).
3. AHL-inactivating enzymes: The third group are oxidoreductases; mainly deactivate signal molecules by oxidation or reduction of acyl chain of AHLs without any degradation of signal. These alterations in acyl chain loss the specificity and inhibit binding between signal and receptor.

15.9 Anti-Quorum-Sensing Molecules in Food Industry

Owing to knowledge on QS in the microbial ecosystem of food may aid in combating the microbial infections in food and food processing industries. Several food-based QS inhibitors (QSIs) as food preservatives have a novel food intervention strategy to ensure food safety and quality. Many studies have shown the potential of animal, plant organic extracts, essential oils rich in phenolic, flavonoid stilbenes, lignin compounds to interfere with QS in different bacteria. These compounds constitute a diverse group of chemical substances, with different chemical activities, also gained importance as potential inhibitors of QS system (Kumar et al. 2014). Ajoene, a sulphur-containing compound extracted from garlic, was found to control QS virulence such as that of rhamnolipid, a heat-stable haemolysin (Jakobsen et al. 2012). Phenolic compounds extracted from different natural sources reported to contain QSI molecules. A phenolic compound extracted from cider of apple, pyrogallol, present in tea acts as QSI in *Cronobacter sakazakii* (Fratianni et al. 2012) and *V. harveyi* (Ni et al. 2008), respectively. The polyphenol compound present in ginger, such as [6]-gingerol, [6]-shogaol and zingerone, exhibited strong QSI activities against pyocyanin and violacein production by *P. aeruginosa* and *C. violaceum*, respectively. An isoxazoline derivative of [6]-gingerol and [6]-azashogaol, a derivative of [6]-shogaol synthesized chemically, was also very effective against the QS activity of P. aeruginosa (Kumar et al. 2014). Different flavonoids such as taxifolin, kaempferol, naringenin, apigenin, baicalein and others have demonstrated their ability to interfere in the QS system of microorganisms such as *P. aeruginosa* PAO1 and *C. violaceum* CV026. Different types of quercetins such as quercetin aglycone, quercetin 4-glucoside, quercetin 3,4-O-diglucoside, quercetin 7,4-diglucoside, quercetin 3-glucosideglucoside and quercetin 5-glucoside are found in onion (Allium cepa Lineu). The anthocyanin cyanidin has also been identified in purple onion cultivars that give reddish or purple colouration to the bulbs. The amount of quercetin in onions varies according to the colour and type of bulb, being distributed mainly in the skins and outer rings. Quercetin, a flavonol present in high concentrations in onion (Allium cepa), presents anti-QS properties against some Gram-negative microorganisms (Quecan et al. 2019).

15.10 Conclusions and Future Perspectives

QS is one of the important advances that were made in the field of microbiology since three decades. Our understanding of bacterial communication has given more opportunities to handle them and opened several challenges. QS coordinates with the help of special microbial metabolites called AIs (AIs). Nowadays spoilage of food is playing a very important role in food safety and security which indeed is a complex process. It is mainly caused by intrinsic biochemical reactions and microbial actions. As a social activity microbial QS is involving in food spoilage besides their involvement in virulence and pathogenesis. AHL- and AI-2-based QS systems associated with Gram-negative bacteria in different food ecosystems have been investigated. Even though QS signalling molecules were detected in spoiled foods, the accurate action performed by them in the process of food spoilage is not clear. The structure and physicochemical attributes of foods by spatial and temporal heterogeneity of microbial community also should be taken into account. The spoilage occurs even if the food is preserved at very low temperatures and in anaerobic conditions means the present-day preservation tools are not adequate to avoid contaminations. Few microbial and plant metabolites obstruct the QS mechanism termed as quorum-sensing inhibitors (QSIs). It could be possible to enhance food safety and shelf life with usage of those molecules as food preservatives and pave way for application of novel food preservation techniques. Most studied QS system in food spoilage is AHL-regulated system which is present in Gram-negative foodborne pathogens. By controlling QS by its inhibition may prevent colonization of food surfaces, toxin formation and proliferation of food-related bacteria. The finding of QS inhibitory compounds from plants raises the possibility of identifying active QS inhibitory compounds from a plethora of natural sources. With the above-said characteristics, the plant-derived QS inhibitors may serve as next-generation ‘magic bullets’ in food preservation.

References

Abee T, Kovács ÁT, Kuipers OP, vander Veen S (2011) Biofilm formation and dispersal in Gram-positive bacteria. Curr Opin Biotechnol 22:172–179. https://doi.org/10.1016/j.copbio.2010.10.016

Abd-Alla MH, Bashandy SR (2012) Production of quorum sensing inhibitors in growing onion bulbs infected with Pseudomonas aeruginosa E (HQ324110). ISRN Microbiol 2012:1–7

Alvarez MV, Moreira MR, Ponce A (2012) Anti-QS and antimicrobial activity of natural agents with potential use in food. J Food Saf 32:379–387

Anand S, Singh D, Avadhanula M, Marka S (2014) Development and control of bacterial biofilms on dairy processing membranes. Compr Rev Food Sci Food Saf 13:18–33

Bassler BL, Losick R (2006) Bacterially speaking. Cell 125:237–246

Boles BR, Horswill AR (2008a) Agr-mediated dispersal of *Staphylococcus aureus* biofilms. PLoS Pathog 4:e1000052

Boles BR, Horswill AR (2008b) AGR-mediated dispersal of *Staphylococcus aureus* biofilms. Plos Pathog 4:4

Burnett SL, Beuchat LR (2001) Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. J Ind Microbiol Biotechnol 27(2):104–110
Chen J, Rossman ML, Pawar DM (2007) Attachment of enterohemorragic Escherichia coli to the surface of beef and a culture medium. LWT- Food Sci Technol 40:249–254
Christensen AB, Riedel K, Eberl L, Flodgaard LR, Molin S, Gram L, Givskov M (2003) QS-directed protein expression in *Serratia proteamaculans* B5a. Microbiology 149:471–483
Chu W, Lu F, Zhu W, Kang C (2011) Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. J Appl Microbiol 110(1):202–208
Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V (2006) The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci U S A 103:10420–10425. https://doi.org/10.1073/pnas.0604343103
Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745
Crump JA, Mintz ED (2010) Global trends in typhoid and paratyphoid. Clin Infect Dis 15 50(2):241–246. https://doi.org/10.1086/649541
Czajkowski R, Jafra S (2009) Quenching of acyl-homoserine lactone-dependent QS by enzymatic disruption of signal molecules. Acta Biochim Pol 56:1–16
Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298
Devine JH, Shadel GS, Baldwin TO (1989) Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744. Proc Natl Acad Sci 86:5688–5692
Dewanti R, Wong AC (1995) Influence of culture conditions on biofilm formation by Escherichia coli O157:H7. Int J Food Microbiol 26(2):147–164
Dhama K, Chakraborthy S, Mahima WMY (2013) Novel and emerging therapies safeguarding health of humans and their companion animals: a review. Pak J Bio Sci 16(3):101–111
Dong YH, Xu JL, Li XZ, Zhang LH (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci 97:3526–3531
Dong H, Rowland I, Yaqoob P (2012) Comparative effects of six probiotic strains on immune function in vitro. Br J Nutr 108(3):459–470
Duanis-Assaf D, Steinberg D, Chai Y, Shemesh M (2015) The LuxS based QS governs lactose induced biofilm formation by *Bacillussubtilis*. Front Microbiol 6:1517. https://doi.org/10.3389/fmicb.2015.01517
EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 10(6):2740
Farber JM, Peterkin PI (1991) Listeria monocytogenes, a food-borne pathogen. Microbiol Rev 55(3):476–511
Flodgaard LR, Dalgaard P, Andersen JB, Nielsen KF, Givskov M, Gram L (2005) Nonbioluminescent strains of *Photobacterium phosphoreum* produce the cell-to-cell communication signal N-(3-hydroxyoctanoyl)homoserine lactone. Appl Environ Microbiol 71:2113–2120
Franck AM (2001) Inulin and oligofructose. In: Gibson G, Angus F (eds) LFRA ingredient handbook: prebiotics and probiotics. Leatherhead Publishing, Surrey, pp 1–18
Frank JF, Ehlers J, Wicker L (2003) Removal of Listeria monocytogenes and poultry soil containing biofilms using chemical cleaning and sanitizing agents under static conditions. Food Prot Trends 23:654–663
Fratianni F, De Giulio A, Sada A, Nazzaro F (2012) Biochemical characteristics and biological properties of annurca apple cider. J Med Food 15(1):18–23
Freitas M, Tavan E, Cayuela C, Diop L, Sapin C, Trugnan G (2003) Host-pathogens cross-talk. Indigenous bacteria and probiotics also play the game. Biol Cell 95(8):503–506
Fuqua WC, Winans SC (1994) A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. J Bacteriol 176:2796–2806
Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone QS. Annu Rev Genet 35:439–468

Givskov M, de Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178:6618–6622

Gopal N, Hill C, Ross PR, Beresford TP, Fenelon MA, Cotter PD (2015) The prevalence and control of *Bacillus* and related spore- forming bacteria in the dairy industry. Front Microbiol 6:1418. https://doi.org/10.3389/fmicb.2015.01418

Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33:121–137

Gram L, Melchiorsen J, Spanggaard B, Huber I, Nielsen T (1999) Inhibition of Vibrio anguillarum by *Pseudomonas fluorescens* strain AH2 - a possible probiotic treatment of fish. Appl Environ Microbiol 65:969–973

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M (2002) Food spoilage—interactions between food spoilage bacteria. Int J Food Microbiol 78:79–97

Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Hoiby N, Givskov M (2003) Attenuation of *Pseudomonas aeruginosa* virulence by QS inhibitors. EMBO J 22:3803–3815

Hoang HH, Becker A, Gonzalez JE (2004) The LuxR homolog ExpR, in combination with the Sin QS system, plays a central role in *Sinorhizobium meliloti* gene expression. J Bacteriol 186:5460–5472

Jackson BR, Griffin PMG, Cole D, Walsh KA, Chai SJ (2013) Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. Emerg Infect Dis 19:1239–1244. https://doi.org/10.3201/eid1908.121511

Jakobsen TH, van Gennip M, Phipps RK, Shanmugham MS, Christensen LD, Alhede M et al (2012) Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrob Agents Chemother 56(5):2314–2325

Jessen B, Lammert L (2003) Biofilm and disinfection in meat processing plants. Int Biodeterior Biodegradation 51:265–269

Jimenez PN, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ (2012) The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. Microbiol Mol Biol Rev 76:46–65

Johnson MR, Montero CI, Conners SB, Shockley KR, Bridger SL, Kelly RM (2005) Population density-dependent regulation of exopolysaccharide formation in the hyperthermophilic bacterium *Thermotoga maritima*. Mol Microbiol 55:664–674

Kalai Chelvam K, Chai LC, Thong KL (2014) Variations in motility and biofilm formation of *Salmonella enterica* serovar Typhi. Gut Pathog 6:1–10. https://doi.org/10.1186/1757-4749-6-2

Kalia VC (2013) QS inhibitors: an overview. Biotechnol Adv 31(2):224–245

Kalia VC, Purohit HJ (2011) Quenching the QS system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

Kerekes E-B, Deak E, Tako M, Tserennadmid R, Petkovits T, Vagvolgyi C, Krisch J (2013) Anti-biofilm forming and anti-QS activity of selected essential oils and their main components on food-related micro-organisms. J Appl Microbiol 115:933–942

Khajanchi BK, Sha J, Kozlova EV, Erova TE, Suarez G, Sierra JC, Popov VL, Horneman AJ, Chopra AK (2009) N-Acylhomoserine lactones involved in QS control the type VI secretion system, biofilm formation, protease production, and *in vivo* virulence in a clinical isolate of *Aeromonas hydrophila*. Microbiol SGM 155:3518–3531

Khiyami MA, Pometto AL, Kennedy WJ (2006) Ligninolytic enzyme production by *Phanerochaete chrysosporium* in plastic composite support biofilm stirred tank bioreactors. J Agric Food Chem 54:1693–1698

Kim TJ, Young BM, Young GM (2008) Effect of flagellar mutations on Yersinia enterocolitica biofilm formation. Appl Environ Microbiol 74(17):5466–5474

Koh CL, Sam CK, Yin WF, Tan LY, Krishnan T, Chong YM et al (2013) Plant-derived natural products as sources of anti-QS compounds. Sensors (Basel) 13:6217–6228

Koutsoumanis K, Nychas G-JE (1999) Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7, and 10_C. Appl Environ Microbiol 65:698–706

Kumar NV, Murthy PS, Manjunatha JR, Bettadaiah BK (2014) Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. Food Chem 159:451–457

Leadbetter JR, Greenberg EP (2000) Metabolism of acylhomoserine lactone quorum-sensing signals by *Variovorax paradoxus*. J Bacteriol 182:6921–6926

Lin YH, Xu JL, Hu J, Wang LH, Ong SL, Leadbetter JR, Zhang LH (2003) Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. Mol Microbiol 47:849–860

Liu M, Gray JM, Griffiths MW (2006) Occurrence of proteolytic activity and *N*-acyl-homoserine lactone signals in the spoilage of aerobically chill-stored proteinaceous raw foods. J Food Prot 69:2729–2737

Liu M, Wang H, Griffiths MW (2007) Regulation of alkaline metalloprotease promoter by *N*-acyl homoserine lactone QS in *Pseudomonas fluorescens*. J Appl Microbiol 103:2174–2184

Llamas IE, Quesada IE, Martinez-Canovas MJ, Gronquist M, Eberhard A, Gonzalez JE (2005) QS in halophilic bacteria: detection of N-acyl-homoserine lactones in the exopolysaccharide-producing species of *Halomonas*. Extremophiles 9:333–341

Lopez-Boado YS, Wilson CL, Parks WC (2001) Regulation of matrilysin expression in airway epithelial cells by Pseudomonas aeruginosa flagellin. J Biol Chem 276:41417

Lu L, Hume ME, Pillai SD (2004) AI-2-like activity associated with foods and its interaction with food additives. J Food Protect 67:1457–1462

Lv J, Wang Y, Zhong C, Li Y, Hao W, Zhu J (2014) The effect of QS and extracellular proteins on the microbial attachment of aerobic granular activated sludge. Bioresour Technol 152:53–58

Ma M, Li J, McClane BA (2015) Structure-function analysis of peptide signaling in the *Clostridium perfringens* Agr-like quorum sensing system. J Bacteriol 197:1807–1818. https://doi.org/10.1128/JB.02614-14

Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg P, Kjelleberg S (1999) Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology 145:283–291

Manefield M, Harris L, Rice SA, de Nys R, Kjelleberg S (2000) Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. Appl Environ Microbiol 66:2079–2084

Manefield M, Welch M, Givskov M, Salmond GP, Kjelleberg S (2001) Halogenated furanones from the red alga, *Delisea pulchra*, inhibit carbapenem antibiotic synthesis and exoenzyme virulence factor production in the phytopathogen *Erwinia carotovora*. FEMS Microbiol Lett 205:131–138

Marchand S, Heylen K, Messens W, Coudijzer K, De Vos P, Dewettinck K, Herman L, De Block J, Heyndrickx M (2009) Seasonal influence on heat-resistant proteolytic capacity of *Pseudomonas lundensis* and *Pseudomonas fragi*, predominant milk spoilers isolated from Belgian raw milk samples. Environ Microbiol 11:467–482

Marchand S, Block JD, Jonghe VD, Coorevits A, Heyndrickx A, Herman L (2012) Biofilm formation in Milk production and processing environments; influence on Milk quality and safety. Comp Rev Food Sci Food Saf 11:133–147

Marta D, Wallin Carlquist N, Schelin J, Borch E, Radstrom P (2011) Extended staphylococcal enterotoxin D expression in ham products. Food Microbiol 28:617–620

Martinelli D, Grossmann G, Sequin U, Brandl H, Bachofen R (2004) Effects of natural and chemically synthesized furanones on QS in *Chromobacterium violaceum*. BMC Microbiol 4:25

Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM (2004) *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol Cell 15:677–687

Montano V DX, Foll M, Linz B, Reinhardt R, Suerbaum S, Jensen JD (2015) Worldwide population structure, longterm demography, and local adaptation of helicobacter pylori. Genetics 200:947–953

Moreira CG, Weinshenker D, Sperandio V (2010) QseC mediates *Salmonella enterica* serovar Typhimurium virulence in vitro and in vivo. Infect Immun 78:914–926. https://doi.org/10.1128/IAI.01038-09

Morten H, Tina M, Rikke LM (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:1–24

Moslehi-Jenabian S, Vogensen FK, Jespersen L (2009) The quorum sensing luxS gene is induced in Lactobacillus acidophilus NCFM in response to *Listeria monocytogenes*. Int J Food Microbiol 149(3):269–273

Nealson K, Platt H, Hastings JW (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 104:313–322

Ni N, Choudhary G, Li M, Wang B (2008) Pyrogallol and its analogs can antagonize bacterial quorum sensing in Vibrio harveyi. Bioorg Med Chem Lett 18(5):1567–1572

Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449

Nychas GJE, Tassou CC, SkandamisAntimicrobials from herbs P, Roller s SM (eds) (2003) Natural antimicrobials for the minimal processing of foods. CRC Press and Wood Head Publishers, New York, pp 176–200

Nychas G-JE, Douglas LM, Sofos JN (2007) Meat, poultry, and seafood. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC, pp 105–140

Otto M (2013) Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Annu Rev Med 64:175–188

Packiavathy I, Sasikumar P, Pandian S, Ravi AV (2013) Prevention of quorum-sensing-mediated biofilm development and virulence factors production in Vibrio spp. by curcumin. Appl Microbiol Biot 97:10177–10187

Paggi RA, Martone CB, Fuqua C, de Castro RE (2003) Detection of QS signals in the haloalkaliphilic archaeon *Natronococcus occultus*. FEMS Microbiol Lett 221:49–52

Palmer JS, Flint SH, Schmid J, Brooks JD (2010) The role of surface charge and hydrophobicity in the attachment of *Anoxybacillus flavithermus* isolated from milk powder. J Ind Microbiol Biotechnol 37:1111–1119. https://doi.org/10.1007/s10295-010-0758-x

Parsek MR, Greenberg EP (2005) Sociomicrobiology: the connections between QS and biofilms. Trends Microbiol 13:27–33

Parsek MR, Val DL, Hanzelka BL, Cronan JE, Greenberg EP (1999) Acyl homoserine-lactone quorumsensing signal generation. Proc Natl Acad Sci U S A 96(8):4360–4365

Pechere JC (2001) New perspectives on macrolide antibiotics. Int J Antimicrob Agents 1:93–97

Pinto UM, de Souza Viana E, Martins ML, Vanetti MCD (2007) Detection of acylated homoserine lactones in gram negative proteolytic psychrotrophic bacteria isolated from cooled raw milk. Food Control 18:1322–1327

Podbielski A, Kreikemeyer B (2004) Cell density-dependent regulation: basic principles and effects on the virulence of gram-positive cocci. Intl J Infect Dis 8:81–95

Quecan BXV, Santos JTC, Rivera MLC, Hassimotto NMA, Almeida FA, Pinto UM (2019) Effect of quercetin rich onion extracts on bacterial quorum sensing. Front Microbiol 10:87

Ragaert P, Devlieghere F, Debevere J (2007) Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biol Technol 44:185–194

Rahman MR, Lou Z, Zhang J, Yu F, Timilsena YP, Zhang C, Zhang Y, Bakry AM (2017) Star Anise (*Illicium verum* Hook. f.) as QS and biofilm formation inhibitor on foodborne bacteria: study in Milk. J Food Prot 80(4):645–653

Rasch M, Buch C, Austin B, Slierendrecht WJ, Ekmann KS, Larsen JL, Johansen C, Riedel K, Eberl L, Givskov M, Gram L (2004) An inhibitor of bacterial QS reduces mortalities caused by vibriosis in rainbow trout (*Oncorhynchus mykiss* Walbaum). Syst Appl Microbiol 27:350–359

Rasch M, Rasmussen TB, Larsen TO, Givskov M, Gram L (2005) Effect of QS inhibitors on food spoilage, 42–3 [abstract]. In: Institute of Food Technologists annual meeting. Institute of Food Technologists, Chicago

Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO, Hentzer M, Eberl L, Hoiby N, Givskov M (2005) Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. Microbiology 151:1325–1340

Rice SA, Givskov M, Steinberg P, Kjelleberg S (1999) Bacterial signals and antagonists: the interaction between bacteria and higher organisms. J Mol Microbiol Biotechnol 1:23–31

Riedel CU, Foata F, Goldstein DR, Blum S, Eikmanns BJ (2006) Interaction of bifidobacteria with Caco-2 cells-adhesion and impact on expression profiles. Int J Food Microbiol 110:62–68

Riedel CU, Monk IR, Casey PG, Waidmann MS, Gahan CG, Hill C (2009) AgrD-dependent QS affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. Mol Microbiol 71:1177–1189

Rivas M, Seeger M, Jedlicki E, Holmes DS (2007) Second acyl-homoserine lactone producing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 73:3225–3231

Rudrappa T, Biedrzycki ML, Bais HP (2008) Causes and consequences of plant-associated biofilms. FEMS Microbiol Ecol 64(2):153–166

Schauder S, Shokat K, Surette MG, Bassler BL (2001) The LuxS family of bacterial AIs: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 41:463–476

Shaw PD, Ping G, Daly SL, Cha C, Cronan JE, Rinehart KL, Farrand SK (1997) Detecting and characterizing *N*-acyl-homoserine lactone signal molecules by thin-layer chromatography. Proc Natl Acad Sci U S A 94:6036–6041

Sifri CD (2008) QS: bacteria talk sense. Healthcare Epidemiol 47:1070–1076

Smith JL, Fratamico PM, Novak JS (2004) QS: a primer for food microbiologists. J Food Protect 67:1053–1070

Somers EB, Wong AC (2004) Efficacy of two cleaning and sanitizing combinations on Listeria monocytogenes biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat-meat residue. J Food Prot 67:2218–2229

Sperandio V, Li CC, Kaper JB (2002) Quorum-sensing *Escherichia coli* regulator A: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. Infect Immun 70:3085–3093

Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB (2003) Bacteria–host communication: the language of hormones. Proc Natl Acad Sci U S A 100(15):8951–8956

Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SCJ (2012) *Salmonella* biofilms: an over view on occurrence, structure, regulation and eradication. Food ResInt 45:502–531. https://doi.org/10.1016/j.foodres.2011.01.038

Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209

Swift S, Lynch MJ, Fish L, Kirke DF, Tomas JM, Stewart G, Williams P (1999) QS-dependent regulation and blockade of exoprotease production in *Aeromonas hydrophila*. Infect Immun 67:5192–5199

Szabo MA, Varga GZ, Hohmann J, Schelz Z (2010) Inhibition of quorum-sensing signals by essential oils. Phytother Res 24:782–786

Tabasco R, García-Cayuela T, Peláez C, Requena T (2009a) *Lactobacillus acidophilus* La-5 increases lactacin B production when it senses live target bacteria. Int J Food Microbiol 132(2–3):109–116

Williams P (2007) QS, communication and cross-kingdom signaling in the bacterial world. Microbiology 153:3923–3938

Waters CM, Bassler BL (2005) QS: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Taga ME, Semmelhack JL, Bassler BL (2001) The LuxS-dependent AI AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol Microbiol 42:777–793

Walters M, Sircili MP, Sperandio V (2006) AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. J Bacteriol 188:5668–5681

Vidal JE, Shak JR, Canizalez-Roman A (2015) The CpAL quorum sensing system regulates production of hemolysins CPA and PFO to build *Clostridium perfringens* biofilms. Infect Immun 83:2430–2442. https://doi.org/10.1128/IAI.00240-15

Whitfield FB, Jensen N, Shaw KJ (2000) Role of *Yersinia intermedia* and *Pseudomonas putida* in the development of a fruity off-flavour in pasteurized milk. J Dairy Res 67:561–569

Van Houdt R, Moons P, Aertsen A, Jansen A, Vanoirbeek K, Daykin M, Williams P, Michiels CW (2007) Characterization of a luxI/luxR-type QS system and *N*-acyl-homoserine lactone–dependent regulation of exo-enzyme and antibacterial component production in *Serratia plymuthica* RVH1. Res Microbiol 158:150–158

Van Houdt R, Moons P, Hueso Buj M, Michiels CW (2006) *N*-acyl-L-homoserine lactone QS controls butanediol fermentation in *Serratia plymuthica* RVH1 and *Serratia marcescens* MG1. J Bacteriol 188:4570–4572

Vert M, Doi Y, Hellwich K-H, Hess M, Hodge P, Kubisa P, Rinaudo M, Schue F (2012) Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). Pure Appl Chem 84:377–410

Vivas J, Padilla D, Real F, Bravo J, Grasso V, Aacosta F (2008) Influence of environmental conditions on biofilm formation by *Hafnia alvei* strains. Vet Microbiol 129:150–155

Tabasco R, García-Cayuela T, Peláez C, Requena T (2009b) *Lactobacillus acidophilus* La-5 increases lactacin B production when it senses live target bacteria. Int J Food Microbiol 132(2–3):109–116

Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Hoiby N (2004) Synthetic furanones inhibit quorumsensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. J Antimicrob Chemother 53:1054–1061

Zhu J, Winans SC (1999) Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. Proc Natl Acad Sci U S A 96:4832–4837

Methods for the Detection and Quantification of Quorum-Sensing Signals in Food Spoilage

16

A. M. V. N. Prathyusha, Harish Annavarapu, and Pallaval Veera Bramhachari

Abstract

Food spoilage is a complex process occurs due to microbial activities. Microorganisms synthesize several proteolytic, pectinolytic, lipolytic and saccharolytic enzymes whose activity is associated with deterioration of foods. The synthesis of these enzymes is controlled by quorum sensing (QS) signifying a possible role of cell-to-cell communication in spoilage of foods. A diverse variety of Gram-negative and Gram-positive bacterial species coordinate communal behaviour based on population density. Microbes communicate among them by producing the signalling molecules, Quorum-sensing molecules (QSM). This review focuses on QS molecules and techniques for their detection and quantification.

Keywords

Quorum-sensing molecules · Food spoilage · Quantitative analysis

16.1 Introduction

Food spoilage is a process, during which food becomes undesirable and uncomfortable for human consumption, which mainly occurs by the biological activity

All authors contributed equally

A. M. V. N. Prathyusha · P. V. Bramhachari (✉)
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
e-mail: veerabramha@gmail.com

H. Annavarapu
Department of Food and Nutritional Sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_16

of microbial community (Skandamis and Nychas 2012). Microorganisms produce several enzymes with proteolytic, saccharolytic, chitinolytic, pectinolytic and lipolytic activities which are associated with the degradation of foods (Ammor et al. 2008). Even after advancement in preservation procedures, excessive amounts of food spoilage strive due to foodborne pathogens, viz. *Bacillus* spp., *Salmonella* spp., *Pseudomonas* spp., *Campylobacter jejuni* and *Yersinia enterocolitica* (Gopu et al. 2015). High level of Quorum sensing (AI-2) activity was reported in frozen fish samples and also in tomato, cantaloupe, carrots, tofu and milk samples (Lu et al. 2004). The role of QS in raw and pasteurized milk spoilage have been determined by identifying AHLs produced by psychrotrophic *Pseudomonas* spp., *Serratia* spp., *Enterobacter* spp., and *Hafnia alvei* (Pinto et al. 2007). Several resaerch groups identified higher level of QS signaling molecules in refrigerated and vaccum packed food products.

Microorganisms use Quorum system (cell-to-cell) communication mediated by extracellular chemical signal that is generated by bacteria to detect exact cell densities. QS is a common phenomenon for bacteria to enable nutrients, adaptation to environmental niches, survival in hostile/growth-restrictive environments, to intensify defensive responses against competing organisms, and to optimize their ability to differentiate into other morphological forms. QS depends on secretion and detection of low molecular weight diffusible signals (auto-inducers) which are synthesized by its own species and other species of bacteria (Nazzaro et al. 2013). Gene expressions are controlled by the Quorum sensing in response to cell densities. When the concentration of auto-inducers reaches threshold level, QS system activates a contextually suitable genetic programme. Bacteria can modulate some complex activities through QS such as biofilm formation, sporulation, bioluminescence, bacitirocin production and virulence response. QS stimulates cooperative behaviour among bacterial populations and therefore develops environmental adaptability. There are three components necessary to modulate bacterial phenotypes for the cell-to-cell communication, i.e.:

- Fusion of chemical signals
- Build-up of the signals
- Recognition of the signal

QS mechanism is present in both +ve and –ve bacterial species. The Gram-positive bacteria utilize peptide derivatives as signalling molecule, while Gram-negative bacteria have fatty acid derivatives as signalling molecules (Bai and Ravishankar Rai 2011). In the development of root nodules symbiotic bacteria use for nitrogen fixation under QS regulatory systems in *Rhizobium* genera (Hoang et al. 2004). Notably, Role of QS in bacterial pathogenicity has been widely documented. This cell-to-cell communication is also involved in bacteria-mediated food spoilage. However, it will be beneficial to design approaches that specifically block these communication systems, which help in reduction or prevention of spoilage reactions.

QS signalling molecules are classified into four categories:

(i) Auto-inducer I (N-acylhomoserine lactones) – it is a fatty acid derivative used by Gram –ve bacteria primarily for interspecies communication.

(ii) Auto-inducer II (furanosyl borate diester) – synthesized by both Gram +ve and –ve bacteria used for both intra- and interspecies communication.
(iii) Auto-inducer III – present in *E. coli* which helps in cross-communication with mammalian epinephrine host cell signalling system.
(iv) Auto-inducer IV (AIPs) – amino acids and short peptides produced by Gram +ve bacteria. In addition, cyclic dipeptides, such as 2-heptyl-3-hydroxy-4-quinolone (PQS) and di-ketopiperazines (DKP), may be involved in cell-to-cell signalling.

Auto-inducers possess elevated biological and pharmacological effects on cells of higher organisms, signifying their function in communication with plant and animal cells. *Cis-11-methyl-2-dodecenoic* acid is an auto-inducer molecule involved in virulence regulation in both eukaryotes and prokaryotes (Henke and Bassler 2004).

16.2 Auto-Inducers

Gram-negative bacteria mainly contain a homoserine lactone ring having various acyl chains coupled with presence or absence of oxo and hydroxyl substitutions at C3 position. QS by AHL (AI-1) is mostly mediated by two proteins (LuxI/LuxR). QS response originates from LuxR-AHL response, and LuxI-AHL synthase is involved in regulating cell response in relation to cell density (Camilli and Bassler 2006). At high concentrations, AHLs interact directly and with the cognate LuxR-type protein which binds to a specific Lux-boxes promoter sequences affecting expression of QS target genes. Generally, LuxR-AHL complex positively controls LuxI AHL synthase gene, generating positive induction loop. Biosensors distinguish and identify a narrow range of AHLs, during the analysis of bacterium with low AHL production (Shaw et al. 1997).

AI-2 is a universal signal inducer thats exists in more than 70 species of both Gram +ve and Gram –ve bacteria (Vendeville et al. 2005). Methionine is a precursor molecule for synthesis of AI-2; which is converted into SAM (S-adenosyl methionine) by SAM synthetase (MetK). Donation of the methyl group of SAM to a variety of methyl acceptors results in SAH, which is recycled back to homocysteine in either a one-step or two-step process. A second enzyme Pfs (Methylthioadenosine/S-adenosylhomocysteine (MTA/SAH) nucleosidase) converts SAM to S-ribosyl-homocysteine (SRH) needed for AI-2 synthesis. The LuxS enzyme acts on S-ribosyl-homocysteine, which results in formation of homocysteine and 4,5-dihydroxy-2,3-pentanedione. 4,5-Dihydroxy-2,3-pentanedione thermodynamically undergoes a favourable cyclization to either *R* or *S* form of 2,4-dihydroxy-2-methyldihydro-3-furanone (DHMF) (Sun et al. 2004, Parveen and Comell 2011). It controls several phenotypic changes, virulence factors production in *V. harveyi*, protease production by *Porphyromonas gingivalis* (Burgess et al. 2002), ABC transporter in *Salmonella typhimurium*, bioluminescence of *Vibrio harveyi* (Bassler et al. 1994), iron acquisition in *Actinobacillus actinomycetemcomitans* (Fong et al. 2001), and

transcript number of genes in *E. coli* (Burgess et al. 2002). *However, only 2 classes of AI-2 specific receptors have been reported and well-characterized yet. Protein structures that act as AI-2 receptors were reported for LuxP family, found in Vibrio spp., and LsrB family, in S. Typhimurium; ribose binding protein (RbsB) family, from Aggregatibacter actinomycetemcomitans and H. influenza* (Pereira et al. 2013).

Auto-inducer-3 is the less studied QS system with structure and synthesis remaining unelucidated (Burgess et al. 2002). AI-3 signals are produced by a number of commensal bacteria such as pathogenic and non-pathogenic bacteria. Furthermore, AI-3 is very complex, probably co-exist with inter-species communiaction and also explains inter-genera interaction among prokaryote and eukaryote. AI-3 can be detected by two-component systems (QseC sensor kinase and QseB response regulator). It is pertinent that, presence of periplasmic AI-3, QseC phosphorylates QseB (autophosphorylation) and triggers expression of genes involved in flagella biosynthesis and motility through upregulation of master flagellar regulator genes (Clarke and Sperandio 2005).

In bacterial cell-to-cell signalling, AIPs are used by Gram +ve bacteria are first identified in *S. aureus* by prototypic Agr system. It is a known fact that bacteria produce various polypeptides that acts as auto-inducers and inhibit the growth of other organism (Dunny and Leonard 1997). These peptides are varying from 5 to 26 amino acid residues, small sized and highly stable, with specificity and diversity, and sometimes they are subjected to post-translational changes which enhance their stability and functionality (Skandamis and Nychas 2012). Two QS activation pathways that differ in signal perception mechanism have been reported for Gram-positive bacteria. The first signaling pathway transduces AIP via a two-component system (TCS) that uses transmembrane histidine kinase (HK) and transcriptional regulator (RR). At high cell density, AIP is detected and leads to autophosphorylation of HK where specific cytoplasmic transcriptional regulator (RR) gets phosphorylated by HK. This phosphorylation modulates the ability of regulator to activate or repress transcription of certain target genes. Yet another activation pathway involves re-importation of the AIP into intracellular medium using an oligopeptide Ami (Opp) permease transport system. Subsequently, AIP interacts directly with a cytoplasmic transcriptional regulator belonging to RNPP family (Rap, NprR, PlcR, and PrgX) and modulates expression of target genes (Kareb & Aïder 2019).

16.3 Methods for Detection of Quorum-Sensing Signals in Spoiled Food

Quorum-sensing molecules were in low concentration when compared to other microbial metabolites and require very advanced methods for their detection. Cell signalling molecules are detected and quantified directly by using cell-free supernatant or from food sample or by using spent cultures which are isolated from various food products (Ammor et al. 2008). Different methods are used for the identification and quantification of cells signalling molecules by using biosensors in olden days; nowadays signalling molecules are identified by using mass spectrometry (MS), liquid chromatography-MS (LC-MS), gas chromatography-MS (GC-MS), nuclear

magnetic resonance spectroscopy (NMR) (Ammor et al. 2008; Rambaugh 2011) and HPLC (high-performance liquid chromatography). Biosensors were used to detect different types of cell signalling molecules quickly and economically. (Cataldi et al. 2007). Different types of AHLs can be detected and quantified with the use of bacterial biosensors which are also easy, economical and faster, and available biosensors mainly sense either AI-1 or AI-2. Biosensors are capable in detection of AI(s) in bacteria, furthermore to screen QS inhibitors (Tello et al. 2013; Anbazhagan et al. 2012). The AHLs can be detected both on solid medium and in spent culture broth. Test strain and biosensor strains are cross-streaked close on suitable solid medium. AHL production by test strain will trigger the expression of reporter gene that encodes for phenotypic responses. The response shows a gradient nature up to the meeting point of the test strain and biosensor strains (Steindler and Venturi 2007). In case of culture broth after the exponential phase, the culture broth can be extracted using solvents, viz. chloroform, ethyl acetate or dichloromethane. The solvent extracts are separated by reverse-phase TLC and then inoculated onto biomonitor systems. Biosensing of exogenous AHL produces a spot, which is identified against AHL standards (McClean et al. 1997; Turovskiy et al. 2007). Highly sophisticated techniques like mass spectrometry and HPLC are now utilized for identification and screening of QS molecules (Rutherford and Bassler 2012).

16.3.1 T-Streak Plate Method

In this method, biosensor and test strains are streaked in T shape on agar plate. Not with standingly, if a biosensor strain produces response tester strain's AI by appearing in specific colour based on reporter gene present in biosensor., the *C. violaceum* CV026 shows a visible purple pigment violacein. Interestingly, the biosensor lacZ encodes enzyme "Beta-galactosidase, which breaks X-gal into a blue colour product, which can be visually detected. T streak is rapid, easy to do and does not require any instrument, but it reports only in presence or absence of AI in tester strain (McClean et al. 1997).

16.3.2 Chromatography Methods

AHLs were extracted from various homogenized food samples by using ethyl acetate and acidified with formic acid. Waste material should be removed and filtered through a Whatman filter paper (Rasch et al. 2005). Under nitrogen flow, filtrate is evaporated for dryness and redissolved in ethyl acetate, transferred to HPLC vial and stored for further analysis. AHL extracts were applied to a TLC plate (Ravn et al. 2001). This method is superior to T streak for the fact that it reported the size and type of AI present in tester strain and more sensitive. Supernatants are loaded on C18-reverse-phase TLC plate and separation is performed using organic solvents. Along with supernatant, AI standards are also loaded onto TLC plate for separation. The plates were developed by using methanol-Milli-Q water. AHLs were estimated by semi-quantitative well diffusion assay with *A. tumefaciens* as an

indicator organism and gentamicin as a standard (Shaw et al. 1997). Then organic solvents from plates are removed and an agarose suspension having a suitable biosensor strain was covered. Biosensor strain produces visible output if AI is present. It has been noted that a combination of specific AI and specific biosensor produces a specific type of visible spots on TLC plate, including circular and tear-shaped spots. Further, AI type is identified by mass spectrometry (Anbazhagan et al. 2012).

By using liquid chromatography and high-resolution mass spectrometry (LC–HR-MS), culture extracts were extracted from various foods by methanol-dichloromethane-ethyl acetate for 2 h and kept for evaporation (Rasch et al. 2005). The samples were collected and dissolved in water-methanol and applied to Strata-X SPE cartridges. The cartridges were washed and eluted with water-methanol containing less amounts of formic acid. The eluates were then evaporated in vacuum and re-dissolved in methanol-water. Samples were injected and analysed by LC-HR-MS (Bruhn et al. 2004).

DPD can be analysed by gas chromatography in a two-step derivatization process. AI-II components are transformed into DPD which is a single, stable, quinoxaline derivative. Firstly AI-II molecules can be transformed into qunoxiline derivatives via DPD (Thiel et al. 2009). Addition of 1,2-phenylenediamine directly to cultural supernatants led to the formation of 1-(3-methyl quinoxaline.2yl)-ethane-1-2diol; this is called as Millard reaction which takes nearly 30 min to complete. The resulting compound can be observed by NMR spectroscopy and pH will be changed by the addition of 1,2-phenylenediamine to the supernatant and adjust the pH to neutral by potassium phosphate buffer to regulate false-positive results. For quantitative derivatization, add buffer to the cultural supernatant, and to this add 1,2-phenylenediamine, and keep in room temperature for 2 hrs (Globisch et al. 2012).

To make polar quinoxaline, water should be removed from the cultural supernatants to get best results for GC-MS analysis. This residue was resuspended with dichloromethane, and it is extracted either by ethyl acetate or dichloromethane which led to an improvement (Meijler et al. 2004). To obtain best results liquid–liquid extraction is carried out with dichloromethane by simultaneous extraction of water and filtrating the insoluble matrix material (Lau et al. 2002).

In the second step of derivatization process, dichloromethane extract is treated with MSTFA (N-methyl-N-(trimethylsilyltrifluoroace-tamide). This is a strong and effective trimethylsilyl donor, furnishing the trimethylsilylated quinoxaline. The sialylation can be completed after 30 min at 60 °C. Finally, the resulting residue was injected to GC and analyse the difference between dichloromethane and their extracts (Thiel et al. 2009).

16.3.3 Calorimetric Assay

Calorimetric assays can be utilized for both qualitative and quantitative analyses of AHLs. This method is suitable for biosensors having lacZ as a reporter gene. Here, a biosensor is grown in extracts of tester strain, supplemented with ortho-nitrophenyl-β-galactoside (ONPG). Galactosidase enzyme (*lacZ* gene producT) breaks ONPG into galactose and orthonitrophenol a yellow product. Ortho-nitrophenol produced

is spectrophotometrically quantified by Miller assay using AHL standard curve. This method provides first hand information on concentration of autoinducers and any information of AHL(s) type that is present in extract of tester strain (Pearson et al. 1994).

16.3.4 Luminescence Assay

Luminescence method is suitable for biosensors by luminescence genes, luxCDABE, as a reporter. Luminometer is used to detect Luminescence. Liquid extracts of test strain along with biosensor strain are used. This method is used for qualitative information for unknown cultures. Luminescence method can also be used to quantitative estimation if AI is already known. This method is not useful to get the size and AI type information (Massai et al. 2011).

16.3.5 Fluorescence Assay

Techniques based on fluorescence property can be used for both detection and quantification of AIs, both at high-density population and single-cell levels. This method using a fluorimeter fluorescence microscope and cytometer is used for analysis. Measurement at single-cell levels is useful to get variability in response at the single-cell level. Highly sensitive florescence reporters, such as gfp and cfp, are used in biosensors to acquire correct results (Rai et al. 2012).

16.3.6 Detection by Biosensors

Bacterial biosensor is a genetically recombinant bacterium, principally including analogous sensing gene network topology:

1. A QS transcriptional activator (LuxR homologue) is expressed either from a constitutive or inducible promoter.
2. Expression of a reporter gene from cognate promoter of LuxR homologue.

Biosensing mechanism of AHL is depicted as follows: AHL binds to its transcriptional activator protein after reaching threshold level generates a readable output by binding to its cognate promoter. This detection and binding of transcriptional activator to AHL is very specific and depends on the length of AHL acyl side chain. A wide range of biosensors were deliberated with different sensitivity and specificity towards AHLs.

Burmølle et al. (2003) developed whole-cell biosensor for detecting AHL production. A green fluorescent protein (gfp) was fused to regulatory region of luxoperon from *V. fischeri* to form luxR-PluxI-gfpmut3-fusion, pAHL-GFP. ECMC4100 harbouring pAHL-GFP responded to AHLs (N-octanoyl homoserine lactone) by emitting green fluorescence. A number of cells expressing fluorescence were

counted using flow cytometry. The biosensor strains containing genes encoding GFP can be used in T-streak TLC assays, and epifluorescent microscope. The bioassay distinguishes only known compounds and warrants the use of several reporters for different kinds of AHL molecules. The reporter strains display a high specificity in the direction of cognate AHL and to a lesser extent to closely related ones. As a consequence, detection of a wide range of AHLs necessitates the use of several different biosensors, each corresponding to specific AHLs.

Together with above specific reporter strain a few are nonspecific reporter strains. The TraR of *A. tumefaciens*, sensitive to most 3-oxo-HSLs (Shaw et al. 1997) and *V. fischeri* LuxR-based reporter plasmids such as pSB403, activated by AHLs with carbon chains of C6 or C8 with or without 3-oxo substitutions (Winson et al. 1998) are examples of reporter strains lacking in specificity. Using these methods biosensors can guide to false-positive and false-negative reactions. Furthermore, screened bacterium produces non-AHL compounds that could perhaps interfere or turns on the response of biosensors (Holden et al. 1999). As different LuxR homologues have varied affinities with different AHLs, it is not precise to evaluate the intensity of a response attained with altered AHLs. Consequently, synthetic AHL should be employed to establish negligible amount of AHL required for response and amount essential for saturated response to plot linear dose-response curve (Steindler and Venturi 2007). It is notable that a high diversity among LuxI and LuxR homologue proteins was anticipated and hence could be a restraining aspect for development of molecular detection methods (Whitehead et al. 2001).

Detection of AI-2 molecules with chemical methods is complex owing to their unsteadiness; thus, bioassay mode is the only alternative. AI-2 are detected using biosensor *V. harveyi* BB170 luxN::Tn5 that synthesizes all 3 AIs. The growth and ability of biosensor to detect AI-2 is inclined by components of culture medium and additives in food sometimes leading to false-negative or false-positive results (Lu et al. 2004). Presently, quantification of BAI-2 is conceded by LuxP-fluorescence resonance energy transfer (FRET)-based reporter strain. The sensor is based on ligand binding-induced changes in FRET between a cyan variant and yellow variant of GFP fused to the termini of BAI-2 receptor, LuxP (Rajamani et al. 2007).

The AI-3 molecule is discovered by β-galactosidase assay using *E. coli* O157:H7 TEVS232 strain (Sperandio et al. 1999) in which LEE1 regulatory regions are fused to lacZ reporter gene in TE2680. TEVS232 is grown in fresh medium or in medium supplemented with *P. cepacia*. Cultures are diluted 1:10 in Z buffer, and β-galactosidase activity is measured by using ONPG as a substrate (Walters et al. 2006). The bioassays that are obtainable for AIPs identification are based on the application of nisin-inducible bioluminescence and engross reporter genes positioned below the regulator nisin-inducible promoter, either bacterial luciferase genes (lux) or GFP gene (gfp). This is exceptionally sensitive and can detect as low as 10 pg/mL nisin present in culture broth. Before measuring fluorescence, supernatant was separated as it absorbs light of GFP with identical wavelengths (Wahlström and Saris 1999; Immonen and Karp 2007) fluorescence (Hakovirta et al. 2006). 2-Alkyl-4-quinolones (AHQs) and 2-heptyl-4-quinolone of *Pseudomonas* spp. are detected and quantified using lux-based *P. aeruginosa* AHQ biosensor strain

(Table 16.1) (Fletcher et al. 2007). However, Occasionally auto-fluorescence of cellular background infer sensitivity. In this approach, the reporter strain is used either in TLC or microtitre plate assays, in which the bioluminescence or green colour of pyocyanin is sensed as endpoints. In the microtitre assay, bacterial extracts or CFS are added to a growth medium containing AHQ biosensor, and resulted light is proportional to the AHQ content of the sample.

Table 16.1 List of Quorum sensing detecting biosensors

AHL biosensors						
Plasmid-based biosensors						
Plasmid	***Host strain***	***Activator***	***Promoter***	***AHL detected***	***Reporter***	***Reference***
pAL105	*E. coli* JLD271	LasR	P*lasI*	3-Oxo-C12-AHL	*luxCDABE*	Lindsay and Ahmer 20,050 = [
pSB1075 3	*E. coli* JM109	LasR	P*lasI*	Oxo-C12-to 3-oxo-C16-AHL, C12-to C16-AHL,	*luxCDABE*	Savka et al. (2011)
pUCP18 P*rsaL*::*lux*	*P. aeruginosa* PA14	LasR	P*rsaL*	3-Oxo-C12-AHL	*luxCDABE*	Massai et al. (2011)
pMS402 P*rsaL*::*lux*	*P. aeruginosa* PA14	LasR	P*rsaL*	3-Oxo-C12-AHL	*luxCDABE*	Massai et al. (2011)
pREC-FF	*E. coli* MG1655 K12Z1	LuxR	P*luxI*	3-Oxo-C6-AHL	*cfp*	Rai et al. (2012)
pUCGMAT1–4	*E. coli*	AhlR	P*ahlI*	3-Oxo-C6-AHL	*mcherry*	Deng et al. (2015)
Chromosomally integrated biosensors						
Strain	***Activator***	***Promoter***	***AHL detected***	***Reporter***	***References***	
S. meliloti sinI::lacZ	SinR	P*sinI*	3-Oxo-C14-AHL, 3-oxo-C16-AHL, 3-oxo-C16:1 AHL	*lacZ*	Llamas et al. (2005)	
P. aeruginosa PA14-R3	LasR	P*rsaL*	3-Oxo-C12-AHL	*luxCDABE*	Massai et al. (2011)	
C. violaceum	CviR	P*vioA*	CV026 C4- to C8-AHL	*vioABCD*	McClean et al. (1997)	
P. aeruginosa M71LZ	LasR	P*lasI*	3-Oxo-C12-AHL	*lacZ*	Dong et al. (2005)	

16.4 Methods for Food Preservation

Food can be consumed by animal and humans in raw, processed, or formulated materials to promote growth, energy and to maintain good health. However, overconsumption of convinced foods such as fats, carbohydrates and salts affects public health. Chemically processed foods contain fat, proteins, carbohydrates and small quantities of minerals and organic compounds. Thus, it will promote growth of microbes since all these compounds are source energy for microbial growth. (Rahman 2007). However, Food quality retension is important to maintain high nutritional values of consumed and processed foods along with our health. Currently, various preservation methods are in use for long period storage either by using conventional or modern techniques during transportation (Devi et al. 2015).

16.4.1 Thermal Processing

Thermal processing is used to preserve fruits and vegetables for long time and also increase their shelf life and effectively decreasing the microbial population and pulverize the natural enzymes (Rosa 2006). This process is used for the preparation of jams, jellies and canned fruits and vegetables. In this process unsterile foods can be heated in containers such as canning. This method is also used in milk pasteurization process (Barrett and Lloyd 2012).

16.4.2 Drying

Drying is used to preserve food material for long time by controlling their moisture contents in foods. Food products can be dried by various techniques either by natural or artificial drying to reduce the microbial contamination (Devi et al. 2015). Natural drying in open surroundings and economical procedures and no need of energy consumption only uses sunlight. Artificial drying food material is dried using fuel, air dyers and brush dryers. This technique is usually used for meat and fish products and can be applied for fruits and vegetables (James 2003). Fruits and vegetable stored using drying are in lighter in weight; therefore, cost of delivery can be reduced. The moisture content of food is reduced to 10–15%, thus, microbial growth can be inhibited and become inactive (Rosa 2006, Sharif et al. 2017).

16.4.3 Pickling

It is a technique where foods are stored in common salts or vinegar. This method is in use from olden days to preserve vegetables and fruits. Pickles are generally consumed as appetizer that promotes the production gastric juice, which helps in digestion. Pickles are less in calories, and nutrient content depends on ingredient used for making them (Devi et al. 2015).

16.4.4 Freezing

One of the most cost-effective ways for preservation foods. Most foods contain enzymes that can destroy colour, flavour, texture, quality, and nutrients in foods during storage (Barrett and Lloyd 2012). Freezing also hinders microbial growth however, cannot kill them. Freezing is used for long-term storage of many foods because it can decrease the water level that will hinder microbial growth and activity, reduces rate of chemical reaction (Gambuteanu, Borda, and Alexe 2013). During freezing and defrosting process the tissue structure of the food will damage due to the rate and temperature applied and will led to the deterioration of its structure, texture, colour and taste (Seon Mi et al. 2015).

16.4.5 Edible Coating

Edible coating is an ecologically friendly innovation that has been applied on many food products to control moisture transfer, and oxidation process. This is another preservation method to preserve fruits and vegetable. Edible coatings with lipids, proteins and some other biopolymers will be applied on the surface of the food products to form a thin layer (Senna, Al-Shamrani, and Al-Arifi 2014). For maintaining the quality of postharvest fruits edible coatings are being used which replaced synthetic waxes to increase customer demand. Currently, edible coating formulations have been applied along with added food additives (Dhall 2013).

16.4.6 Food Additives

Food additives such as antimicrobial, antioxidant agents and stabilizer are used to preserve foods by lowering its redox potential and pH value. These additives also hinder the development of microbial growth in food products or avoid food spoilage (Inetianbor et al. 2015). Preservatives maintain its nutritional value and food consistency, protecting the food from microbial and to enhance its flavour. Food preservatives can be divided into two classes – natural preservatives and synthetic preservatives. Synthetic preservatives like sulphites, benzoates, and sorbates are in use in particular food products but can harm people on overconsumption (Sharif et al. 2017). Many non-traditional preservation techniques are being developed to fulfil customer demand for nutritious and healthy food. In recent years, natural antimicrobial agents that constrain fungal and bacterial growth have been of considerable interest for better quality and shelf-life (Nazir et al. 2018). A few potential antimicrobials from plant and animal origin can be used as food additives viz. Lactoferrin, Chitosan, Lysozyme, Nisin, etc. Some of milk-derived bioactive compounds such as casein and whey proteins also act as food additives.

(a) *Lactoferrin* – Milk contains an iron-binding compound Lactoferrin that possesses antimicrobial property and acts against bacteria and viruses (Hintz et al.

2015). In some meat products, lactoferrin can be applied as antimicrobial and attained approval in some countries. Lactoferrin shows antimicrobial activity against some foodborne pathogens like *E. coli*, *carnobacterium* and *L. monocytogens* (Juneja et al. 2012). However, in bifidobacteria, iron acts as a growth-promoting factor which binds to bacteria when pathogens show negative impact on their growth (Bezkorovainy 1989). Under less Fe^{+2} conditions, bacterium produces anti-microbial bifidogenic compounds which inhibit the growth of other microorganisms and improve its quality (Ahmed et al. 2013).

(b) *Chitosan* – Polycationic biopolymer occur in exoskeletons of arthropods and crustaceans (Tikhonov et al. 2006). Chitosan is insoluble at higher and neutral pH and acts as food preservative in some food products. Using Maillard reaction, water-soluble chitosan derivatives are reported to have antimicrobial activity against *B. cereus*, *L. monocytogenes*, *Shigella dysenteriae* and *E. coli*, and are promising industrial substitute for acid-soluble chitosan (Chung, Yeh, and Tsai 2011). Antimicrobial activity of chitosan can be against Gram +ve and –ve bacteria.

(c) *Antimicrobials from Plant Origin* **–** Plants contain many bioactive compounds (phenols, alkaloids and terpenes) which are used as antimicrobial agents. Plants synthesize secondary metabolites for protection from the pathogen of which polyphenols and phenols are the largely produced (Pandey and Kumar 2013). Oils which are extracted from plant seeds, leaves and flowers have volatile properties and also produce phytochemical compounds used as food preservatives. Essential oils with effective antimicrobial properties include flavonoids, linalool, thymol, citral, carvacrol, eugenol, saponins and terpenes. Several methods are used for the extraction like distillation, supercritical fluid extraction (Bassolé and Juliani 2012). Oils containing hydrophilic properties will react with the lipids at cell membrane of the microorganism and degrade the cell wall of the microorganism and increase the cell wall permeability and degrade the cytoplasmic membrane (Hintz et al. 2015). Cinnamon and clove oil contains cinnamonaldehyde, eugenol and linalool which are capable of stopping the development of microorganisms (Sharif et al. 2017). In olden days, herbs and spices are also used as antimicrobial agents for various purposes.

(d) *Antimicrobials from Microbial Origin*

Bacteria produce many active compounds to inhibit the growth of pathogenic microorganisms (Nazir et al. 2018). Gram-positive and Gram-negative bacterium contains bacteriocins which are proteinaceous antimicrobial compounds that contain heterologous subgroups of ribosomal synthesized antimicrobial peptides. Bacteriocins are cationic hydrophobic peptides which mostly target bacterial membrane (De Vugst and Vandamme 1994). Nisin (natural antimicrobial peptide) most

widely used bacteriocin in preservation of many food products, which is also approved by FDA. Nisin is effective at low pH and has an inadequate spectrum of activity which does not limit the growth of Gram-negative bacteria and fungi (Barnby-Smith 1992). Nisin is produced from modified fermentation milk and contains lactic acid bacterial and *Lactococcus lactic* strains. Nisin interacts with the phospholipids of cytoplasmic bacteria and distracting function of cell membrane and prevents the spore formation on outer surface, inhibiting the swelling process of germination (Nazir et al. 2018). It can also be used to preserve different food products and acts against various food pathogens and reduces the growth of microorganisms in beef, sausages and poultry (Henning et al. 1986). Nisin decreases the initial growth of *Listeria monocytogenes* and also suppresses subsequent growth in ready-to-eat (RTE) meat foods (Nassar and Farrag 1995).

16.5 Conclusion and Future Perspectives

Quorum sensing plays a prime role in regulating food spoilage and food borne infections. Thus proper and in depth understanding of QS system in food associated microbes is very much essential. Undeniably the biosensor technology has been involved in rapid isolation and detection of AHL in Gram –ve bacteria. There are drawbacks and one has to be careful in the interpretation of data obtained with AHL biosensors. Nevertheless, a broad spectrum reporter strain possesses the ability to detect diverse QS ranging from AHLs, AIs and AIP is highly needed. TLC is particularly involved in analysis of bacterial growth, quantification of amount of signal molecules which express more than one independent signalling system. There is increasing demand for food free of synthetic preservatives; hence it is essential to analyse and identify unconventional and safe methods in regulation of foodborne pathogens. The quantity of natural antimicrobials in foods remains limited principally because of side effects (undesirable flavour or aroma). Henceforth, research has to be focused on determination of optimum levels of natural antimicrobials usage in foods without unconventionally altering any sensory characteristics.

Acknowledgments Dr. Bramhachari and colleagues are grateful to Krishna University, Machilipatnam, for the extended support.

Conflict of Interest The authors declare that they have no competing interests.

References

Ahmed N, Singh J, Kour H, Gupta P (2013) Naturally occurring preservatives in food and their role in food preservation. Int J Pharm Biol Arch 4(1):22–30

Ammor MS, Michaelidis C, Nychas G-JE (2008) Insights into the role of quorum sensing in food spoilage. J Food Prot 71(7):1510–1525. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-71.7.1510

Anbazhagan D, Mansor M, Yan GO, Md Yusof MY, Hassan H, Sekaran SD (2012) Detection of QS signal molecules and identification of an AI synthase gene among biofilm forming clinical isolates of *Acinetobacter* spp. PLoS One 7(7):e36696

Bai AJ, Ravishankar Rai V (2011) Bacterial quorum sensing and food industry. Compr Rev Food Sci Food Saf 10(3):183–193

Barnby-Smith FM (1992) Bacteriocins: applications in food preservation. Trends Food Sci Technol 3:133–137

Barrett DM, Lloyd B (2012) Advanced preservation methods and nutrient retention in fruits and vegetables. J Sci Food Agric 92(1):7–22

Bassler BL, Wright M, Silverman MR (1994) Multiple signalling systems controlling expression of luminescence in Vibrio Harveyi: sequence and function of genes encoding a second sensory pathway. Mol Microbiol 13(2):273–286

Bassolé IHN, Juliani HR (2012) Essential oils in combination and their antimicrobial properties. Molecules 17(4):3989–4006

Bezkorovainy A (1989) Biochemistry and physiology of bifidobacteria. CRC Press, Boca Raton

Bruhn JB et al (2004) Presence of Acylated Homoserine lactones (AHLs) and AHL-producing bacteria in meat and potential role of AHL in spoilage of meat. Appl Environ Microbiol 70(7):4293–4302

Burgess NA et al (2002) LuxS-dependent quorum sensing in Porphyromonas Gingivalis modulates protease and Haemagglutinin activities but is not essential for virulence. Microbiology 148(3):763–772

Burmølle M, Hansen LH, Oregaard G, Sørensen SJ (2003) Presence of N-acyl homoserine lactones in soil detected by a whole-cell biosensor and flow cytometry. Microb Ecol 45(3):226–236

Camilli A, Bassler BL (2006) Bacterial small-molecule signaling pathways. Science 311(5764):1113–1116

Cataldi TRI, Bianco G, Palazzo L, Quaranta V (2007) Occurrence of N-acyl-l-homoserine lactones in extracts of some gram-negative bacteria evaluated by gas chromatography-mass spectrometry. Anal Biochem 361(2):226–235

Chung YC, Yeh JY, Tsai CF (2011) Antibacterial characteristics and activity of water-soluble chitosan derivatives prepared by the Maillard reaction. Molecules 16(10):8504–8514

Clarke MB, Sperandio V (2005) Transcriptional autoregulation by quorum sensing Escherichia Coli regulators B and C (QseBC) in Enterohaemorrhagic E. Coli (EHEC). Mol Microbiol 58(2):441–455

De Vugst L, Vandamme EJ (1994) Bacteriocins of lactic acid Bacteria: microbiology. Genetics and Applications Blackie Academics and Professional, London

Deng Y, Zhao Y, Padilla-Zakour O, Yang G (2015) Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. Ind Crop Prod 74:803–809

Devi MP, Bhowmick N, Bhanusree MR, Ghosh SK (2015) Preparation of value-added products through preservation. In Value addition of horticultural crops: recent trends and future directions. Springer, New Delhi, pp 13–41

Dhall RK (2013) Advances in edible coatings for fresh fruits and vegetables: a review. Crit Rev Food Sci Nutr 53(5):435–450

Dong YH, Zhang XF, Soo HML, Greenberg EP, Zhang LH (2005) The two-component response regulator PprB modulates quorum-sensing signal production and global gene expression in *Pseudomonas aeruginosa*. Mol Microbiol 56(5):1287–1301

Dunny GM, Leonard BAB (1997) Cell-cell communication in gram-positive bacteria. Microbiology 51:–527, 564

Fletcher MP, Diggle SP, Cámara M, Williams P (2007) Biosensor-based assays for PQS, HHQ and related 2-alkyl-4-quinolone quorum sensing signal molecules. Nat Protoc 2(5):1254

Fong K, Chung W, Lamont R (2001) Intra-and interspecies regulation of gene expression by actinobacillus actinomycetemcomitans LuxS. Infect Immun 69(12):7625–7634. http://iai.asm.org/cgi/content/abstract/69/12/7625

Gambuteanu C, Borda D, Alexe P (2013) The effect of freezing and thawing on technological properties of meat: review. J Agroaliment Process Technol 19(1):88–93

Globisch D, Lowery CA, McCague KC, Janda KD (2012) Uncharacterized 4,5-Dihydroxy-2,3-Pentanedione (DPD) molecules revealed through NMR spectroscopy: implications for a greater signaling diversity in bacterial species. Angew Chemie Int Ed 51(17):4204–4208

Gopu V, Meena CK, Shetty PH (2015) Quercetin influences quorum sensing in food borne bacteria: in-vitro and in-silico evidence. PLoS One 10(8):1–17

Hakovirta J, Reunanen J, Saris PEJ (2006) Bioassay for nisin in milk, processed cheese, salad dressings, canned tomatoes, and liquid egg products. Appl Environ Microbiol 72(2):1001–1005

Henke JM, Bassler BL (2004) Three parallel quorum-sensing systems regulate gene expression in Vibrio harveyi. J Bacteriol 186(20):6902–6914

Henning S, Metz R, Hammes WP (1986) Studies on the mode of action of nisin. Int J Food Microbiol 3(3):121–134

Hintz T, Matthews KK, Di R (2015) Review: the use of plant antimicrobial compounds for food preservation. Hindawi (BioMed Res Int) 2015:1–12

Hoang HH, Becker A, González JE, Gonza JE (2004) The LuxR homolog ExpR, in combination with the sin quorum sensing system, plays a central role in Sinorhizobium Meliloti gene expression. J Bacteriol 186(16):5460–5472

Holden MT, Sr C, de Nys R, Stead P, Bainton NJ, Hill PJ, Manefield M, Kumar N, Labbate M, England D, Rice S, Givskov M, Salmond GPC, Stewart GSAB, Bycroft BW, Kjellberg S, Williams P (1999) Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. Mol Microbiol 33:1254–1256

Immonen N, Karp M (2007) Bioluminescence-based bioassays for rapid detection of nisin in food. Biosens Bioelectron 22(9–10):1982–1987

Inetianbor JE, Yakubu JM, Ezeonu SC (2015) Effects of food additives and preservatives on man–a review. Asian J Sci Technol 6(2):1118–1135

James IF (2003) AD03E preservation of fruit and vegetables. Agromisa Foundation

Juneja VK, Dwivedi HP, Yan X (2012) Novel natural food antimicrobials. Annu Rev Food Sci Technol 3(1):381–403. http://www.annualreviews.org/doi/10.1146/annurev-food-022811-101241

Kareb O, Aïder M (2019) Quorum sensing circuits in the communicating mechanisms of Bacteria and its implication in the biosynthesis of Bacteriocins by lactic acid Bacteria: a review. In: Probiotics Antimicrobial Proteins, pp 1–13

Lingeng L, Hume ME, Pillai SD (2004) Autoinducer-2–like activity associated with foods and its interaction with food additives. J Food Prot 67(7):1457–1462

Llamas I, Quesada E, Martínez-Cánovas MJ, Gronquist M, Eberhard A, Gonzalez JE (2005) Quorum sensing in halophilic bacteria: detection of N-acyl-homoserine lactones in the exopolysaccharideproducing species of Halomonas. Extremophiles 9(4):333–341

Lu L, Hume ME, Pillai SD (2004) Autoinducer-2–like activity associated with foods and its interaction with food additives. J Food Prot 67(7):1457–1462

Massai F, Imperi F, Quattrucci S, Zennaro E, Visca P, Leoni L (2011) A multitask biosensor for micro-volumetric detection of N-3-oxo-dodecanoylhomoserine lactone QS signal. Biosens Bioelectron 26(8):3444–3449

Meijler MM, Hom LG, Kaufmann GF, McKenzie KM, Sun C, Moss JA et al (2004) Synthesis and biological validation of a ubiquitous quorum-sensing molecule. Angew Chem Int Ed 43(16):2106–2108

McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW, Stewart GS, Williams P (1997) QS and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology 143(Pt 12):3703–3711

Nassar A, Farrag SA (1995) Nisin as inactivator to Listeria Monocytogenes in broth and in ground beed. Assiut Vet Med J 32:198–198

Nazir F et al (2018) Natural antimicrobials for food preservation. Journal of Pharmacognosy and Phytochemistry JPP 6(66):2078–2082. http://www.phytojournal.com/archives/2017/vol6issue6/PartAC/6-6-335-952.pdf

Nazzaro F, Fratianni F, Coppola R (2013) Quorum sensing and phytochemicals. Int J Mol Sci 14(6):12607–12619

Pandey AK, Kumar S (2013) Perspective on plant products as antimicrobials agents: a review. Pharmacologia 4(7):469–480. http://pharmacologia.com/abstract.php?doi=pharmacologia.2013.469.480

Parveen N, Cornell KA (2011) Methylthioadenosine/S-adenosylhomocysteine nucleosidase, a critical enzyme for bacterial metabolism. Mol Microbiol 79(1):7–20

Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, Iglewski BH, Greenberg EP (1994) Structure of the AI required for expression of *Pseudomonas aeruginosa* virulence genes. Proc Natl Acad Sci U S A 91(1):197–201

Pereira CS, Thompson JA, Xavier KB (2013) AI-2-mediated signalling in bacteria. FEMS Microbiol Rev 37(2):156–181

Pinto UM, de Souza Viana E, Martins ML, Vanetti MCD (2007) Detection of acylated homoserine lactones in gram-negative proteolytic psychrotrophic bacteria isolated from cooled raw milk. Food Control 18(10):1322–1327

Rahman MS (2007) Food preservation: overview. In: Handbook of food preservation. CRC Press, pp 21–36

Rai N, Anand R, Ramkumar K, Sreenivasan V, Dabholkar S, Venkatesh KV, Thattai M (2012) Prediction by promoter logic in bacterial QS. PLoS Comput Biol 8(1):e1002361

Rajamani S, Zhu J, Pei D, Sayre R (2007) A LuxP-FRETbased reporter for the detection and quantification of AI-2 bacterial quorum-sensing signal compounds. Biochemistry 46:3990–3997

Rambaugh KP (2011) Qorum sensing: methods and protocols. Springer, New York/Dordrecht/Heidelberg/London

Rasch M et al (2005) Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. Appl Environ Microbiol 71(6):3321–3330

Ravn L et al (2001) Methods for detecting acylated homoserine lactones produced by gram-negative Bacteria and their application in studies of AHL-production kinetics. J Microbiol Methods 44(3):239–251

Rosa SR (2006) Postharvest management of fruit and vegetables in the Asia-Pacific, 1st edn. Tokyo, Asian Productivity Organization

Rutherford ST, Bassler BL (2012) Bacterial QS: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2(11)

Savka MA, Le PT, Burr TJ (2011) LasR receptor for detection of long-chain quorum-sensing signals: identification of N-acyl-homoserine lactones encoded by the avsI locus of agrobacterium vitis. Curr Microbiol 62(1):101–110

Senna MMH, Al-Shamrani KM, Al-Arifi AS (2014) Edible coating for shelf-life extension of fresh banana fruit based on gamma irradiated plasticized poly(vinyl alcohol)/carboxymethyl cellulose/tannin composites. Mater Sci Appl 05(06):395–415. http://www.scirp.org/journal/PaperDownload.aspx?DOI=10.4236/msa.2014.56045

Seon Mi Y et al (2015) Effects of various freezing and thawing techniques on pork quality in ready-to-eat meals. Afr J Food Sci 9(11):525–533. http://academicjournals.org/journal/AJFS/article-abstract/0DE7A8D55979

Sharif ZIM et al (2017) Review on methods for preservation and natural preservatives for extending the food longevity. Chem Eng Res Bull 19(September):145. https://www.banglajol.info/index.php/CERB/article/view/33809

Shaw PD et al (1997) Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. Proc Natl Acad Sci 94(12):6036–6041. http://www.pnas.org/cgi/doi/10.1073/pnas.94.12.6036

Skandamis PN, Nychas GJE (2012) Quorum sensing in the context of food microbiology. Appl Environ Microbiol 78(16):5473–5482

Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB (1999) Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. Proc Natl Acad Sci 96(26):15196–15201

Steindler L, Venturi V (2007) Detection of quorumsensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266(1):1–9

Sun J, Daniel R, Wagner-Döbler I, Zeng AP (2004) Is autoinducer-2 a universal signal for interspecies communication: a comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. BMC Evol Biol 4:1–11

Tello E, Castellanos L, Duque C (2013) Synthesis of cembranoid analogues and evaluation of their potential as QS inhibitors. Bioorg Med Chem 21(1):242–256

Thiel V et al (2009) Identification, quantification, and determination of the absolute configuration of the bacterial quorum-sensing signal autoinducer-2 by gas chromatography-mass spectrometry. Chembiochem 10(3):479–485

Tikhonov VE et al (2006) Bactericidal and antifungal activities of a low molecular weight chitosan and its N−/2(3)-(Dodec-2-Enyl)Succinoyl/−derivatives. Carbohydr Polym 64(1):66–72

Turovskiy Y, Kashtanov D, Paskhover B, Chikindas ML (2007) QS: fact, fiction, and everything in between. Adv Appl Microbiol 62:191–234

Vendeville A et al (2005) Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. Nat Rev Microbiol 3(5):383–396

Wahlström G, Saris PEJ (1999) A nisin bioassay based on bioluminescence. Appl Environ Microbiol 65(8):3742–3745

Walters M, Sircili MP, Sperandio V (2006) AI-3 synthesis is not dependent on luxS in *Escherichia coli*. J Bacteriol 188(16):5668–5681

Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP (2001) Quorum-sensing in gram-negative bacteria. FEMS Microbiol Rev 25:365–404

Winson MK, Swift S, Fish L, Throup JP, Jorgensen F, Chhabra SR, Bycroft BW, Williams P, Stewart GS (1998) Construction and analysis of luxCDABE-based plasmid sensors for investigating *N*-acyl homoserine lactone–mediated QS. FEMS Microbiol Lett 163:185–192

17 Novel Perspectives on the Quorum Sensing Inhibitors (QSIs)/Quorum Quenchers (QQs) in Food Preservation and Spoilage

Saqib Hassan, Tanveer Ahmad, Mudasir Bashir, G. Seghal Kiran, and Joseph Selvin

Abstract

Quorum sensing (QS) regulates major bacterial behaviors such as virulence, antibiotic resistance biofilm formation, and bioluminescence when the population reaches high density. The role of QS in biofilm formation and virulence is an extreme problem for food safety, biofilm-related infectious diseases, etc. Food spoilage is a consequence of degrading enzymatic activities, viz., proteolytic, lipolytic, chitinolytic, and pectinolytic, of some food-associated bacteria. Several activities associated with the deterioration of goods are regulated by QS, suggesting a potential role of such cell-to-cell communication in food spoilage. Therefore, interrupting QS mechanism might be an alternative strategy to develop novel QS-based antibacterial/anti-biofilm drugs. QS-based antibacterial/anti-biofilm agents can be used to manage foodborne pathogens and biofilm formation in food industries. Efforts to disrupt biofilms have enabled the identification of bioactive molecules produced by prokaryotes and eukaryotes. Production and bioactivity of mushroom polysaccharide was enhanced by using microbial QS

S. Hassan
Department of Medicine, Imperial College London, London, UK

T. Ahmad
Department of Biotechnology, School of Life Sciences, Pondicherry University, Puducherry, India

M. Bashir · G. S. Kiran
Department of Food Science and Technology, School of Life Sciences, Pondicherry University, Puducherry, India

J. Selvin (✉)
Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India
e-mail: josephselvinss@gmail.com

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_17

molecules. Plant food extracts and phytochemicals were found to have anti-QS properties. Organic acids can act as effective potential sanitizers in reducing microbial load associated with fresh fruits and vegetables. Moreover, organic acids are known to be used as food preservatives due to their antimicrobial potential. Most approaches to use QQ as an anti-virulence strategy are still in initial phase; the increasing number of organisms and enzymes known to interfere with QS opens new perspectives for the development of innovative antibacterial strategies to prevent food spoilage.

Keywords
Quorum · Enzymes · Food · Biofilms · Pathogenicity

17.1 Introduction

Quorum sensing (QS) is a cell-to-cell communication system in bacteria directed by production and secretion of small signaling molecules known as autoinducers (AIs). AIs accumulate in extracellular environment and increase their concentration as a function of cell density (Romero et al. 2011). QS assists bacteria in communication, regulating gene expression, production of virulence factors, synchronizing phenotypic expression, motility, biofilm development, and bioluminescence (Tang and Zhang 2014; Kiran et al. 2017). Formerly, bacteria were assumed to survive rather solitary life; however, with advancements in research, it is revealed that bacteria possess complex chemical signaling system to communicate among intraspecies and interspecies. QS is ubiquitous in many human and plant bacterial species as well as in extremophiles, viz., *Natronococcus occultus*, *Thermotoga maritima*, *Acidithiobacillus ferrooxidans*, and *Halomonas* genus. (Johnson et al. 2005; Llamas et al. 2004; Paggi et al. 2003; Rivas et al. 2007) Both Gram-positive and Gram-negative bacteria possess QS activity. Some of the QS molecules which were studied in detail include acyl-homoserine lactones (AHL), autoinducer 2 (AI-2), and peptide signals; however, some other signal molecules also exist, viz., indole and cholera autoinducer (CAI) (Nazzaro et al. 2013). Three major sets of QS systems based on signaling molecules and sensing mechanism include (1) Gram-negative LuxI/LuxR system with AHLs as signaling molecules (Fuqua et al. 1994); (2) Gram-negative *V. harveyi* like two-component signaling circuits that distinguish three different AIs, AHLs, FBD, and an uncharacterized Cal −1 molecule (Bassler et al. 1993, 1994; Henke and Bassler 2004). (3); and Gram-positive two-component signaling systems that use modified oligopeptides as AIs (Lazazzera and Grossman 1998).

As far as food microbiology is concerned, it was well established that food ecosystems and, therefore, food properties are altered by microbial communication that takes place among the microorganisms they contain. The production of signaling molecules by food microorganisms was found to affect food sensory quality and safety. It was reported that certain foods such as meats (Blana and Nychas 2014)

contain dairy products (Gori et al. 2012) or vegetables (Lau et al. 2013) contain AHLs and AI-2. Food microbiologists are now considerably suggesting the role of QS in bacterial food spoilage (Ammor et al. 2008). Understanding the QS mechanisms in food microbes is essential to develop food products, and to increase their shelf lives. Disrupting the QS process can help to repress microbial gene expression associated with food spoilage. Synthesis of cell signaling molecules can be blocked thus interruption of signaling system in these organisms by using quorum sensing inhibitors (QSIs) also known as quorum quenchers (QQs) can help in prevention of biofilm formation and food spoilage.

Though it is perplexing to understand how different factors in foods effect cell-to-cell signaling and how different pathogens respond to various signals generated by other associated bacteria (Smith et al. 2004). Exploring these things further may possibly lead to the discovery of species-specific molecules, and therefore possible interventions can be sought to regulate QS-regulated food spoilage, food quality, and safety.

17.2 Role of QS in Food Spoilage

QS plays an important role in food spoilage. Various autoinducer compounds like AI-1 and AI-2 which act as signaling molecules were found to prevail in different foods such as meat, milk, fruits, and vegetables. (Bruhn et al. 2004; Liu et al. 2006; Lu et al. 2004; Ammor et al. 2008). It is believed that these signaling molecules produced by certain microorganism which are initially associated with these foods and such organisms were named as "spoilage specific organisms" (SSOs) (Huis in't Veld 1996). Most of the SSOs belong to the family *Enterobacteriaceae*, but other organisms which have also associated and act as SSOs include *Shewanella (Alteromonas) putrefaciens*, lactic acid bacteria (LAB) *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Aeromonas* spp. (Gram et al. 2002). These bacteria are thought to be major contributors for vegetable and muscle food spoilage based on food type and other conditions.

A concept called "ephemeral spoilage organism" (ESO) was proposed which is a smaller fraction of SSO. ESOs are thought to dominate via selection during the process of food storage (Huis in't Veld 1996). *Pseudomonas* spp. are the SSO commonly associated with storage of muscle foods (e.g., fish and meat) stored in aerobic conditions at cold temperature with high relative humidity, while *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas fragi* are ESOs for the same food products. Both biotic (i.e., implicit) and abiotic (i.e., intrinsic, extrinsic, or processing) factors are responsible for the establishment of ESOs as well as their growth rate (Nychas and Skandamis 2005). All these factors together form the dimensions of an ecological niche, where an organism dwell (Boddy and Wimpenny 1992). The QS molecules that are concerned in food spoilage may differ or are significant in some foods while less prevalent in others even when the SSOs or ESOs are the same in certain cases (Medina-Martinez et al. 2007).

17.3 QS in Seafood

Seafoods are one of the most abundantly perishable food due to chemical effects of oxygen present in the atmosphere and microbial spoilage. Microbial spoilage relies on density of microbial population present in seafood which is involved in QS (Wang et al. 2017). Seafood spoilage can occur due to various reasons which may include contamination, dehydration, enzymes, oxidation, or even physical damage (Wang et al. 2017). Microbial growth is a major source of seafood spoilage resulting in the formation of compounds like aldehydes, alcohols, amines, organic acids, ketones, and sulfides with unpleasant smell and distasteful off-flavors. There are several microorganisms associated with seafood known as "specific spoilage organisms (SSOs)" which are found to dominate over the other microbiota as they are favored by various parameters like food product, atmosphere, temperature, type of preservation, salt content, etc. and reach higher populations and produce various metabolites (Parlapani et al. 2015). This SSO concept has made it possible for us to understand the process of seafood spoilage quite significantly (Dalgaard 1995). In the past few years, QS (QS) a term describing bacterial communication mechanism was thought to play a role in microbial food spoilage via regulating chitinolytic, lipolytic, and proteolytic activities related to degradation of food and spoilage (Ammor et al. 2008).

The role of QS in food spoilage was predominantly studied via biofilm formation, metabolic activities, and siderophore synthesis (Wang et al. 2017). It was found that *Aeromonas veronii* is capable of generating total volatile base nitrogen (TVB-N) and putrescine apart from producing four types of AHLs, viz., N-butanoyl-L-homoserine lactone (C4-HSL), N-hexanoyl-L-homoserine lactone (C6-HSL), N-heptanoyl-L-homoserine lactone (C7-HSL), and N-octanoyl-L-homoserine lactone (C8-HSL) may be concerned in regulation of spoilage potential. Therefore, understanding QS process in *Aeromonas* may provide new strategy for regulating spoilage in food industry (Zhao et al. 2017a, b). Refrigeration is the universal method to store and transport fresh shrimp. It is quite interesting to explore how spoilage floras in refrigerated shrimp developed. Presence of characteristic QS systems in *Shewanella* spp. has been reported that are involved in spoilage of refrigerated *Litopenaeus vannamei* (Zhu et al. 2015).

The spoilage process of fish is like that of meat and is associated with the presence of SSOs (Gram et al. 2002; Ammor et al. 2008). *S. putrefaciens* and *Pseudomonas* spp. were described as SSOs for frozen marine and freshwater fish, respectively, obtained from the Mediterranean Sea (Gram and Huss 1996; Gram et al. 2002; Koutsoumanis and Nychas 1999). *P. phosphoreum* were identified as SSO for modified-atmosphere-packaged cod (Dalgaard et al. 1997; Gram et al. 2002). *Enterobacteriaceae*, *Carnobacterium* sp., and *Lactobacillus* sp. act as SSOs for spoilage of vacuum-packaged cold-smoked salmon (Jorgensen et al. 2000.) Gram and colleagues (Gram et al. 2002) have detected AHLs (acyl-homoserine lactones) in many spoiled commercial fish products, viz., cold-smoked salmon, fish fillets, and minced fish. However, significance of QS systems in cold-smoked salmon spoilage has not been described. Gram and coworkers (Gram et al. 1999; Gram et al. 2002;

Flodgaard et al. 2005) reported AHLs (mainly 3-hydroxy-C8-HSL) produced by non-bioluminescent *P. phosphoreum* and *Aeromonas* spp. strains isolated as dominant spoilage causing microbes from packed cod fillets, thus suggesting that an AHL-based system may be playing a crucial role in the regulation of chitinase activity, which is responsible for enhancing degradation of crustaceans (Flodgaard et al. 2005). AHLs (3-oxo-C6-HSL, C6-HSL, C8-HSL, and C12-HSL) were mainly detected in *H. alvei* and *S. liquefaciens* belonged to the family *Enterobacteriaceae* as well as in *P. fluorescens* and *P. putida* from the family *Pseudomonadaceae* which are spoilage causing microorganisms for rainbow trout fillets with significant proteolytic activity as well (Ammor et al. 2008). It was found in *S. proteamaculans* B5a that activities of several exoenzymes (chitinase, lipase, and protease) were affected by 3-oxo-C6-HSL which suggests a possible QS-based regulation of food spoilage in this strain. Using whole genome sequencing, Fu et al. (2018) identified three potential genes of *Shewanella baltica* which are involved in spoilage of fish products through QS-regulated mechanism. These three genes include *torT*, *cysM*, and *trxB*, and among these three, *torT* was found to be the most predominant (Fu et al. 2018). In this study, authors also reported that diketopiperazine (DKP)-mediated QS circuit is associated with expression of spoilage genes and biofilm formation, which enhance spoilage. Large yellow cracker (*Pseudosciaena crocea)* is highly nutritious and perishable fish species. At low-temperature storage *Shewanella baltica* acts as SSO for the large yellow cracker (*Pseudosciaena crocea)*; however, various *S. baltica* strains exhibit diverse spoilage ability. The authors suggest that DPKs and spoilage genes can be used as targets for developing novel food antiseptics. Zhu et al. (2017) observed that *S. putrefaciens* and *S. baltica* isolated from spoiled and refrigerated *Litopenaeus vannamei* exhibited distinctspoilage activities both in vitro and in vivo; furthermore, it was identified that *S. baltica* has a higher spoilage potential as compared to *S. putrefaciens* (Zhu et al. 2017). During the spoilage process of *Litopenaeus vannamei*, it was observed that both *S. putrefaciens* and *S. baltica* cooperate in shrimp spoilage and cyclo (L-Pro-L-Leu)-dependent QS between them plays a vital role in this cooperation. The authors have suggested that to reduce the postharvest loss of shrimp, novel preservation techniques (biological or chemical) must be developed which can help to disrupt cyclo-(L-Pro-L-Leu)-dependent QS. Studies have also reported that AHL-based signal molecules can affect vital metabolic properties in *Shewanella* spp. (Zhang et al. 2017).

17.4 QS in Milk and Dairy Products

Pseudomonads comprising Gram-negative, proteolytic, psychrotrophic bacteria are mainly associated with spoilage of milk and other dairy products. *Pseudomonads* produce various compounds which cause spoilage of dairy products that include extracellular proteinases, glycosidases, lipases, and lecithinases (Dogan and Boor 2003). Spoilage of some dairy products was also associated with hydrolases (phospholipases) from Gram-positive psychrotrophic aerobic *Bacillus* spp. Despite the fact, hydrolases mainly secreted at the end of stationary phase of microbial growth

during which a high cell density is achieved, it may be suggested that QS might be playing a role in the modulation of such enzymatic processes. Different AHLs were found to be produced by several proteolytic psychrotrophic bacteria isolated from raw and pasteurized milk such as *Enterobacter* sp., *Hafnia alvei*, *Pseudomonas* sp., and *Serratia* sp., thus suggesting that QS may have a significant role in the spoilage of milk and dairy products (Lindberg et al. 1998; Whitfield et al. 2000).

Researchers have observed that the production of an alkaline metalloprotease, a strong proteolytic milk spoiler in *P. fluorescens* 395, is controlled by C4-HSL and 3OC8-HSL, and moreover it was found that the protease production was affected on disruption of AHL production via degradation or mutation (Liu and Griffths 2003). *S. proteamaculans* strain B5a was found to produce extracellular lipolytic and proteolytic enzymes controlled by an AHL-based QS system, suggesting the involvement of QS in milk spoilage process (Christensen et al. 2003). QS was also found to influence the growth of *P. fluorescens* (Whan et al. 2000). QS signaling molecules were significantly detected in regular milk with a low bacterial population which suggests the possible involvement of interspecies communication and modulation in spoilage (Lu et al. 2004). Some QS signal molecules like AI-2 can withstand high temperatures of 80 °C; thus, pasteurization does not help in such cases as it is ineffective in destroying endogenous proteases, which cause milk spoilage.

17.5 QS in Meats and Meat Products

Fresh meat has many microorganisms associated with it and includes genera from the family *Enterobacteriaceae.* Commonly found microbes are *S. putrefaciens*, *B. thermosphacta*, and *Pseudomonas* spp., and occasionally lactic acid bacteria (*Lactobacillales*) were also present. *Pseudomonas* spp., *P. fluorescens*, and *P. putida* were found in spoiled meat or meat products that are stored under aerobic conditions at very low temperatures of 3–8 °C. During the storage of fresh meat, *P. fragi* can be the ESO and becomes dominant later through selection. Psychrotrophic members belonging to the family *Enterobacteriaceae*, like *H. alvei*, were found to be mainly associated with meat products (Nychas et al. 1988). Appearance of biofilms on surfaces of fresh meat products stored under aerobic refrigerated conditions was noticed during spoilage process suggesting the involvement of QS (Jay et al. 2003). AHL production as well as proteolytic activity was detected in meat products stored in aerobic conditions at low temperatures (Liu et al. 2006). Significant proteolytic activity along with a broad range of AHL signals like C4-HSL, 3-oxo-C6-HSL, C6-HSL, C8-HSL, and C12-HSL was noticed in aerobically chill-stored ground beef and chicken (Liu et al. 2006). LAB and *Enterobacteriaceae* were found to interact in chill-stored vacuum-packed meat during its spoilage process (Bruhn et al. 2004). In vacuum-packed meat *H. alvei* and *Serratia* spp. were identified as dominating species among AHL-producing *Enterobacteriaceae* (Bruhn et al. 2004; Gram et al. 1999). However, prevalence of AHLs in vacuum-packed meat does not play a significant role in spoilage of these

products (Bruhn et al. 2004). Low levels of BAI-2 were reported to be present in various meat products like beefsteak, beef patties, chicken breast, etc. even though the presence of indigenous bacteria was in higher numbers (Lu et al. 2004). This team also found that such meat products are involved in the inhibition of AI-2 activity either partially or completely (Lu et al. 2004). Beef-derived fatty acids were found to inhibit AI-2-based cell signaling (Soni et al. 2008). *Enterobacteriaceae* and *Pseudomonadaceae* were associated with AHL production in fresh ground pork. AHLs were identified in minced beef stored aerobically under modified atmospheric conditions, and these AHLs were associated with the presence of *Pseudomonads* and *Enterobacteriaceae* sp. Moreover, throughout storage of minced beef, low levels of AI-2 activity were observed (Blana and Nychas 2014).

17.6 QS in Fruits and Vegetables

The spoilage of fruits and vegetables is often caused by the enzymatic activity (pectinolytic activity) of organisms belonging to *Pseudomonadaceae* or *Enterobacteriaceae* (Lund 1982). A broad range of AHLs (mainly 3-oxo-C6-HSL and C6-HSL) are produced by *Erwinia* and *Pseudomonas* strains possessing pectinolytic and proteolytic activity and account for spoiling various ready-to-eat vegetables like bean sprout. This suggests that modulation of vegetables and fruit spoilage may be mediated via AHL-based QS systems (Rasch et al. 2005). Rasch et al. (2005) observed that there was a faster spoilage when bean sprouts where inoculated with AHL-producing pectinolytic *P. carotovorum* (*E. carotovora*) (Rasch et al. 2005). Bean sprout spoilage bacterium *Pectobacterium* sp. A2JM was described to exhibit the same type of AHL (3-oxo-C6-HSL) mediation for the regulation of pectinase, protease, and cellulase. It was also observed in the same bacterium that siderophore-mediated iron chelation is also regulated by 3-oxo-C6-HSL (Rasch et al. 2005).

S. plymuthica RVH1, isolated from raw vegetable, were reported to produce LuxI homolog, which controls the production of three AHLs (C4-HSL, C6-HSL, and 3-oxo-C6-HSL) (Van Houdt et al. 2007). The production of extracellular lipase, S-layer protein, PrtA metalloprotease, and butanediol fermentation in cucumber rot-associated *S. marcescens* strain MG1 (previously *S. liquefaciens* MG1) were regulated by SwrI/SwrR QS system and its cognate C4-HSL (Riedel et al. 2001; Van Houdt et al. 2006). In *Serratia* sp. ATCC 39006, the exoenzymes cellulase and pectate lyase were reported to be under the control of SmaI/SmaR QS system and its cognate C4-HSL and C6-HSL (Thomson et al. 2000). QS inhibitory compounds were reported to be present in bean sprouts, carrot, garlic, habanera, and chamomile. Like AHL-based QS, AI-2 activity was observed on surfaces of some fruits and vegetables, viz., tomato, cantaloupe, and carrots, andon tofu (Lu et al. 2004). However, it is not yet clear whether this activity is involved in the spoilage of such products.

17.7 Biofilm Formation and Foodborne Pathogens

Foodborne diseases are a burden worldwide. The Global Foodborne Infections Network (GFN) functions to estimate and mitigate the problem of foodborne diseases (WHO, 2011) and the Department of Food Safety and Zoonoses (FOS) of the World Health Organization (WHO) has taken initiatives to measure the global burden of mortality and morbidity caused by foodborne diseases (WHO 2012). National Institutes of Health (NIH, USA) has reported that 80% of all microbial diseases, including foodborne illnesses, are caused by microbes in biofilms (National Institutes of Health, USA 1997). During the past three decades, there was a significant increase in the incidence of foodborne diseases associated with fresh fruits and vegetables (Sivapalasingam et al. 2004).

Biofilms comprise of an aggregation of microorganisms that form on the surfaces and are surrounded by extracellular polymeric substances (EPSs) (Sutherland 2001). Biofilms are formed by foodborne pathogens on food and food contact surfaces, thus a concern for food safety (Jahid and Ha 2012). Biofilms on surfaces have a characteristic structure consisting of microcolonies enclosed in a hydrated matrix of microbially produced proteins, nucleic acids and polysaccharides (Donlan 2002; Annous et al. 2009). Biofilms on food surfaces were found to exhibit resistance to common disinfectants (Jahid and Ha 2012). Biofilm formation on produce surfaces has reported to be one of the main factors in the failure of washing treatments to remove or inactivate human pathogens on such surfaces (Annous and others 2001, 2004, 2005).

Most of the research related to biofilms was mainly focused on monospecies (or pure cultures). Biofilms present in natural environments are comprised of multiple bacterial species as well as algae, fungi, and protozoa (Percival et al. 2000). Biofilms constituting mixed-species have recently gained importance in food microbiology and in food safety due to high resistance to disinfectants and sanitizers as compared to biofilms containing monospecies. Various factors determine the formation of mixed-species biofilms on food and food processing surfaces which include physical, chemical, and biological processes, species composition, indigenous microbiota and nutrients, food types, temperature, QS, EPS production, biofilms maturation, and dispersal steps, respectively. Mixed-species are highly resistant to antimicrobials, most likely due to higher EPS production, internalization into food, fitness of species, denser and thicker biofilms maturation, and interspecific protection. Based on the genetic background of species involved in biofilm formation, the fitness of mixed-species biofilms populations can imply whether there is cooperative, competitive, or neutral type of interaction in mixed-species biofilms. Nonetheless, various methods are currently used for detection and to better comprehend mixed-species biofilms which include microarray, confocal microscopy, and proteomics.

Among various foods, cantaloupe melons, apples (as unpasteurized juice or cider), and leafy greens were repeatedly linked to outbreaks, and each of these has been associated to a different human pathogen. Since 1990, six multistate outbreaks of "salmonellosis" were linked to the consumption of cantaloupe melons. It was reported that a variety of sanitizers have proven ineffective to remove or inactivate

S. enterica on cantaloupe (Annous et al. 2005). Moreover, it was documented that when the organism resides on rind surface for more than 24 h, the efficacy of sanitizers on cantaloupes depicted a significant decrease. This suggests that increased residence time led to the formation of biofilm prior to application of a sanitizer; thus further studies were carried out to explore the ability of *S. enterica* to form biofilms on whole cantaloupe surfaces (Annous et al. 2004). *E. coli* O157:H7 is a significant causative agent of severe gastrointestinal disease in humans (Silagyi et al. 2009). Between 1982 and 2002, seven *E. coli* O157:H7 outbreaks associated with apple juice or cider occurred; there are no reports of foodborne illnesses linked to consumption of fresh apples (Rangel et al. 2005).

Prepackaged salads and minimally processed leafy greens have emerged as important vehicles for transmission of foodborne pathogens. The FDA identified 18 *E. coli* O157:H7 outbreaks since 1995, associated with lettuce and one outbreak associated with spinach. Many other outbreaks accountable for hundreds of illnesses and some deaths due to foodborne illness were linked to basil, cabbage, cilantro, green onions, and parsley (DeWaal and Barlow 2002; MMWR 2005).

An investigation into the ability of *Shigella* to persist and form biofilms on the surface of parsley plants was taken into consideration after an outbreak in March 1999, involving *Shigella boydii* linked to bean salad (Agle 2003). Such investigations help elucidate the interactions between enteric pathogens and plant tissues and the ability of these pathogens to form biofilms.

Cell-to-cell signaling, known as QS, was shown to play a role in biofilm formation in foodborne pathogens. Knowledge about the chemical structures of different types of signaling molecules helps in the identification of compounds that can be used for modulating the QS, including biofilm formation. Further research is warranted to understand how QS signaling influences the virulence and antimicrobial resistance of biofilm communities. This helps design strategies that control biofilm formation on industrial, medical, food, and food processing surfaces. Plethora of studies examining the role of QS signaling systems in biofilm formation in foodborne pathogens include *Campylobacter jejuni*, it is a spiral rod-shaped Gram-negative microaerophilic bacterium and causes gastroenteritis that is commonly associated with foodborne illness. Though the AHLs production was not recognized in *C. jejuni* (Smith et al. 2004), enzyme LuxS as well as a product resembling AI-2 was evidenced in *C. jejuni* (Cloak et al. 2002; Jeon et al. 2005). The production of AI-2 activity in milk and chicken broth by *C. jejuni* and *C. coli* was demonstrated and luxS gene was sequenced in both organisms (Cloak et al. 2002). *C. jejuni* was also found to form non-AHL or AI-2-dependent biofilms on surfaces used in animal production watering systems. Non-AHL or AI-2-dependent biofilm formation was evidenced in Gram-negative isolates from a vegetable-processing facility (Van Houdt et al. 2004). Some strains belonging to *A. hydrophila* can cause illness in fish and amphibians as well as in humans. *A. hydrophila* uses C4-HSL for the formation of mature biofilms on stainless steel. Flagella formation in *A. hydrophila* is probably thought to be regulated through QS though was reported.

Helicobacter pylori is a microaerophilic helical-shaped Gram-negative bacterium found in the stomach and duodenum and is one of the causal agents of chronic

gastritis, gastric ulcers, and stomach cancer. Humans get infected with *H. pylori* through the ingestion of contaminated food and water. A strain of *H. pylori* with a mutation in LuxS gene exhibited a threefold increase in the formation of biofilms on glass surfaces as compared to its wild type (Cole et al. 2004).

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, facultatively anaerobic, motile, beta hemolytic bacterium commonly found in soil and food. Some strains of *B. cereus* cause foodborne illness, while other strains can be beneficial as probiotics for animals (Ryan and Ray 2004). It was reported that QS system of *B. cereus* consists of PlcR and *PapR*. *PlcR* gene functions as an encoder for a transcriptional regulator while as PapR gene encodes a QS signaling peptide. LuxS/AI-2 system was reported in *B. cereus* (Slamti and Lereclus 2002, 2005). It was observed that a plcR-negative mutant of *B. cereus* produces approximately fourfold more biofilm on polystyrene as compared to its isogenic wild type (Hsueh and group 2006). Furthermore, the addition of exogenous AI-2 to wild-type *B. cereus* was shown to decrease biofilm formation. Biofilm formation by *Listeria monocytogenes*, a facultative intracellular pathogenic Gram-positive rod-shaped bacterium, was demonstrated on PVC microtiter plates, glass slides, stainless steel, polyethylene, teflon coupons, conveyer belt materials, and floor drains of food processing facilities (Zhao et al. 2004; Pan et al. 2006). *L. monocytogenes* was found to be associated with foods like cheese, fermented ice cream, milk, raw meat sausages, raw meat, raw poultry, and raw and smoked fish. *L. monocytogenes* grows even at temperatures as low as 3 °C. Like *B. cereus*, *L. monocytogenes* also has a LuxS/AI-2 system; the *luxS* gene was found to repress biofilm formation (Sela et al. 2006), while a mutation in *luxS* gene resulted in a fourfold thicker biofilm than in the wild type. Moreover, the addition of in vitro synthesized AI-2 to mutant cultures did not show repression in biofilm formation (Challan et al. 2006). It was concluded that during biofilm formation in *L. monocytogenes*, there is no indication of QS role for AI-2. It was suggested that peptide QS compounds may exist in *L. monocytogenes* though have not been reported.

E. coli is a Gram-negative, facultative anaerobic, rod-shaped coliform bacterium commonly found in the lower intestine of warm-blooded organisms (Tenaillon et al. 2010). Most *E.coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts leading to cause gastrointestinal and extra-intestinal infections (CDC National Center for Emerging and zoonotic Infectious Diseases 2012; Vogt and Dippold 2005). *E. coli* O157:H7 was shown to cause hemorrhagic colitis and hemolytic uremic and such outbreaks were linked to contaminated food and water. Common foods leading to such infections include minced beef and raw milk, etc. *E. coli* strains produce biofilms on a wide range of surfaces such as glass, glass wool, glass coverslips, high-density polyethylene, polyamide-6, polyvinyl chloride, polystyrene microtiter plates, stainless steel, and teflon coupons (Faille et al. 2002). Although the LuxS/AI-2 system is present in *E. coli* (Ahmer 2004), the role of the LuxS/AI-2 system in biofilm formation by *E. coli* is still not clear (Yoon and Sofos 2008).

Many strains of *Salmonella enterica* are associated with foodborne and waterborne gastroenteritis. *S. enterica* are facultative anaerobic Gram-negative bacteria. Numerous *S. enterica* isolates were found to form biofilms on meat (Solomon et al. 2005). *S. enterica* containing SdiA has a homolog of LuxR, but they lack LuxI

homolog and thus are unable to synthesize AHLs. LuxS/AI-2 is also present in *Salmonella*. However, the relationship between biofilm formation and the presence of an active LuxS system and AI-2 in *S. enterica* is not yet clear (Yoon and Sofos 2008). *Yersinia pestis*, *Yersinia pseudotuberculosis*, *and Yersinia enterocolitica* are facultative anaerobic Gram-negative rods. Out of the three examples, *Y. enterocolitica* and *Y. pseudotuberculosis* are associated with foodborne illness (Joshua et al. 2003; Patel et al. 2006). *Y. pestis* was evidenced to contain two luxI/luxR-like genes yspI/yspR and ypeI/ypeR (Kirwan et al. 2006). It was observed that AHL synthase YspI in *Y. pestis* synthesizes mainly N-3-oxo-octanoyl-L-homoserine lactone (3-oxo-C8-HSL) and N-3-oxo-hexanoyl-L-homoserine lactone (3-oxo-C6-HSL) in the ratio of 1: 1 (Kirwan and others 2006). *Y. pseudotuberculosis* was also established to contain two luxI synthase genes, *ypsI* and *ytbI* (Ortori et al. 2007). Twenty-four different AHLs were reported to be produced by wild-type *Y. pseudotuberculosis*. It was reported that YenI present in *Y. enterocolitica* leads to the synthesis of 3-oxo-C6-HSL and C6-HSL as well as smaller amounts of 3-oxo-C10-HSL, 3-oxo-C12-HSL, and 3-oxo-C14-HSL (Atkinson et al. 2006). *Y. enterocolitica* was demonstrated to produce AHLs in milk and in liquid extracts of beef, fish, and pork (Medina-Martínez et al. 2006), while no AHL production was found in the case of liquid extracts of mixed lettuce, cucumber, or soy bean. The luxS gene was reported to be present in *Y. pestis*, and researchers also suggested that this gene might be present in *Y. pseudotuberculosis* and *Y. enterocolitica* as well (Jarrett et al. 2004).

Few *Vibrio* spp. are associated with waterborne and foodborne diseases. *Vibrio* are facultative anaerobic Gram-negative bacteria including *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. It is noteworthy that QS in vibrios was examined in plethora of studies (Milton 2006). It was anticipated that all three organisms mentioned in examples can form biofilms on various surfaces (Hammer and Bassler 2003). In *V. cholerae*, it was observed that biofilm formation is regulated and controlled by several QS systems operating concurrently to regulate the transcription of genes that are involved in the production of EPS. It is noteworthy that this organism can form biofilms at low cell densities rather than high when QS signal molecules get accumulated (Hammer and Bassler 2003). LuxS/AI-2 system was reported in *V. parahaemolyticus*, and it can synthesize AHLs via LuxM synthase, and these AHLs are detected by LuxN (Henke and Bassler 2004; Defoirdt and others 2006; Henke and Bassler 2004). *V. vulnificus* was also found to contain LuxS/AI-2 system as well as SmcR which is a homolog of LuxR (Shao and Hor 2001). Several AHLs were isolated from cultures of *V. vulnificus* such as C4-, C6-, 3-oxo-C8-,3-oxo-C10-, 3-oxo-C12-, and 3-oxo-C14-HSL (Morin et al. 2003). A Gram-positive coccus can produce highly heat-stable enterotoxin which can cause food poisoning. *S. aureus* strains can form biofilms on different surfaces like polystyrene and glass microtiter plates, teflon catheters, and other medical devices, and these biofilms formed by *S. aureus* were associated with human and animal infections (Gross et al. 2001). Biofilm-associated infections caused by *S. aureus* include endocarditis, osteomyelitis, skin infections, etc. (Yarwood and Schlievert 2003). QS in *S. aureus* was reported to be based on secretion of a short peptide attached to a 5-membered thiolactone ring autoinducing peptide (AIP) encoded by *agr* locus (Table 17.1).

Table 17.1 Numerous examples of mixed-species in biofilm formation, their interactions, and relevant key findings

Microorganisms	Biofilm formation on surface of food	Interaction	Keys findings	References
Salmonella typhimurium and cultivable natural microflora from lettuce	Stainless steel (SS) and lettuce	Competitive	Resistance to UV-C by mixed-culture biofilms compared to mono-cultures on lettuce but not on SS	Jahid et al. (2014)
Escherichia coli and Gram-negative species isolated from fresh produce-processing facilities	Microtiter plate and glass surface	Both neutral and stimulatory	Environmental isolates enhance or remain neutral to biofilms of pathogenic bacteria	Liu et al. (2014)
Acylated homoserine lactones-containing chicken breast muscle broth from *Pseudomonas aeruginosa*	Microtiter plate with chicken broth	Competitive	Inhibition of *P. aeruginosa* biofilms by quorum sensing compound-containing broth	Zhang et al. (2014)
Listeria monocytogenes, *Pseudomonas fluorescens*, *Serratia proteamaculans*, or *Shewanella baltica*	SS surface	Inhibitory or stimulatory	More EPS, enhanced survival against desiccation and distinct biofilm structures	Alavi and Hansen (2013)
P. aeruginosa and *E. coli*	Silicon coupons	Inhibitory	*P. aeruginosa* outnumbered *E. coli* after 48 h	Cerqueira et al. (2013)
Commensal *E. coli* with pathogenic *E. coli*, and *Klebsiella pneumoniae*	Micro-fermenter glass slide and mouse model intestinal colonization	Inhibitory	Mixed-species biofilms express different genes than single species, and commensal species reduce pathogens biofilms	Da Re et al. (2013)
L. monocytogenes and *Pseudomonas putida*	SS surface	Competitive, 90% of population was *P. putida*	Resistance to benzalkonium chloride	Giaouris et al. (2013)
E. coli and *P. aeruginosa*		Competitive	Mixed-species biofilms have greater mass than monoculture biofilms	Kuznetsova et al. (2013)
P. aeruginosa, Pseudomonas protegens, and *K. pneumonia*	Three-channel flow cells	Mixed biofilm formation delayed than single biofilm	Mixed-species biofilm compact structure and resistance to antibiotic	Lee et al. (2013a, b)

Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans, and *Paenibacillus amylolyticus*	Microtiter plate with peg	Synergistic	Strong synergistic interactions in a 4-species biofilm model, more than threefold increase in biofilm formation and strong dominance of 2 strains, *Xanthomonas retroflexus* and *Paenibacillus amylolyticus*	Ren et al. (2013)
E. coli, Enterobacteriaceae cloacae, P. aeruginosa, Stenotrophomonas maltophilia, *Variovorax* sp., *Cupriavidus respiraculi*, *Bradyrhizobium* sp., *Sediminibacterium* sp., *Novosphingobium subterraneum*, *Sphingomonas* sp., *Blastomonas natatoria*, and *Methylobacterium isbiliense*, *Mycobacterium* sp.			Mixed-species are 50- to 300-fold more resistant compared to single-species biofilms, and distinct 3-D structures are observed for multispecies biofilms	Schwering et al. (2013)
Shiga toxin-producing *E. coli* and *Salmonella enterica* serovar, Typhimurium		Competition	Mixed-species form more EPS, and are more resistant to sanitizers	Wang et al. (2013b)
P. aeruginosa and *S. enterica* from meat-processing plants	Polystyrene microplate	Competitive	Cell-free supernatant inhibits biofilms of *S. enterica* at exponential phase but not at stationary phase	Wang et al. (2013a)
Burkholderia cepacia and *P. aeruginosa*	Silicone tubing	Competitive	Mixed biofilms are more resistant compared to monocultures	Behnke and Camper (2012)
Bacillus subtilis and *Staphylococcus aureus*	Polystyrene 96-well microtiter plates	Cooperative	Mixed biofilms are more resistant because *B. subtilis* protects *S. aureus* isolates in mixed biofilms with higher EPS production	Bridier et al. (2012)
L. monocytogenes and *P. putida*	Stainless steel and polypropylene sheets	Competitive	Mixed biofilms form more complex and dense structures, causing resistance to BAC	Ibusquiza et al. (2012)

(continued)

Table 17.1 (continued)

Microorganisms	Biofilm formation on surface of food	Interaction	Keys findings	References
Campylobacter jejuni and *P. aeruginosa*	Flat plate flow reactor	Positive	*C. jejuni* survives better compared to mono-culture due to oxygen consumption by *P. aeruginosa*	Ica et al. (2012)
S. aureus, L. monocytogenes, and *Salmonella enteritidis*	Polystyrene microplate	ND	Mixed culture is more resistant compared to monocultures	do Valle Gomes and Nitschke (2012)
Aeromonas hydrophila and *Flavobacterium* sp.	Chitin surface	Competitive	*Flavobacterium* sp. outcompetes *A. hydrophila* in chitin biofilm	Jagmann et al. (2012)
L. monocytogenes and *S. enterica*	Stainless steel	None	Different strains show different ability to form biofilms in mixed culture and monocultures, mixed cultures are equally sensitive	Kostaki et al. (2012)
S. aureus and *E. coli*	Polypropylene coupons	Competitive	Dual species are more resistant than single-species biofilms	Millezi et al. (2012)
S. enterica serovar Thompson or Newport or with *P. fluorescens*	SS surface	Competitive	Increase in resistance of *Salmonella* sp. when it forms mixed-species biofilms with *P. fluorescens*	Shen et al. (2012)
S. enterica, E. coli, and *L. monocytogenes*	Glass, polypropylene, polyethylene, polyvinyl chloride, copper, silicone rubber, and stainless steel	Competitive	Top layers by *E. coli* and bottom layers by *S. enterica* and *L. monocytogenes*	Almeida et al. (2011)
S. typhimurium and *Aspergillus niger*	Fungal hyphae act as biotic surfaces	*S. typhimurium* forms biofilm on hyphae of *A. niger*	Bacteria form biofilms on hyphae using cellulose and chitin interaction	Brandl et al. (2011)
E. coli and *P. aeruginosa*	Silicone rubber	Neutral	Phage can penetrate inside both monoculture and mixed-culture biofilms, which act as reservoir for phage	Kay and others (2011)

L. monocytogenes and *P. aeruginosa*	CBD	Neutral	Formation of mixed-culture biofilms shows lowered susceptibility at lower temperature	Lourenco et al. (2011)
L. monocytogenes and *Lactobacillus plantarum*	Polystyrene microtiter plates	Almost neutral, a slightly lower count in mixed culture for *L. monocytogenes*	Mixed-species biofilms are more resistant compared to monospecies biofilms	van der Veen and Abee (2011)
P. aeruginosa and *Candida comprising Candida albicans, C. glabrata, C. krusei, C. tropicalis, C. parapsilosis*, and *C. dubliniensis*	Polystyrene surface	Inhibitory	Mixed-species form scanty biofilms while single-species form dense biofilms	Bandara et al. (2010)
Cell-free supernatant of *Hafnia alvei* and *Salmonella enterica* serovar Enteritidis	SS coupons	Inhibitory		Chorianopoulos et al. (2010)
Resident microflora from feed industry and *Salmonella enterica*	SS coupons	Synergistic and neutral	Synergistic effect found for *Pseudomonas* and *Staphylococcus* spp.	Habimana et al. (2010a)
E. coli O157:H7 and *Acinetobacter calcoaceticus* from meat industry	Sealed glass coverslip	Synergistic	*E. coli* cells embedded and covered by *A. calcoaceticus* cells in mixed-species biofilms	Habimana et al. (2010b)
E. coli O-:H4, *E. coli* O157:H7, *Salmonella* spp., *P. aeruginosa*, *Citrobacter* spp., *Serratia liquefaciens*, and *B. subtilis*	Glass microscope slides	Positive	*E. coli* O157:H7 enhances the biofilm formation with dual-species biofilms as well as resistance	Uhlich et al. (2010)
Dekkera bruxellensis, *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, *Acetobacter aceti*, and *Lactobacillus hilgardii*	CBD	Both neutral and stimulatory	*D. bruxellensis* is neutral with *A. aceti* while *A. aceti* forms threefold higher biofilms with *L. hilgardii*	Tristezza et al. (2010)
Enterococcus faecalis, E. coli, P. aeruginosa, Salmonella agona, Staphylococcus simulans, and *C. jejuni*	Polystyrene microtiter plate	Both neutral and stimulatory	*E. faecalis and S. simulans* enhance while *P. aeruginosa and S. agona* are neutral to formation of mixed-species biofilms	Teh et al. (2010)

(continued)

Table 17.1 (continued)

Microorganisms	Biofilm formation on surface of food	Interaction	Keys findings	References
L. monocytogenes and Staphylococcus epidermis	Polystyrene microtiter plate	Synergistic	Higher and stronger biofilms formed for mixed culture	Zameer et al. (2010)
E. coli O157:H7 and resident flora from meat processing plants	Polyurethane conveyor belt	Synergistic or neutral	Resident microflora shows favorable effect on biofilm formation of *E. coli* O157:H7	Marouani-Gadri et al. (2009)
Serratia plymuthica and *E. coli*	Microscope glass coverslip	Competitive	Wild-type shows more competitive interaction than deficient mutant of *Serratia plymuthica* with *E. coli*	Moons et al. (2006)
L. monocytogenes serotypes 2a and 4b	SS coupons	Synergistic	Mixed-culture forms more biofilms than single-culture	Pan et al. (2009)
S. simulans and Lactobacillus fermentum, P. putida, Salmonella enterica, and *L. monocytogenes*	SS coupons	Competitive	Both monoculture and mixed-cultures show same effectiveness	Chorianopoulos et al. (2008)
L. monocytogenes and biofilms natural microflora on wooden shelves used in the ripening of a soft and smear cheese	Glass fiber filters (GFF) deposited on sterile smear cheese	Competitive, natural microflora inhibit *L. monocytogenes* growth and biofilm formation	Natural microflora inhibits the biofilm formation of *L monocytogenes*	Guillier et al. (2008)
L. monocytogenes and *S. aureus* from dairy industry	SS coupons	Stimulatory, inhibitory, and neutral	Effect is strain-dependent and supernatant also shows the effect	Rieu et al. (2008)
C. jejuni and bacteria collected from a saline rinse of poultry-processed broiler chicken carcasses	SS	Neutral effect	*C. jejuni* can form biofilms on pre-existing biofilms on SS	Sanders et al. (2008)
Lactobacillus casei and *S. cerevisiae*	Both glass slide and microtiter plate	Synergistic	Enhanced biofilm formation with yeast cells with distinct morphology for mixed biofilms	Kawarai et al. (2007)
Staphylococcus equorum, *Staphylococcus succinus*, and *Lactobacillus sakei* as monoculture, and *S. aureus* with *L. monocytogenes* and *Pseudomonas fragi* with *E. coli* as mixed cultures	Glass fiber filter	Inhibitory	Antimicrobials have similar effects on both mono and mixed cultures	Lebert et al. (2007)

L. monocytogenes, *Yersinia enterocolitica, Salmonella enterica, E. coli* O157:H7, *Pseudomonas marginalis*, and native microflora recovered from fresh peeled baby carrots	Baby carrot	Inhibitory	Inhibitory effect caused due to antagonistic effect of associated microflora and pathogens	Liao (2007)
Microbacterium phyllosphaerae, *Shewanella japonica*, *Dokdonia donghaensis*, and *Acinetobacter lwoffii*	Polystyrene	Synergistic	Multispecies form more biomass than single-species biofilms, also show resistance to antimicrobials	Burmølle et al. (2006)
E. coli, *P. putida*, and *Staphylococcus epidermidis*	Glass tube	Synergistic and neutral	Coadhesion mechanisms stimulate biofilms of *E. coli*	Castonguay et al. (2006)
S. typhimurium and *E. coli*	Epithelial cells	Inhibitory	*S. typhimurium* displaces *E. coli* biofilms	Esteves and others (2005)
L. monocytogenes and resident microflora from food industry	SS coupons	Stimulatory, inhibitory, and neutral, depending on strains	Bacterial strains or supernatant have the positive effect on biofilms of *L. monocytogenes*	Carpentier and Chassaing (2004)
C. albicans and *S. epidermidis*	Catheter disks	Synergistic	Mixed-species, that is, fungi and bacteria protect each other from antibiotics	Adam et al. (2002)
Shewanella putrefaciens and *P. fluorescens*	SS coupons	Inhibitory	Both species form biofilms and inhibit the growth of *S. putrefaciens*	Bagge et al. (2001)
Pseudomonas sp., *P. putida*, and *E. coli*	Glass surface	Commensal	Tower-like biofilm structure and mixed-culture forms higher EPS	Cowan et al. (2000)
P. putida, *Acinetobacter* sp., and 5 other Gram-positive bacteria	Four-channel flow cells	Synergistic	Thick biofilms form and degrade toluene, rather than monocultures	Møller et al. (1998)

Table adapted from Jahid and Ha (2014)

17.8 Role of QS Inhibitors (QSIs)/Quorum Quenchers (QQs) in Food Preservation

The term "quorum quenching" was coined to describe all the processes that interfere with QS. QQ is a promising innovative tool for the development of novel therapeutics to control microbial infections in many cases. In few cases, one bacterial species may hold advantage over the other in its ability to disrupt QS; this is mostly found in niches where the bacterial populations compete for limited resources (Kiran et al. 2017). Similarly, the host's ability to step-in bacterial QS communication plays an indispensable role in averting colonization of pathogenic bacteria that use QS to coordinate virulence. Thus, QQ evolved as a promising mechanism to interfere and inhibit the bacterial cell-cell communication (Waters and Bassler 2005).

QQ or inhibition of QS can occur in several ways, which include (1) enzymatic degradation of signal molecule, (2) blocking signal generation, and/or (3) blocking signal reception (Hentzer and Givskov 2003). AHLs can be degraded both enzymatically as well as nonenzymatically. AHLs present in bacterial cultures can get degraded nonenzymatically at pH values above 7, and this process of degradation of AHLs at alkaline pH values occurs due to lactonolysis (Byers et al. 2002). In the process of lactonolysis, the lactone ring upon hydrolysis of the ester bond of the ring gets opened yielding an AHL molecule. Several bacteria were found to produce lactonases that can hydrolyze the ester bond of homoserine lactone ring (Dong and Zhang 2005). Degradation of AHLs via acylase action was observed in a few bacteria. During the acylase action, the cleavage of amide bond joining the lactone ring to acyl chain occurs; it results in the release of homoserine lactone and fatty acid which is further broken down by bacteria (Dong and Zhang 2005).

Inhibition of QS activity by stable food-grade spice oil nanoemulsions was evaluated against QS-dependent phenotypes of preferred foodborne pathogens and observed that formulated spice oil nanoemulsions effectively regulated bacterial phenotypes like violacein pigmentation, biofilm formation, and exopolysaccharide (EPS) production in comparison to untreated controls. Out of three formulations, i.e., pepper oil, cumin oil, and fennel oil nanoemulsions, cumin oil and fennel oil nanoemulsions exhibited better QQ activity as compared to pepper oil nanoemulsion (Venkadesaperumal et al. 2016). Hence, such nanoemulsions may have various applications as antimicrobials.

Studies have shown that phenolic compounds present as secondary metabolites in plants can also inhibit bacterial communication. Phenolic extract acquired from wild strawberry *Rubus rosaefolius* was shown to inhibit all the phenotypes regulated by QS in bacteria, together with violacein production, swarming motility, and biofilm formation. *R. rosaefolius* serves as good source of novel bioactive compounds possessing QQ activity which can be used to produce anti-virulence drugs and new additives for food industry in future (Oliveira et al. 2016). Some studies reported that organic acid-based antimicrobials can reduce bacterial populations via inhibition of (AI-2) activity or QS. Almasoud et al. (2016) first demonstrated the ability of organic acids (malic and lactic acid) which act as natural antimicrobials and were

shown to inhibit the expression of AI-2 molecule in *E. coli* O157:H7 and *S. typhimurium*, thereby checking the QS ability of such pathogenic bacteria. It was observed that lactic acid alone as well as in combination with malic acid was able to inhibit AI-2 activity by *E. coli* O157:H7 and *Salmonella* by 80% on spinach and cantaloupe respectively (Almasoud et al. 2016). As such, these natural antimicrobials can be used to enhance food protection and safety in minimally processed produce industry dealing with frozen or refrigerated vegetables, fruits, and salads.

Antimicrobial properties were demonstrated in many flavonoids obtained from various plants and spices, and these may serve as sources of antimicrobials. Naringin, the main constituent of pummelo peel flavonoid extracts, was reported to cause significant reduction in QS-dependent phenotypes, viz., violacein production, biofilm formation, swimming, swarming motility, and protease production (Liu et al. 2017). Thus, pummelo peel, a QQ, can be used to produce antibacterial agents and to overcome antimicrobial resistance. In one of the studies involving the disruption of QS activity in *Aeromonas sobria*, an opportunistic pathogen commonly found in environment and food, curcumin liposomes were found to significantly inhibit siderophore production, swimming, swarming motility, extracellular proteases, biofilm formation, and AHLs production in *A. sobria*.

Furthermore, it was revealed through in silico molecular docking studies that QS inhibited by curcumin liposomes were mainly owing to curcumin liposome's interaction with LuxI type protein via hydrogen bonding, which hindered the production of AHLs in *A. sobria* (Ding, Wang and Li, Ding et al. 2017). Outbreaks from fresh fruits and vegetables involving pathogens, viz., *E. coli* and *Salmonella* sp., are a major concern in the world nowadays. Despite following the routinely practiced sanitizing methods in food industries, no method has proven to be successful to thwart outbreaks associated with fresh produce.

Organic acids were employed as food preservatives because of their antimicrobial potential. The ability of three organic acids, namely, acetic acid, citric acid, and lactic acid, was evaluated to manage *E. coli* and *Salmonella* sp. from fresh fruits and vegetables. Furthermore, the biofilm forming ability and QS inhibitory potential was also studied. It was observed that the swimming and swarming patterns in *E. coli* were significantly affected by both acetic and lactic acid. However, acetic acid and lactic acid showed higher anti-QS activity as compared to citric acid. It was reported that among all the three mentioned organic acids, lactic acid exhibited the most significant anti-QS ability. It was observed that when a cucumber is treated 2% lactic acid, it inactivates the *E. coli* and *Salmonella* sp. (Amrutha et al. 2017).

Organic acids can therefore help in sinking the microbial load linked with fresh fruits and vegetables and serve as potential QQ agents in food industry. Cinnamaldehyde, an aldehyde that gives cinnamon its flavor and odor, was found to partially inhibit the transcription induced by AHL and cause reduced bioluminescence in two different *V. harveyi* reporter strains that acted in response to AHL and AI-2, respectively (Niu et al. 2006). Cinnamaldehyde was found to affect AI-2 activity in foods like beef and turkey patties, chicken breast, and beefsteak (Lu et al. 2004). Various food additives such as sodium propionate, sodium benzoate, and sodium acetate were reported to inhibit of AI-2 activity (Lu et al. 2004).

Some products isolated from garlic were found to act as inhibitors of QS most probably via competitive binding with LuxR, and these compounds could not exhibit antimicrobial properties (Persson et al. 2005). N-(heptylsulfanylacetyl)-L-homoserine lactone is regarded as the most effective QS inhibitor present in garlic. AI-2-like activity was observed in several types of fruits, vegetables, and frozen fish (Lu et al. 2004, 2005) (Table 17.2).

Table 17.2 Examples of QSIs obtained from natural sources

Inhibitor	Source	Inhibition activity	Reference
Natural QSI: Enzymes AHL-acylase	A wide range of bacteria, fungi, plants, and legumes	Cleaves HSL ring or hydrolyze the amide linkage	Lade et al. (2014)
AHL-lactonase	A wide range of bacteria, fungi, plants, and legumes	Open HSL ring	Lade et al. (2014)
AHL-oxidoreductase	A wide range of bacteria, fungi, plants, and legumes	Oxidizes or reduces the acyl chain of HSL	Lade et al. (2014)
Blueberry extracts	–	Violacein production of *C. violaceum* CV026	Vattem et al. (2007)
Brassica oleracea, basil, thyme, rosemary, ginger, and turmeric	Extracts from herbs and spices	Violacein production of *C. violaceum* CV026	Vattem et al. (2007)
Grape extracts	Fruit extract	Violacein production of *C. violaceum* CV026	Vattem et al. (2007)
Rosmarinic acid	Sweet basil (*Ocimum basilicum*)	Protease and elastase production, biofilm formation, and virulence factors in *P. aeruginosa*	Vattem et al. (2007)
Raspberry extracts	Seedling	Violacein production in *C. violaceum*	Vattem et al. (2007)
Scorzonera sandrasica	Chloroform extract	Violacein production in ATCC12472 and CV026 and carbapenem antibiotic production in *Erwinia carotovora*	Kalia (2013); Bosgelmez-Tinaz et al. (2007)
Vanillin (4-hydroxy-3 methoxybenzaldehyde)	Vanilla beans extract (*Vanilla planifolia* Andrews)	Interferes with AHL receptors. Inhibits C4-HSL, C6-HSL, C8-HSL, 3-oxo-C8-HSL. Inhibits biofilm formation in *Aer. hydrophila* and Violacein production in *C. violaceum*	Ponnusamy et al. (2009)
Scorzonera sandrasica	Chloroform extract	Violacein in *C. violaceum* and carbapenem antibiotic production in *Erwinia carotovora*	Kalia (2013)

(continued)

Table 17.2 (continued)

Inhibitor	Source	Inhibition activity	Reference
Hamamelis virginiana	Hamamelitannin	Biofilm formation and cell attachment in *Staphylococcus* spp.	Kalia (2013)
Tecoma capensis (Thunb.) Lindl. (leaves, flowers), *Sonchus oleraceus* L. (aerial parts), *R. officinalis* L. (leaves)	–	Violacein production in *C. violaceum*	Kalia (2013)
Satureja thymbra L.	Essential oil and hydrosol fraction	Biofilm formation in *P. aeruginosa* PA01	Kalia (2013)
Halogenated furanones	*Delisea pulchra* (marine alga)	Antagonistic towards AHL-controlled processes, prevent binding of AHLs to luxR, inhibit AI-1 and AI-2-mediated QS in *V. harveyi*, inhibit biofilm formation and swarming of *E. coli*	Givskov et al. (1996); Ren et al. (2001)
γ-Hydroxybutyrate (GHB)	Tomato seedlings exudate	AHL signaling in *A. tumefaciens*	Chai et al. (2007)

17.9 Conclusion

It is quite obvious that QS enhances the bacterial ability to access nutrients or more flattering environmental niches and to increase bacterial defenses against eukaryotic hosts, competing bacteria, and environmental stresses. QS has received considerable attention as far as the physiological and clinical aspects of QS are concerned. However, QS in the context of food microbiology needs to be explored further. For better understanding, further research is warranted to identify the mechanism of biofilm formation in foodborne pathogens. Compared to clinical settings, works on QS related to food systems were relatively less. It was reported in some studies that definite food components may affect QS molecules produced by bacteria. With the advent of techniques like proteomics and genomics, it is possible to elucidate the phenotypes linked with QS and thereby identify the mechanisms wherein the said pathways are activated or repressed. Several molecules which act as QSIs or QQs were isolated or synthesized, and these compounds were quite effective in inhibiting growth, virulence mechanisms, and biofilm formation of bacteria in varied food environments. Time is not far when we can think of developing formulated foods that can interfere with QS and thus help inhibit the growth of biofilm forming pathogenic spoilage organisms which would facilitate in checking food spoilage and will be a boon for food production quality and safety.

References

Adam B, Baillie GS, Douglas LJ (2002) Mixed-species biofilms of *Candida albicans* and Staphylococcus epidermidis. J Med Microbiol 51:344–349

Agle ME (2003) *Shigella boydii* 18: characterization and biofilm formation [PhD thesis]. University of Illinois, Urbana

Ahmer BMM (2004) Cell-to-cell signaling in *Escherichia coli* and *Salmonella enterica*. Mol Microbiol 52:933–945

Alavi HED, Hansen LT (2013) Kinetics of biofilm formation and desiccation survival of Listeria monocytogenes in single and dual species biofilms with Pseudomonas fluorescens, *Serratia proteamaculans* or *Shewanella baltica* on food-grade stainless steel surfaces. Biofouling 29(10):1253–1268

Almasoud A, Hettiarachchy N, Rayaprolu S, Babu D, Kwon YM, Mauromoustakos A (2016) Inhibitory effects of lactic and malic organic acids on autoinducer type 2 (AI-2) QS of *Escherichia coli* O157: H7 and *Salmonella typhimurium*. LWT-Food Sci Technol 66:560–564

Almeida C, Azevedo NF, Santos S, Keevil CW, Vieira MJ (2011) Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence in situ hybridization (PNA FISH). PLoS One 6(3):e14786

Ammor MS, Michaelidis C, Nychas GJE (2008) Insights into the role of quorum sensing in food spoilage. J Food Prot 71(7):1510–1525

Amrutha B, Sundar K, Shetty PH (2017) Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. Microb Pathog 111:156–162

Annous BA, Sapers GM, Mattrazzo AM, Riordan DCR (2001) Efficacy of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. J Food Prot 64:159–163

Annous BA, Burke A, Sites JE (2004) Surface pasteurization of whole fresh cantaloupes inoculated with *Salmonella Poona* or *Escherichia coli*. J Food Prot 67:1876–1885

Annous BA, Sapers GM, Jones DM, Burke A (2005) Improved recovery procedure for evaluation of sanitizer efficacy in disinfecting contaminated cantaloupes. J Food Sci 70:M242–M247

Annous BA, Fratamico PM, Smith JL (2009) Quorum sensing in biofilms: why bacteria behave the way they do. J Food Sci 74(1):R1–R14

Atkinson S, Chang C-Y, Sockett RE, Cámara M, Williams P (2006) QS in *Yersinia enterocolitica* controls swimming and swarming motility. J Bacteriol 188:1451–1461

Bagge D, Metthe H, Charlotte J, Ingrid H, Lone G (2001) *Shewanella putrefaciens* adhesion and biofilm formation on food processing surfaces. Appl Environ Microbiol 67(5):2319–2325

Bandara HMHN, Yau JYY, Watt RM, Jin LJ, Samaranayake LP (2010) *Peudomonas aeruginosa* inhibits in-vitro *Candida* biofilm development. BMC Microbiol 10:125

Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Intercellular signalling in Vibrio harveyi: sequence and function of genes regulating expression of luminescence. Mol Microbiol 9(4):773–786

Bassler BL, Wright M, Silverman MR (1994) Sequence and function of LuxO, a negative regulator of luminescence in Vibrio harveyi. Mol Microbiol 12(3):403–412

Behnke S, Camper AK (2012) Chlorine dioxide disinfection of single and dual species biofilms, detached biofilm and planktonic populations. Biofouling 28(6):635–647

Blana VA, Nychas GJE (2014) Presence of QS signal molecules in minced beef stored under various temperature and packaging conditions. Int J Food Microbiol 173:1–8

Boddy L, Wimpenny JW (1992) Ecological concepts in food microbiology. Soc Appl Bacteriol Symp Ser 21:23S–38S

Brandl MT, Carter MQ, Parker CT, Chapman MR, Huynh S, Zhou Y (2011) *Salmonella* biofilm formation on *Aspergillus niger* involves cellulose–chitin interactions. PLoS One 6(10):e25553

Bridier A, Sanchez-Vizuete M del P, Le Coq D, Aymerich S, Meylheuc T, Maillard JY, Thomas V, Dubois-Brissonnet F, Briandet R (2012) Biofilms of a *Bacillus subtilis* hospital isolate protect Staphylococcus aureus from biocide action. PLoS One 7(9):e44506

Bruhn JB, Christensen AB, Flodgaard LR, Nielsen KF, Larsen TO, Givskov M, Gram L (2004) Presence of acylated homoserine lactones (AHLs) and AHL-producing bacteria in meat and potential role of AHL in spoilage of meat. Appl Environ Microbiol 70:4293–4302

Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S (2006) Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol 72:3916–3923

Byers JT, Lucas C, Salmond GPC, Welch M (2002) Nonenzymatic turnover of an *Erwinia carotovora* quorum-sensing signaling molecule. J Bacteriol 184:1163–1171

Carpentier B, Chassaing D (2004) Interactions in biofilms between *Listeria monocytogenes* and resident microorganisms from food industry premises. Intl J Food Microbiol 97(2):111–122

Castonguay MH, van der Schaaf S, Koester W, Krooneman J, van der Meer W, Harmsen H, Landini P (2006) Biofilm formation by Escherichia coli is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. Res Microbiol 157(5):471–478

CDC National (2012) Center for Emerging and Zoonotic Infectious Diseases. Retrieved on 2 October

Cerqueira L, Oliveira JA, Nicolau A, Azevedo NF, Vieira MJ (2013) Biofilm formation with mixed-species of Pseudomonas aeruginosa/Escherichia coli on silicone using artificial urine to mimic urinary catheters. Biofouling 29(7):829–840

Chai Y, Tsai CS, Cho H, Winanas SC (2007) Reconstitution of the biochemical activities of the AttJ repressor and the AttK, Atl and AttM catabolic enzymes of *Agrobacterium tumefaciens*. J Bacteriol 2(189):3674–3679

Challan Belval S, Gal L, Margiewes S, Garmyn D, Piveteau P, Guzzo J (2006) Assessment of the roles of LuxS, S-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by *Listeria monocytogenes* EGD-e. Appl Environ Microbiol 72:2644–2650

Chorianopoulos NG, Giaouris ED, Skandamis PN, Haroutounian SA, Nychas GJE (2008) Disinfectant test against monospecies and mixed-species biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid–base sanitizers. J Appl Microbiol 104:1586–1596

Chorianopoulos NG, Giaouris ED, Kourkoutas Y, Nychas GJE (2010) Inhibition of the early stage of Salmonella enterica serovar Enteritidis biofilm development on stainless steel by cell-free supernatant of a *Hafnia alvei* culture. Appl Environ Microbiol 76(6):2018–2022

Christensen AB, Riedel K, Eberl L, Flodgaard LR, Molin S, Gram L, Givskov M (2003) Quorum-sensing-directed protein expression in *Serratia proteamaculans* B5a. Microbiology 149:471–483

Cloak OM, Solow BT, Briggs CE, Chen C-Y, Fratamico PM (2002) QS and production of autoinducer-2 in *Campylobacter* spp., *Escherichia coli* O157:H7 and *Salmonella enterica* serovar *Typhimurium* in foods. Appl Environ Microbiol 68:4666–4671

Cole SP, Harwood J, Lee R, She R, Guiney DG (2004) Characterization of monospecies biofilm formation by *Helicobacter pylori*. J Bacteriol 186:3124–3132

Cowan SE, Gilbert E, Liepmann D, Keasling JD (2000) Commensal interactions in a dual-species biofilm exposed to mixed organic compounds. Appl Environ Microbiol 66(10):4481–4485

Da Re S, Valle J, Charbonnel N, Beloin C, Latour-Lambert P, Faure P, Turlin E, Le Bougu'enec C, Renauld-Mong'enie G, Forestier C, Ghigo JM (2013) Identification of commensal Escherichia coli genes involved in biofilm resistance to pathogen colonization. PLoS One 8(5):e61628

Dalgaard P (1995) Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int J Food Microbiol 26(3):319

Dalgaard P, Mejlholm O, Christiansen TJ, Huss HH (1997) Importance of *Photobacterium phosphoreum* in relation to spoilage of modified-atmosphere–packed fish products. Lett Appl Microbiol 24:373–378

Defoirdt T, Crab R, Woods TK, Sorgeloos P, Verstraete W, Bossier P (2006) Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii* and *Vibrio parahaemolyticus* isolates. Appl Environ Microbiol 72:6419–6423

DeWaal CS, Barlow K (2002) Outbreak alert! Closing the gaps in our federal food safety net, 5th edn. Center for Science in the Public Interest, Washington, DC

Ding T, Li T, Wang Z, Li J (2017) Curcumin liposomes interfere with QS system of *Aeromonas sobria* and in silico analysis. Sci Rep 7(1):8612

do Valle Gomes MZ, Nitschke M (2012) Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. Food Control 25(2):441–447

Dogan B, Boor KJ (2003) Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. Appl Environ Microbiol 69:130–138

Dong YH, Zhang LH (2005) Quorum sensing and quorum-quenching enzymes. J Microbiol 43(special issue #S):101–109

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890

Esteves CL, Jones BD, Clegg S (2005) Biofilm formation by Salmonella enterica serovar Typhimurium and Escherichia coli on epithelial cells following mixed inoculations. Infect Immun 73(8):5198–5203

Faille C, Jullien C, Fontaine F, Bellon-Fontaine M-N, Slomianny C, Benezech T (2002) Adhesion of *Bacillus* spores and *Escherichia coli* cells to inert surfaces: role of surface hydrophobicity. Can J Microbiol 48:728–738

Flodgaard LR, Dalgaard P, Andersen JB, Nielsen KF, Givskov M, Gram L (2005) Nonbioluminescent strains of *Photobacterium phosphoreum* produce the cell-to-cell communicationsignal N-(3-hydroxyoctanoyl) homoserine lactone. Appl Environ Microbiol 71:2113–2120

Fu L, Wang C, Liu N, Ma A, Wang Y (2018) QS system-regulated genes affect the spoilage potential of *Shewanella baltica*. Food Res Int 107:1–9

Fuqua WC, Winans SC, Greenberg EP (1994 Jan) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176(2):269

Giaouris E, Chorianopoulos N, Doulgeraki A, Nychas GJ (2013) Co-culture with Listeria monocytogenes within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. PLoS One 8(10):e77276

Givskov M, de Nys R, Manefield M, Gram L, Maximilien RIA, Eberl LEO et al (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178(22):6618–6622

Gori K, Moslehi-Jenabian S, Purrotti M, Jespersen L (2012) Autoinducer-2 activity produced by bacteria found in smear of surface ripened cheeses. Int Dairy J 21:48–53

Gram L, Huss HH (1996 Nov 1) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33(1):121–137

Gram L, Melchiorsen J, Spanggaard B, Huber I, Nielsen TF (1999) Inhibition of Vibrio anguillarum byPseudomonas fluorescens AH2, a possible probiotic treatment of fish. Appl Environ Microbiol 65(3):969–973

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M (2002) Food spoilage—interactions between food spoilage bacteria. Int J Food Microbiol 78:79–97

Gross M, Cramton SE, Götz F, Peschel A (2001) Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect Immun 69:3423–3426

Guillier L, Stahl V, Hezard B, Notz E, Briandet R (2008) Modelling the competitive growth between *Listeria monocytogenes* and biofilm microflora of smear cheese wooden shelves. Intl J Food Microbiol 128(1):51–57

Habimana O, Møretrø T, Langsrud SS, Lene K, Vestby LK, Live L, Nesse LL, Heir E (2010a) Micro ecosystems from feed industry surfaces: a survival and biofilm study of *Salmonella* versus host resident flora strains. BMC Vet Res 6:48

Habimana O, Heir E, Langsrud S, Asli AW, Møretrø T (2010b) Enhanced surface colonization by Escherichia coli O157:H7 in biofilms formed by an *Acinetobacter calcoaceticus* isolate from meat-processing environments. Appl Environ Microbiol 76(13):4557–4559

Hammer BK, Bassler BL (2003) QS controls biofilm formation in *Vibrio cholerae*. Mol Microbiol 50:101–114

Henke JM, Bassler BL (2004) QS regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. J Bacteriol 186:3794–3805
Hentzer M, Givskov M (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 112(9):1300–1307
Huis in't Veld JHJ (1996) Microbial and biochemical spoilage of foods: an overview. Int J Food Microbiol 33:1–18
Ibusquiza PS, Herrera JJ, V'azquez-S'anchez D, Cabo ML (2012) Adherence kinetics, resistance to benzalkonium chloride and microscopic analysis of mixed-species biofilms formed by *Listeria monocytogenes* and *Pseudomonas putida*. Food Control 25:202–210
Ica T, Caner V, Istanbullu O, Nguyen HD, Ahmed B, Call DR, Beyenal H (2012) Characterization of mono and mixed-species *Campylobacter jejuni* biofilms. Appl Environ Microbiol 78(4):1033–1038
Jagmann N, von Rekowski KS, Philipp B (2012) Interactions of bacteria with different mechanisms for chitin degradation result in the formation of a mixed-species biofilm. FEMS Microbiol Lett 326(1):69–75
Jahid IK, Ha SD (2012) A review of microbial biofilms of produce: future challenge to food safety. Food Sci Biotechnol 21(2):299–316
Jahid IK, Ha SD (2014) The paradox of mixed-species biofilms in the context of food safety. Compr Rev Food Sci Food Saf 13(5):990–1011
Jahid IK, Han N, Srey S, Ha SD (2014) Competitive interactions inside mixed-species biofilms of *Salmonella typhimurium* and cultivable indigenous microorganisms on lettuce enhance microbial resistance of their sessile cells to ultraviolet C (UV-C) irradiation. Food Res Int 55:445–454
Jarrett CO, Deak E, Isherwood KE, Oyston PC, Fisher ER, Whitney AR, Kobayashi SD, DeLeo FR, Hinnebusch BJ (2004) Transmission of *Yersinia pestis* from an infectious biofilm in the flea vector. J Infect Dis 190:783–792
Jay JM, Vilai JP, Hughes ME (2003) Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5–7 °C. Int J Food Microbiol 81:105–111
Jeon B, Itoh K, Ryu S (2005) Promoter analysis of cytolethal distending toxin genes (cdtA, B and C) and effect of a luxS mutation on CDT production in *Campylobacter jejuni*. Microbiol Immunol 49:599–603
Johnson MR et al (2005) Population density-dependent regulation of exopolysaccharide formation in the hyperthermophilic bacterium *Thermotoga maritima*. Mol Microbiol 55:664–674
Jorgensen LV, Huss HH, Dalgaard P (2000) The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. J Appl Microbiol 89:920–934
Joshua GWP, Karlyshev AV, Smith MP, Isherwood KE, Titball RW, Wren BW (2003) A *Caenorhabditis elegans* model of Yersinia infection: biofilm formation on a biotic surface. Microbiology 149:3221–3229
Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31(2):224–245
Kawarai T, Furukawa S, Ogihara H, Yamasaki M (2007) Mixed-species biofilm formation by lactic acid bacteria and rice wine yeasts. Appl Environ Microbiol 73:4673–4676
Kay MK, Erwin TC, McLean RJ, Aron GM (2011) Bacteriophage ecology in *Escherichia coli* and Pseudomonas aeruginosa mixed-biofilm communities. Appl Environ Microbiol 77(3):821–829
Kiran GS, Hassan S, Sajayan A, Selvin J (2017) Quorum quenching compounds from natural sources. In: Bioresources and bioprocess in biotechnology. Springer, Singapore, pp 351–364
Kirwan JP, Gould TA, Schweizer HP, Bearden SW, Murphy RC, Churchill MEA (2006) Quorum-sensing signal synthesis by the *Yersinia pestis* acyl-homoserine lactone synthase YspI. J Bacteriol 188:784–788
Kostaki M, Chorianopoulos N, Braxou E, Nychas GJ, Giaouris E (2012) Differential biofilm formation and chemical disinfection resistance of sessile cells of Listeria monocytogenes strains under monospecies and dual-species (with Salmonella enterica) conditions. Appl Environ Microbiol 78(8):2586–2595
Koutsoumanis K, Nychas G-JE (1999) Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7, and 10°C. Appl Environ Microbiol 65:698–706

Kuznetsova MV, Maslennikova IL, Karpunina TI, Nesterova LY, Demakov VA (2013) Interactions of Pseudomonas aeruginosa in predominant biofilm or planktonic forms of existence in mixed-species with Escherichia coli in vitro. Can J Microbiol 59(9):604–610

Lade H, Paul D, Kweon JH (2014) Quorum quenching mediated approaches for control of membrane biofouling. Int J Biol Sci 10(5):550

Lau YY, Sulaimane J, Chen JW, Yin WF, Chan KG (2013) Quorum sensing activity of *Enterobacter asburiae* isolated from lettuce leaves. Sensors 13:14189–14199

Lazazzera BA, Grossman AD (1998 Jul 1) The ins and outs of peptide signaling. Trends Microbiol 6(7):288–294

Lebert I, Leroy S, Talon R (2007) Effect of industrial and natural biocides on spoilage, pathogenic and technological strains grown in biofilm. Food Microbiol 24(3):281–287

Lee JH, Cho HS, Joo SW, Regmi SC, Kim JA, Ryu CM, Ryu SY, Cho MH, Lee J (2013a) Diverse plant extracts and trans-resveratrol inhibit biofilm formation and swarming of Escherichia coli O157:H7. Biofouling 29(10):1189–1203

Lee KW, Periasamy S, Mukherjee M, Xie C, Kjelleberg S, Rice SA (2013b) Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. ISME J 8. https://doi.org/10.1038/ismej.2013.194

Liao CH (2007) Inhibition of foodborne pathogens by native microflora recovered from fresh peeled baby carrot and propagated in cultures. J Food Sci 72(4):M134–M139

Lindberg AM, Ljungh A, Ahrne S, Lofdahl S, Molin G (1998) *Enterobacteriaceae* found in high numbers in fish, minced meat and pasteurised milk or cream and the presence of toxin encoding genes. Int J Food Microbiol 39:11–17

Liu M, Griffths MW (2003) The role of QS in the regulation of protease by *Pseudomonas fluorescens* and its relation to food spoilage. Abstracts of Institute of Food Technologies Annual Meeting 2003. Institute of Food Technologists, Chicago, pp 85, 29G–26

Liu M, Gray JM, Griffiths MW (2006) Occurrence of proteolytic activity and N-acyl-homoserine lactone signals in the spoilage of aerobically chill-stored proteinaceous raw foods. J Food Prot 69:2729–2737

Liu NT, Nou X, Lefcourt AM, Shelton DR, Lo YM (2014) Dual-species biofilm formation by Escherichia coli O157:H7 and environmental bacteria isolated from fresh-cut processing facilities. Intl J Food Microbiol 171:15–20

Liu Z, Pan Y, Li X, Jie J, Zeng M (2017) Chemical composition, antimicrobial and anti-QS activities of pummelo peel flavonoid extract. Ind Crop Prod 109:862–868

Llamas I, Keshavan N, Gonzalez JE (2004) Use of *Sinorhizobium meliloti* as an indicator for specific detection of long-chain N-acyl homoserine lactones. Appl Environ Microbiol 70:3715–3723

Lourenco A, Machado H, Brito L (2011) Biofilms of Listeria monocytogenes produced at 12 °C either in pure culture or in co-culture with Pseudomonas aeruginosa showed reduced susceptibility to sanitizers. J Food Sci Chicago 76(2):M143–M148

Lu L, Hume ME, Pillai SD (2004) Autoinducer-2-like activity associated with foods and its interaction with food additives. J Food Prot 67:1457–1462

Lu L, Hume ME, Pillai SD (2005) Autoinducer-2-like activity on vegetable produce and its potential involvement in bacterial biofilm formation on tomatoes. Foodborne Path Dis 2:242–249

Lund BM (1982) The effect of bacteria on post-harvest quality of vegetables and fruits, with particular reference to spoilage. Soc Appl Bacteriol Symp Ser 10:133–153

Marouani-Gadri N, Augier G, Carpentier B (2009) Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection – influence of isolated strains on biofilm formation by Saka¨ı and EDL 933 E. coli O157:H7. Int J Food Microbiol 133(1–2):62–67

Medina-Martínez MS, Uyttendaele M, Meireman S, Debevere J (2006) Relevance of Nacyl-L-homoserine lactone production by *Yersinia enterocolitica* in fresh foods. J Appl Microbiol 102:1150–1158

Medina-Martinez MS, Uyttendaele M, Meireman S, Debevere J (2007) Relevance of N-acyl-L-homoserine lactone production by *Yersinia enterocolitica* in fresh foods. J Appl Microbiol 102(4):1150–1158

Millezi FM, Pereira MO, Batista NN, Camargos N, Auad I, Cardoso MDG, Piccoli RH (2012) Susceptibility of monospecies and dual-species biofilms of *Staphylococcus aureus* and Escherichia coli to essential oils. J Food Saf 32:351–359

Milton DL (2006) Quorum sensing in vibrios: complexity for diversification. Int J Med Microbiol 296:61–71

MMWR, Morbidity and Mortality Weekly Report (2005) Outbreaks of *Salmonella* Infections Associated with eating roma tomatoes—United States and Canada. MMWR 54(13):325–328.

Møller S, Sternberg C, Andersen JB, Christensen BB, Ramos JL, Givskov M, Molin S (1998) In situ gene expression in mixed-species biofilms: evidence of metabolic interactions between community members. Appl Environ Microbiol 64(2):721–732

Moons P, Van Houdt R, Aertsen A, Vanoirbeek K, Engelborghs Y, Michiels CW (2006) Role of quorum sensing and antimicrobial component production by *Serratia plymuthica* in formation of biofilms, including. Appl Environ Microbiol 72(11):7294–7300

Morin D, Grasland B, Vallée-Réhel K, Dufau C, Haras D (2003) On-line high performance liquid chromatography-mass spectrometric detection and quantification of N−acylhomoserine lactones, quorum sensing signal molecules, in the presence of biological matrices. J Chromatogr A 1002:79–92

National Institutes of Health, USA (1997) Minutes of the National Advisory Dental and Craniofacial Research Council—153rd Meeting, Bethesda, MD

Nazzaro F, Fratianni F, Coppola R (2013) Quorum sensing and phyto-chemicals. Int J Mol Sci 14:12607–12619

Niu C, Afre S, Gilbert ES (2006) Subinhibitory concentrations of cinnamaldehyde interfere with quorum sensing. Lett Appl Microbiol 43:489–494

Nychas G-JE, Skandamis P (2005) Fresh meat and modified atmosphere packaging (MAP). In: Sofos JN (ed) Improving the safety of fresh meat. Woodhead Publishing Ltd, Cambridge, pp 461–502

Nychas G-JE, Dillon VM, Board RG (1988) Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. Biotechnol Appl Biochem 10:203–231

Oliveira BDÁ, Rodrigues AC, Cardoso BMI, Ramos ALCC, Bertoldi MC, Taylor JG et al (2016) Antioxidant, antimicrobial and anti-QS activities of *Rubus rosaefolius* phenolic extract. Ind Crop Prod 84:59–66

Ortori CA, Atkinson S, Chhabra SR, Cámara M, Williams P, Barrett DA (2007) Comprehensive profiling of N-acylhomoserine lactones produced by *Yersinia pseudotuberculosis* using liquid chromatography coupled to hybrid quadrupole-linear ion trap mass spectrometry. Anal Bioanal Chem 387:497–511

Paggi RA, Martone CB, Fuqua C, de Castro RE (2003) Detection of quorum sensing signals in the haloalkaliphilic archaeon *Natronococcus occultus*. FEMS Microbiol Lett 221:49–52

Pan Y, Breidt F, Kathariou S (2006) Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a stimulated food processing environment. Appl Environ Microbiol 72:7711–7717

Pan Y, Breidt F Jr, Kathariou S (2009) Competition of Listeria monocytogenes serotype 1/2a and 4b strains in mixed-species biofilms. Appl Environ Microbiol 75(18):5846–5852

Parlapani FF, Haroutounian SA, Nychas GJ, Boziaris IS (2015 Sep 1) Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 C. Food Microbiol 50:44–53

Patel CN, Wortham BW, Lines JL, Fetherston JD, Perry RD, Oliveira MA (2006 Apr 1) Polyamines are essential for the formation of plague biofilm. J Bacteriol 188(7):2355–2363

Percival SL, Walker JT, Hunter PR (2000) Microbiological aspects on biofilms and drinking water. CRC Press, Boca Raton

Persson T, Hansen TH, Rasmussen TB, Skinderso ME, Givskov M, Nielsen J (2005) Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. Org Biomol Chem 3:253–262

Ponnusamy K, Paul D, Kweon JH (2009) Inhibition of quorum sensing mechanism and *Aeromonas hydrophila* biofilm formation by vanillin. Environ Eng Sci 26(8):1359–1363

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL (2005) Epidemiology of *Escherichia coli* outbreaks, United States, 1982–2002. Emerg Infect Dis 11:603–609

Rasch M, Andersen JB, Nielsen KF, Flodgaard LR, Christensen H, Givskov M, Gram L (2005) Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. Appl Environ Microbiol 71:3321–3330

Ren D, Sims JJ, Wood TK (2001) Inhibition of biofilm formation and swarming of Escherichia coli by (5Z) -4-bromo-5-(bromomethylene) -3-butyl-2 (5H) -furanone. Environ Microbiol 3(11):731–736

Ren D, Madsen JS, Cruz-Perera CI, Bergmark L, Sørensen SJ, Burmølle M (2013) High-throughput screening of multispecies biofilm formation and quantitative PCR-based assessment of individual species proportions, useful for exploring interspecific bacterial interactions. Microb Ecol. https://doi.org/10.1007/s00248-013-0315-z

Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, Wu H, Høiby N, Givskov M, Molin S, Eberl L (2001 Dec 1) N-acylhomoserine-lactone-mediated communication between Pseudomonas aeruginosa and Burkholderia cepacia in mixed biofilms. Microbiology 147(12):3249–3262

Rieu A, Lemaître JP, Guzzo J, Piveteau P (2008) Interactions in dual species biofilms between Listeria monocytogenes EGD-e and several strains of Staphylococcus aureus. Intl J Food Microbiol 126(1–2):76–82

Rivas M, Seeger M, Jedlicki E, Holmes DS (2007) Second acylhomoserine lactone-producing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. Appl Environ Microbiol 73:3225–3231

Romero M, Martin-Cuadrado AB, Roca-Rivada A, Cabello AM, Otero A (2011) Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. FEMS Microbiol Ecol 75:205–217

Ryan KJ, Ray CG (eds) (2004) Sherris medical microbiology, 4th edn. McGraw Hill. ISBN 0-8385-8529-9

Sanders SQ, Frank JF, Arnold JW (2008) Temperature and nutrient effects on *Campylobacter jejuni* attachment on multispecies biofilms on stainless steel. J Food Prot 71(2):271–278

Schwering M, Song J, Louie M, Turner RJ, Ceri H (2013) Multi-species biofilms defined from drinking water microorganisms provide increased protection against chlorine disinfection. Biofouling 29(8):917–928

Sela S, Frank S, Belausov E, Pinto R (2006) A mutation in the luxS gene influences *Listeria monocytogenes* biofilm formation. Appl Environ Microbiol 72:5653–5658

Shao CP, Hor LI (2001) Regulation of metalloprotease gene expression in *Vibrio vulnificus* by a *Vibrio harveyi* LuxR homologue. J Bacteriol 183:1369–1375

Shen C, Luo Y, Nou X, Bauchan G, Zhou B, Wang Q, Millner P (2012) Enhanced inactivation of *Salmonella* and *Pseudomonas* biofilms on stainless steel by use of T-128, a fresh-produce washing aid, in chlorinated wash solutions. Appl Environ Microbiol 78(19):6789–6798

Silagyi K, Kim SH, Lo YM, Wei CI (2009) Production of biofilm and quorum sensing by Escherichia coli O157: H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. Food Microbiol 26(5):514–519

Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV (2004) Fresh produce; a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot 67:2342–2353

Slamti L, Lereclus D (2002) A cell-cell signaling peptide activates the PlcR virulence regulon in bacteria of the *Bacillus cereus* group. EMBO J 21:4550–4559

Slamti L, Lereclus D (2005) Specificity and polymorphism of the PlcR-PapR quorum sensing system in the *Bacillus cereus* group. J Bacteriol 187:1182–1187

Smith JL, Fratamico PM, Novak JS (2004) QS: a primer for food microbiologists. J Food Prot 67:1053–1070

Solomon EB, Niemira BA, Sapers GM, Annous BA (2005) Biofilm formation, cellulose production, and curli biosynthesis by *Salmonella* originating from produce, animal and clinical sources. J Food Prot 68:906–912

Soni KA, Jesudhasan P, Cepeda M, Widmer K, Jayaprakasha GK, Patil BS, Hume ME, Pillai SD (2008) Identification of ground beef derived fatty acid inhibitors of autoinducer-2 based cell signaling. J Food Prot 71:134–138

Sutherland IW (2001) The biofilm matrix– an immobilized but dynamic microbial environment. Trends Microbiol 9:222–227

Tang K, Zhang XH (2014) Quorum quenching agents: resources for antivirulence therapy. Mar Drugs 12(6):3245–3282

Teh KH, Flint S, French N (2010) Biofilm formation by *Campylobacter jejuni* in controlled mixed-microbial populations. Intl J Food Microbiol 143(3):118–124

Tenaillon O, Skurnik D, Picard B, Denamur E (2010) The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol 8(3):207–217. https://doi.org/10.1038/nrmicro2298. ISSN 1740-1526

Thomson NR, Crow MA, McGowan SJ, Cox A, Salmond GP (2000 May) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in Serratia is under quorum sensing control. Mol Microbiol 36(3):539–556

Tristezza M, Lourenco A, Barata A, Brito L, Malfeito-Ferreira M, Loureiro V (2010) Susceptibility of wine spoilage yeasts and bacteria in the planktonic state and in biofilms to disinfectants. Ann Microbiol 60:549–556

Uhlich GA, Rogers DP, Mosier DA (2010) Escherichia coli serotype O157: H7 retention on solid surfaces and peroxide resistance is enhanced by dual-strain biofilm formation. Foodborne Pathog Dis 7(8):935–943

van der Veen S, Abee T (2011) Mixed-species biofilms of *Listeria monocytogenes* and Lactobacillus plantarum show enhanced resistance to benzalkonium chloride and peracetic acid. Intl J Food Microbiol 144(3):421–431

Van Houdt R, Aertsen A, Jansen A, Quintana AL, Michiels CW (2004) Biofilm formation and cell-to-cell signalling in Gram-negative bacteria isolated from a food processing environment. J Appl Microbiol 96:177–184

Van Houdt R, Moons P, Buj MH, Michiels CW (2006 Jun 15) N-acyl-L-homoserine lactone quorum sensing controls butanediol fermentation in Serratia plymuthica RVH1 and Serratia marcescens MG1. J Bacteriol 188(12):4570–4572

Van Houdt R, Moons P, Aertsen A, Jansen A, Vanoirbeek K, Daykin M, Williams P, Michiels CW (2007) Characterization of a luxI/luxR-type quorum sensing system and N-acyl-homoserine lactone–dependent regulation of exo-enzyme and antibacterial component production in *Serratia plymuthica* RVH1. Res Microbiol 158:150–158

Vattem DA, Mihalik K, Crixell SH, McLean RJC (2007) Dietary phytochemicals as quorum sensing inhibitors. Fitoterapia 78(4):302–310

Venkadesaperumal G, Rucha S, Sundar K, Shetty PH (2016) Anti-QS activity of spice oil nanoemulsions against food borne pathogens. LWT Food Sci Technol 66:225–231

Vogt RL, Dippold L (2005) *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June–July 2002. Publ Health Rep 120(2):174–178. PMC 1497708. PMID 15842119

Wang H-H, Ye K-P, Zhang Q-Q, Yang D, Xu X-L, Zhou G-H (2013a) Biofilm formation of meat-borne *Salmonella enterica* and inhibition by the cell-free supernatant from Pseudomonas aeruginosa. Food Control 32:650–658

Wang R, Kalchayanand N, Schmidt JW, Harhay DM (2013b) Mixed biofilm formation by Shiga toxin-producing *Escherichia coli* and Salmonella enterica serovar Typhimurium enhanced bacterial resistance to sanitization due to extracellular polymeric substances. J Food Prot 76(9):1513–1522

Wang F, Fu L, Bao X, Wang Y (2017) The spoilage microorganisms in seafood with the existed quorum sensing phenomenon. J Food Microbiol 1(1)

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Whan L, Dunstall G, Rowe MT (2000) A study of the growth kinetics of two pseudomonads from pasteurized milk and the possible role of quorum sensing. Milchwissenschaft 55:371–373

Whitfield FB, Jensen N, Shaw KJ (2000 Nov) Role of Yersinia intermedia and Pseudomonas putida in the development of a fruity off-flavour in pasteurized milk. J Dairy Res 67(4):561–569

World Health Organization (WHO) (2011) Global Foodborne Infections Network (GFN). http://www.who.int/gfn/publications/strategic_plan_2011/en/index.html

World Health Organization (WHO) (2012) Initiative to estimate the Global Burden of Foodborne Diseases. http://www.who.int/foodsafety/foodborne_disease/ferg/en/

Yarwood JM, Schlievert PM (2003) QS in *Staphylococcus* infections. J Clin Invest 112:1620–1625

Yoon Y, Sofos JN (2008) Autoinducer-2 activity of gram-negative foodborne pathogenic bacteria and its influence on biofilm formation. J Food Sci 73:M140–M147

Zameer F, Kreft J, Gopal S (2010) Interaction of *Listeria monocytogenes* and *Staphylococcus epidermis* in dual species biofilms. J Food Saf 30:954–968

Zhang QQ, Ye KP, Wang HH, Xiao HM, Xu XL, Zhou GH (2014) Inhibition of biofilm formation of Pseudomonas aeruginosa by an acylated homoserine lactones-containing culture extract. LWT Food Sci Technol 57:230–235

Zhang C, Zhu S, Jatt AN, Pan Y, Zeng M (2017) Proteomic assessment of the role of N-acyl homoserine lactone in *Shewanella putrefaciens* spoilage. Lett Appl Microbiol 65(5):388–394

Zhao T, Doyle MP, Zhao P (2004) Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. Appl Environ Microbiol 70:3996–4003

Zhao D, Lyu F, Liu S, Zhang J, Ding Y, Chen W, Zhou X (2017a) Involvement of bacterial quorum sensing signals in spoilage potential of *Aeromonas veronii* bv. veronii isolated from fermented surimi. J Food Biochem 42:e12487

Zhao X, Zhao F, Wang J, Zhong N (2017b) Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. RSC Adv 7(58):36670–36683

Zhu S, Wu H, Zeng M, Liu Z, Wang Y (2015) The involvement of bacterial quorum sensing in the spoilage of refrigerated *Litopenaeus vannamei*. Int J Food Microbiol 192:26–33

Zhu S, Zhang C, Wu H, Jie J, Zeng M, Liu Z et al (2017) Spoilage of refrigerated (4° C) *Litopenaeus vannamei* cooperation between *Shewanella* species and contribution of cyclo-(L-Pro-L-Leu)-dependent quorum sensing. Int J Food Sci Technol 52(6):1517–1526

Implications of Quorum Sensing and Quorum Quenching in Aquaculture Health Management

18

Mani Jayaprakashvel and Ramesh Subramani

Abstract

The world human population is growing on an exponential phase and pace. Aquaculture, raising of aquatic animals in artificial or facilitated ecosystem, is evolving as the rapidly growing food production sector globally. The growth of aquaculture industry has been speculated to be inevitable that may certainly contribute toward meeting the food security of growing global population. India, with a vast coastline and enormous marine resources, is having greater potential to build up this industry as a productive economic sector. However, the bacterial infections in aquaculture hatcheries and farms cause a huge loss in productivity and remain a major challenge for the growth of this vital industry. Considering the ill effects to environment and public health, risk of development of antibiotic resistance, and persistence of antibiotic residues in aquaculture animal foods, it has necessitated the regulatory bodies across the globe to restrict the usage of antibiotics for aquaculture disease management. Hence, finding alternate measures for the aquaculture disease management in both hatcheries and forms is the current need. It has been well documented that exhibition of virulence factors and formation of biofilms are the major factors for the establishment of disease in aquaculture animals by the bacterial pathogens. Both these factors are being regulated by quorum sensing (QS), which is a population density-dependent expression of selected phenotypes in a coordinated manner through

M. Jayaprakashvel (✉)
Department of Marine Biotechnology, AMET deemed to be University, Kanathur, Chennai, Tamilnadu, India
e-mail: jayaprakashvel@gmail.com

R. Subramani
School of Biological and Chemical Sciences, Faculty of Science, Technology & Environment, The University of the South Pacific, Suva, Republic of Fiji
e-mail: subramani_r@usp.ac.fj

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_18

the production of autoinducers (AI). Quorum quenching (QQ) is a disruption of quorum sensing. Thus, QQ is considered as one of the most preferred preventive strategies for the ecofriendly management of aquaculture infections. The AI molecules involved in gram-positive and gram-negative QS system and also the enzymes and molecules involved in QQ are also widely studied in aquaculture systems. This chapter would provide an overview of QS and QQ systems being operated among aquaculture pathogens and other beneficial organisms in the aquaculture system with more emphasis on shrimp aquaculture. This chapter also emphasizes the recent developments on the impact of QS and QQ with special reference to the virulence of bacterial pathogens both in vivo and in vitro with a short focus on future perspectives of QQ and QS for the disease management in aquaculture systems.

Keywords
Aquaculture systems · Quorum sensing (QS) · Quorum quenching (QQ) · Marine bacteria · Disease management

18.1 Introduction

Aquaculture is one of the major emerging industries in the past few decades. The global population is growing on an uncontrollable speed and technologies are to be focused on the food security. Thus, aquaculture has emerged to complement the supply effect of fisheries sector. During 2018, it was estimated that all around the world, close to 800 million people are depending either directly or indirectly to the aquaculture and wild fisheries sector. Though the aquaculture sector is growing at an average growth rate of 6% per year, overfishing activities necessitate the increased production through aquaculture. A plethora of studies approximated that the aquaculture production needs to be increased by 20–30% to compensate the declining natural fishery wealth in our seas (Gupta 2018). Many review works emphasized to enlighten the unquestionable need for aquaculture with respect to food security. It was envisaged that rapid growth in human population along with prosperity in the economic status, nonetheless the need for seafood, especially fish foods, has increased manyfold in Africa. Few modeling studies have concluded that only the aquaculture sector could help in meeting the food security in Africa. Furthermore, it is also envisaged that more indigenous aquaculture techniques would reduce the reliance of imports (Chan et al. 2019). In low- and medium-income countries, fin fishes and their products contribute to food security and nutritional requirements. Aquaculture sector, in these countries, contributes not only for food security but also for the economic uplift of low-income people (Little and Bunting 2016). During 2016, Béné et al. (2016) have considered 202 research and review articles published during 2013–2014. They have concluded that the

growth of aquaculture and sustained fishery activities would help in managing the food security issues. These developments could also improve the nutritional requirements and development of economy in the developing and upcoming nations. Crustaceans such as crabs and shrimps could also contribute for the nutrition and food security. Bondad-Reantaso et al. (2012) have also emphasized that aquaculture of shrimps, prawns, crabs, and mussels would be helpful in complementing the protein nutrition. Hence, their growth is very vital for the developing nations.

Humans have used fish and other aquatic animals as foods over many millennia. Though oceanic environment covers 70% of the surface of Earth, the overexploitation through wild catch fisheries may not offer viable resource to meet the global food security. Hence, it has necessitated the emergence of aquaculture (Ahmed and Thompson 2019; Stevens et al. 2018). Aquaculture has been done in both freshwater and marine ecosystems. In both the ecosystems, the technologies are almost same. They grow brood stocks of chosen animal, facilitate hatching of eggs, nurture the seedlings, acclimatize the juveniles into artificial ponds, and finally grow them until harvest. Technologies related to brood stock maintenance, hatchery techniques, feed formulation, pond management, etc. are found to be very crucial in getting aspired success in aquaculture. Indeed, these crucial technologies are also in one way named as challenges.

18.2 Bacterial Pathogens in the Aquaculture Systems

Though the viral diseases contribute to the huge economic losses in aquaculture, there has been greater emphasis on viral diseases. However, less emphasis is being paid toward bacterial diseases of aquaculture animals. They are emerging as most serious microbial communities which affect the health and prosperity of aquaculture sector worldwide. Major diseases caused by various bacteria in aquaculture animals are summarized in Table 18.1 (Haenen 2017). Relatively, bacterial diseases of shrimps have attracted less attention in comparison with viral infections. However, the losses due to bacterial diseases are severe in terms of both capital and quality. The diseases can affect the shrimps both at hatchery and aquaculture farms. Among them, the diseases of shrimps at hatchery are more serious and cause huge economic losses, and at worse situations, they may entirely halt the industry. Luminescent bacteria, especially *Vibrio* spp., cause extensive damage in shrimp hatcheries and farms across the world. Vibrios are the proven sources of disease outbreaks in cultured tiger shrimp (Sung et al. 2001). However, strategies for the management of bacterial diseases are to be explored more extensively and comprehensively. People have tried to breed penaeid shrimps for disease resistance which has been reviewed by Cock et al. (2009). However, it is observable that bacterial diseases of aquaculture animals have been paid less attention.

Table 18.1 List of major bacterial diseases of aquaculture animals (summarized from Haenen 2017)

Sl. no.	Bacterial disease	Causative agent
1	Vibriosis	1. *Vibrio* spp. including *V. anguillarum, V. harveyi clade, V. parahaemolyticus, Aliivibrio salmonicida (V. salmonicida), V. vulnificus*
		2. *Photobacterium damselae*
2	Aeromonasis	*Aeromonas* spp. including *Aeromonas caviae, A. hydrophila, A. sobria, A. veronii, A. jandaei, A. salmonicida*
3	Edwardsiellosis	*Edwardsiella anguillarum, E. ictaluri, E. piscicida, E. tarda, Yersinia ruckeri*
4	Pseudomonasis	*Pseudomonas anguilliseptica, P. fluorescens*
5	Flavobacteriosis	*Flavobacterium branchiophilum, F. columnare, F. psychrophilum, Tenacibaculum maritinum*
6	Mycobacteriosis	Caused by *Mycobacterium* spp. and other filamentous actinobacteria:
		1. *Mycobacterium fortuitum, M. marinum*
		2. *Nocardia asteroides, N. crassostreae (ostreae), N. seriolae*
7	Streptococcosis	*Streptococcus agalactiae, S. iniae, Lactococcus garvieae, Aerococcus viridans*
8	Other bacterial diseases	1. *Renibacterium salmoninarum*
		2. Anaerobic bacteria such as *Clostridium botulinum, Enterobacterium catenabacterium*
		3. Intracellular infections by *Piscirickettsia salmonis, Hepatobacter penaei, Francisella noatunensis, Chlamydia* spp.

18.3 Various Disease Control Strategies in Practice

Both prevention and control strategies are being followed in aquaculture disease management practices. Aquaculture is a very rapidly developing food industry all over the world. It is one of the most needed industrial development for managing the food security issues in the developing countries. It is expected to meet the demands of overexploding human population in both Asia and Africa. India, with a vast coastline and enormous marine resources, is having greater potential to build up this industry as a productive economic sector. Shrimp culture is the most voluminous and important sector in the aquaculture industry of our country. Like agriculture, diseases caused by many organisms are the main challenge for shrimp aquaculture industry. Recently, analyses have shown that the economic losses because of infectious diseases in the shrimp aquaculture alone would come around three billion US dollars (Lundin 1996; Karunasagar et al. 2004). Assefa and Abunna (2018) emphasized that not a single disease control or disease prevention strategy could provide complete disease management. These researchers depicted to adapt integrated disease management practices by combining different strategies that would be more productive. Figure 18.1 summarizes the various strategies being followed for the management of aquaculture diseases. Each of the disease management strategies has its own merits and demerits. Though these methods are following two strategies,

Fig. 18.1 Summary of aquaculture disease management practices

i.e., prevention and control, the preventive strategies are most preferred due to avoidance of risk factors. Table 18.2 also summarizes various aspects of these disease management practices in aquaculture.

Among various disease control strategies, use of antibiotics has been studied quite extensively since it has been widely used by aquaculture farmers. Roque et al. (2001) have demonstrated the control of vibriosis in penaeid shrimps by using 15 antibiotics with different levels of microbial susceptibility in Mexico. Heuer et al. (2009) extensively reviewed about environmental and human health hazards, resulting in response to overuse of antibiotics in aquaculture. There is increasing interest in using probiotics for the control of shrimp disease in both farm and hatcheries (Gatesoupe 1999; Jayaprakashvel et al. 2014). Additionally, Das et al. (2010) have isolated and characterized three *Streptomyces* spp. from oceanic environment for their effective use as aquaculture probiotic organisms. They have also demonstrated satisfactory control in the population strength of vibrios and reduced the disease severity. Though probiotics are very good preventive agents, their curative effect is very poor (Wang et al. 2008). In this scenario, research may be focused to develop a novel disease control strategy by utilizing bacterial intercellular communication systems.

Table 18.2 Summary of disease management practices in aquaculture hatcheries and farms

Sl. no.	Strategy	Advantage	Disadvantage
1	Antibiotic therapy: use of antibiotics	1. It is a control strategy 2. It could produce disease control in shorter period 3. It can provide curative effect as well 4. It could save the harvest which is already infected	1. Leads to development of antibiotic resistance in aquaculture pathogens 2. Leads to pose risks of resistance spread through other organisms 3. The use of antibiotics is restricted to save environment and public health
2	Vaccination: use of immune boosting methods	1. Use of: i. Heat-killed vaccine ii. Live attenuated vaccines iii. DNA vaccines iv. Recombinant vaccines v. Synthetic peptide vaccines 2. It is a preventive strategy	It cannot provide curative effect The immune system of aquaculture animals is comparatively poorly developed
3	Pond management and cultural practices	(a) Preventive strategy (b) Less expensive (c) Uses improved husbandry/ management practices, movement restrictions, genetically resistant stock, dietary supplements	(a) Time consuming (b) Labour intensive (c) Cannot provide curative effect
4	Prevention by bioagents	(a) It can provide effective prevention (b) Uses probiotics, prebiotics, and medicinal plants (c) Safe to environment	(a) Cannot provide curative effect (b) Success rate is subject to environmental conditions (c) Strain variations are there which question the uniform efficacy
5	Chemical control	(a) It uses chemical with biocide potential (b) Immediate curative effect (c) Much effective and less cost intensive	(a) Spoil environment (b) Usage is banned/ highly restricted (c) Cannot be preventive (d) Residue effects in the animals leads to long-term health effects in consumers
6	Biosecurity methods	(a) Biosecurity measures include stringent animal quarantine protocols, disinfection of eggs, water sanitation, appropriate feed, and destroying dead animals (b) Much effective	(a) Needs much technical expertise (b) High cost involved (c) Curative effect is not possible (d) Labour intensive

18.4 QS and QQ as Inevitable Attributes for an Alternative Disease Control Strategy

Antibiotic agents are the common choice for the management of bacterial infections in aquaculture hatcheries and farms. These agents were applied as feed fortified with antibiotics and also by adopting the immersion therapy (adding directly to pond water) (Rodgers and Furones 2009). However, frequent and intense use of antibiotics in aquaculture hatcheries and farms develops a selective pressure on the aquaculture animals. It also adversely affects the environment, creating reservoirs of drug-resistant bacteria and transferable resistance genes in pathogens and other bacteria in aquatic environment. From these reservoirs, resistant genes may disseminate by horizontal gene transfer and reach human pathogens or drug-resistant pathogens from aquatic environment which ultimately would be biomagnified and affect public health (Heuer et al. 2009). So, alternative strategies, which will not induce resistance in pathogens, are desirable (Defoirdt et al. 2007) in the current scenario on controlling shrimp diseases. Inhibition of quorum sensing (QS) has been proposed as an alternative strategy for the management of bacterial infections. Several approaches were being proposed to negatively affect the bacterial communication to stop their quorum sensing system (Jayaprakashvel and Shanmugaiah 2015). Researchers in other parts of the world have speculated and demonstrated the effectives of interfering QS in aquaculture (Defoirdt et al. 2007). However, comprehensive and conclusive studies on the inhibition or modification of bacterial QS system still hold much promise.

Most of the pathogens regulate their virulence factors such as biofilm formation, peptide synthesis, and production of certain enzymes through a population density-dependent intercellular bacterial communication system called QS (de Kievit and Iglewski 2000). When these QS systems are disrupted, the bacterial pathogens lose their disease-causing potential (Defoirdt et al. 2006, 2010). Autoinducer (AI) molecules are produced as signaling molecules to sense the bacterial populations in the vicinity by the QS organisms. However, the organisms that combat the QS are reported to produce quite a few QSI enzymes and higher organisms are proved to disrupt this QS phenomenon in many systems (Teplitski et al. 2000; Bauer and Robinson 2002; Bauer and Mathesius 2004). Many researchers elsewhere in the world (Manefield et al. 2000; Defoirdt et al. 2006; You et al. 2007) have studied this novel and impressive way of disease control in aquaculture. Nonetheless, this promising strategy has not been comprehensively attempted in India. So, it is much anticipated that one could get some potent novel QS inhibitors from our marine resources like marine microbes and other organisms from the vast coastal and marine resources of India. The information about the different bacterial pathogens associated with the shrimp diseases is not yet conclusive. So, it is very essential to isolate pathogenic bacteria associated with shrimp larvae in the hatchery. This would provide information about the dominance of bacterial pathogens in shrimp hatcheries and the same would help to form unique strategies for their management.

18.5 Shrimp Diseases and QS of Aquaculture Bacterial Pathogens

Until very recently, it was strongly believed that bacteria were existing as monocellular forms only to find their nutrients from the environment and also to reproduce by binary fission. During the 1970s, scientists have discovered a cell-to-cell communication mediated by signaling molecules among bacteria. This has prompted scientists across the globe that bacteria could exist as microbial communities to evade the harsh environmental conditions or to exhibit their virulence factors in a coordinated manner. The discovery of intercellular communication among bacteria has led to a realization that bacteria are capable of exhibiting coordinated activities such as biofilm formation, luminescence, virulence factor production, nodule formation, etc. The phenomenon of quorum sensing or autoinducer-mediated intercellular bacterial communication has been found to exist among many environmental bacteria, especially in the aquatic environments. The concept of quorum sensing strongly depends on the principle that at low cell densities, the QS operating bacterium produces lesser quantity of the AIs which can be hardly detected by bacteria of the same nature. Similarly, when the bacterial community reaches a threshold population, their production of AIs increases considerably so that all the similar bacteria in the vicinity effectively sense the levels of AIs in turn the direct proportionate population of the producing bacteria. This allows the bacteria to sense a threshold cell mass, and in response, to activate or repress set of genes involved in the exhibition of certain phenotypes, for example, biofilm formation (Taga and Bassler 2003). Interestingly, acylated homoserine lactones (AHLs) and AI peptides are the most studied signaling molecules of gram-negative and gram-positive bacteria, respectively (Taga and Bassler 2003). Most of the bacteria so far identified as QS system operators are found to have some synergistic or pathogenic association with higher-order organisms such as plants and animals (de Kievit and Iglewski 2000; You et al. 2006). The genes identified in relation to QS system are found very critical for their virulence, biofilm formation, and colonization of eukaryotic hosts (Bauer and Robinson 2002).

It has been evidently understood that the QS system in pathogenic bacteria regulates the synthesis of virulence factors. Hence, it is now proposed and successfully demonstrated in many host systems that if we could alter or inactivate AIs and suppress QS signal generation, we could modify the gene response in pathogens to operate virulence factors and thus could avoid the pathogen to express its virulence mechanism. Thus, it could be useful in controlling infection development and persistence of human, animal, and plant bacterial pathogens (Mäe et al. 2001; Fray 2002; Hentzer et al. 2002; Zhang 2003; Ozer et al. 2005).

Hence, researchers targeted QS systems of pathogens as a novel strategy to combat the infectious diseases in aquaculture animals (Defoirdt et al. 2004) such as *Macrobrachium rosenbergii* (Baruah et al. 2009); Artemia (van Cam et al. 2009), rotifer *Brachionus plicatilis* (Tinh et al. 2007), and on first-feeding turbot larvae

Scophthalmus maximus L. (Tinh et al. 2008). In this scenario, it can be concluded that the disruption of QS has been considered as a novel disease control strategy in aquaculture in which the international researchers are progressing steadily. However, this novel disease control strategy is yet to be studied more comprehensively with special reference to Indian aquaculture.

In India, the profiling of shrimp pathogens at hatchery and farms has been carried out to some extent. But our literature survey has suggested that there has not been much work on QS of aquaculture pathogens. Moreover, the use of QS inhibitors as disease control molecules in shrimp aquaculture has been a relatively unexplored strategy in India. Nayak et al. (2010) identified unregulated immune-related genes in *P. monodon* postlarvae which are produced against artificial infection of *V. harveyi* (Bramhachari and Dubey 2006). Remarkably, Sharma et al. (2010) evaluated the immune response and resistance to diseases in tiger shrimp, *P. monodon.* They have initially fed the animals with biofilm forming *V. alginolyticus* and concluded that the immunization of animals resulted in the quick reduction of *V. alginolyticus* and WSSV from the internal systems of the shrimps and thus had provided effective resistance by shrimp juveniles against viral and bacterial pathogens. Probiotics for aquaculture are studied extensively in India. Very recently, Soundarapandian and Babu (2010) studied the effect of probiotics on the hatchery seed production of black tiger shrimp, *Penaeus monodon* (Fabricius). Selvin and Lipton (2004) have worked on *Dendrilla nigra*, a marine sponge, and demonstrated its effective usage as a resource of antibacterial substances for managing shrimp diseases.

Vaseeharan et al. (2007) isolated a novel *Photobacterium damselae* ssp. *damselae* strain from the infected *P. monodon*, a tiger shrimp, in India. Immanuel et al. (2004) have isolated solvent extracts (butanol extracts) from some of the land-based herbal plants and seaweeds. They have demonstrated the effect of those extracts on the survival, growth, and pathogen (*V. parahaemolyticus*) load on shrimp *Penaeus indicus* at larval stages. Chrisolite et al. (2008) have found the presence of bioluminescent *V. harveyi* from the operational shrimp hatcheries in the southern part of India. They also have isolated and established the inhibitory effect of bacteriophages against shrimp pathogenic bacteria. Gopala et al. (2005) have studied the presence of pathogenic vibrios from the shrimp industry and demonstrated their ill effects in the aquaculture environments. It is noteworthy that Karunasagar et al. (2007) worked extensively on biocontrol of pathogens in shrimp hatcheries using bacteriophage which is one of the recent disease control strategies. In almost all types of shrimp hatcheries, *Vibrio* infections are found in India including the semi-intensive penaeid shrimp hatcheries of Tamil Nadu (Abraham and Palaniappan 2004). Tyagi et al. (2007) envisaged the transgene technology to control the vibriosis of shrimps. They have developed a lysozyme to be expressed from *P. monodon* through recombinant DNA technology. These recombinant lysozymes have been reported to have antibiotic potential against vibrios in shrimp.

18.6 Quorum Quenching in Aquaculture Pathogens as a New Disease Management Strategy

Aquaculture and fisheries are considered to be the best approaches for the sustenance of global food security in the population explosion era. Aquaculture, especially shrimp aquaculture, has been followed intensively owing to technical advancements. However, the bacterial and viral diseases of shrimps are found to be the chief restraining factors of shrimp aquaculture. Among the various diseases caused by pathogens, bacterial diseases of shrimps at their larval stages in hatcheries are reported to be very tough to manage. Several species of luminescent *Vibrio* spp. and quite a few *Bacillus* spp. are found associated with devastating bacterial infections in shrimp larvae (Vaseeharan et al. 2007; Sung et al. 2001). Nonetheless, antibiotics could offer better control against bacterial infections. However, due to increasing awareness against chemical usage, regulations against antibiotics, emergence of antimicrobial-resistant bacteria in fish and other aquatic animals, and fear over antibiotic residues in animals necessitate developing novel disease management technologies for the effective management of shrimp production through aquaculture.

It has been demonstrated that the major bacterial pathogens of shrimps were found to operate the quorum sensing system to exhibit their virulence factors and pathogenicity. They were found to operate quorum sensing for the establishment of biofilms. Hence, it could be understood that quorum sensing could be the potential target to inhibit the disease-causing ability of bacterial pathogens in shrimp (Zhao et al. 2015). Moreover, QS is a classical gene regulation system of many pathogens in humans, plants, and animals. Disruption of QS molecules in the pathogens by antiquorum sensing metabolites such as lactonoses, halogenated furanones, etc. are considered as a novel disease control strategy in many ecosystems. Moreover, in a more controlled environments such as shrimp hatcheries, the disruption of QS could be a reliable and ecofriendly disease management strategy. The disruption of QS by AI inhibitors completely blocks the QS system in bacterial pathogens. They not only prevent the expression of virulence-associated genes but also could attenuate the disease-causing ability of shrimp pathogenic bacteria. Hence, inhibition of QS has been proposed as a new anti-infective strategy. Several techniques that could be used to disrupt QS were investigated with reference to shrimp aquaculture in other parts of the world (Defoirdt et al. 2007). However, not much work has been done on this novel disease control strategy in India. Scores of currently studied QS inhibitors are from marine resources. Actinobacteria, especially marine actinobacteria, are prolific producers of effective antibiotics. Nearly 70% of recently discovered antibiotics are from actinobacteria. The marine actinobacteria are considered as a sustainable and effective bioresources for the search source of novel bioactive metabolites (Subramani and Aalbersberg 2012). Since the marine actinobacteria are relatively less explored microbial community, chances are plenty to obtain potent antimicrobial metabolites that could disrupt the QS system and attenuate the virulence factors of shrimp pathogens.

18.7 Future Perspectives

The aquaculture sector is the fastest growing agriculture sector which is directly related to future food security. The heavy use of antibiotics in this sector for control of bacterial infections has been proven to have environmental and human health hazards. Recent advancement in scientific research has paved the way to use alternative strategies for disease management in aquaculture. The bacterial infections in aquaculture can be controlled with QS inhibitors, a relatively effective yet a novel disease control strategy. This alternative, new strategy would pave way for the use of environment-friendly disease control strategy in aquaculture sector with no direct or indirect negative impacts on human health and environment.

18.8 Conclusion

Based on the fact that nearly the virulence factors of bacterial pathogens in aquaculture are being controlled by QS, the microbial metabolites with the ability to block the QS can be used to prevent bacterial infection in aquaculture.

Acknowledgments Author MJ would like to record his acknowledgments to the Management of AMET University for encouragement and financial support in the form of seed money. Author RS thanks the University of South Pacific, Fiji, for support and encouragement.

References

Abraham TJ, Palaniappan R (2004) Distribution of luminous bacteria in semiintensive penaeid shrimp hatcheries of Tamil Nadu, India. Aquaculture 232:81–90

Ahmed N, Thompson S (2019) The blue dimensions of aquaculture. Sci Total Environ 20(652):851–861

Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Vet Med Int 2018:10 pages. Article ID 5432497. https://doi.org/10.1155/2018/5432497

Baruah K, Cam DTV, Dierckens K, Wille M, Defoirdt T, Sorgeloos P, Bossier P (2009) In vivo effects of single or combined N-acyl homoserine lactone quorum sensing signals on the performance of Macrobrachium rosenbergii larvae. Aquaculture 288:233–238

Bauer WD, Mathesius U (2004) Plant responses to bacterial quorum sensing signals. Curr Opin Plant Biol 7:429–433

Bauer WD, Robinson JB (2002) Disruption of bacterial quorum sensing by other organisms. Curr Opin Biotechnol 13:234–237

Béné C, Arthur R, Norbury H, Allison EH, Beveridge M, Bush S, Campling L, Leschen W, Little D, Squires D, Thilsted SH, Troell M, Williams M (2016) Contribution of fisheries and aquaculture to food security and poverty reduction: assessing the current evidence. World Dev 79:177–196

Bondad-Reantaso MG, Subasinghe RP, Josupeit H, Cai J, Zhou X (2012) The role of crustacean fisheries and aquaculture in global food security: past, present and future. J Invertebr Pathol 110(2):158–165

Bramhachari PV, Dubey SK (2006) Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. Lett Appl Microbiol 43(5):571–577

Chan CY, Tran N, Pethiyagoda S, Crissman SC, Sulser TB, Phillips MJ (2019) Prospects and challenges of fish for food security in Africa. Glob Food Sec 20:17–25

Chrisolite B, Thiyagarajan S, Alavandi SV, Abhilash EC, Kalaimani N, Vijayan KK, Santiago TC (2008) Distribution of luminescent Vibrio harveyi and their bacteriophages in a commercial shrimp hatchery in South India. Aquaculture 275:13–19

Cock J, Gitterle T, Salazar M, Rye M (2009) Breeding for disease resistance of Penaeid shrimps. Aquaculture 286:1–11. 10.1016

Das S, Adams L, Burke C (2010) Screening of marine Streptomyces spp. for potential use as probiotics in aquaculture. Aquaculture 305:32–41

Defoirdt T, Boon N, Bossier P, Verstraete W (2004) Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. Aquaculture 240:69–88

Defoirdt T et al (2006) Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp Artemia franciscana from pathogenic Vibrio harveyi, Vibrio campbellii, and Vibrio parahaemolyticus isolates. Appl Environ Microbiol 72:6419–6423

Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P (2007) Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. Trends Biotechnol 25:472–479

Defoirdt T, Boon N, Bossier P (2010) Can bacteria evolve resistance to quorum sensing disruption. PLoS Path e1000989:6

de Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849

Fray RG (2002) Altering plant–microbe interaction through artificially manipulating bacterial quorum sensing. Ann Bot 89:245–253

Gatesoupe FJ (1999) The use of probiotics in aquaculture. Aquaculture 180:147–165. https://doi.org/10.1016/S0044-8486(99)00187-8

Gopala S, Ottaa SK, Kumar S, Karunasagar I, Nishibuchi M, Iddya Karunasagar T (2005) The occurrence of Vibrio species in tropical shrimp culture environments; implications for food safety. Int J Food Microbiol 102:151–159

Gupta MV (2018) Contribution of aquaculture to global food security. In: Reference module in food science. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.22361-5

Haenen O (2017, April) Major bacterial diseases affecting aquaculture. Aquatic AMR Workshop, Mangalore, India, vol 1, 10–11 April 2017. Weblink: http://www.fao.org/fi/static-media/MeetingDocuments/WorkshopAMR/presentations/07_Haenen.pdf

Hentzer M, Riedel K, Rasmussen TB et al (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology 148:87–102

Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ (2009) Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis 49:1248–1253

Immanuel G, Vincybai VC, Sivaram V, Marian MP (2004) Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (Vibrio parahaemolyticus) load on shrimp Penaeus indicus juveniles. Aquaculture 236(1–4):53–65

Jayaprakashvel M, Shanmugaiah V (2015) Quorum sensing in plant pathogenic and plant associated bacteria. In: Rajeshkannan V, Kurtulus BK (eds) Sustainable approaches on controlling of plant pathogenic bacteria. CRC Press, pp 223–240

Jayaprakashvel M, Guru R, Surendiran G, Chelvan Y, Ashok Kumar P, Selvanayagi K, Jaffar Hussain A (2014) Antagonistic mechanism of probiotic Lactobacillus against sea food and human pathogenic Bacteria. Biosci Biotechnol Res Asia 11.(Spl. Edn. 1:45–52

Karunasagar I, Karunasagar I, Umesha RK (2004) Microbial diseases in shrimp aquaculture, pp 121–134. https://pdfs.semanticscholar.org/fd5c/a1854f713f1f6572b506d3b77b75ab1f501c.pdf?_ga=2.159626749.1139818412.1567775712-327379712.1560235569

Karunasagar I, Shivu MM, Girisha SK, Krohne G, Karunasagar I (2007) Biocontrol of pathogens in shrimp hatcheries using bacteriophages. Aquaculture 286:288–292

Little DC, Bunting SW (2016) 5 – Aquaculture technologies for food security. In: Madramootoo C (ed) In Woodhead publishing series in food science, technology and nutrition, emerging technologies for promoting food security. Woodhead Publishing, pp 93–113

Lundin CG (1996) Global attempts to address shrimp diseases. Marine Environmental Paper No. 4, Land, Water and Natural Habitats Division, Environmental Department, The World Bank. 45 pp

Mäe A, Montesano M, Koiv V, Palva ET (2001) Transgenic plants producing the bacterial pheromone N-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen Erwinia carotovora. Mol Plant-Microbe Interact 14:1035–1042

Manefield M, Harris L, Rice SA, de Nys R, Kjelleberg S (2000) Inhibition of luminescence and virulence in the black tiger prawn (Penaeus monodon) pathogen Vibrio harveyi by intercellular signal antagonists. Appl Environ Microbiol 66:2079–2084

Nayak S, Singh SK, Ramaiah N, Sreepada RA (2010) Identification of upregulated immune-related genes in Vibrio harveyi challenged Penaeus monodon postlarvae. Fish Shellfish Immunol 29:544–549

Ozer EA, Pezzulo A, Shih DM, Chun C, Furlong C, Lusis AJ, Greenberg EP, Zabner J (2005) Human and murine paraoxonase 1 are host modulators of *Pseudomonas aeruginosa* quorum-sensing. FEMS Microbiol Lett 253:29–37

Rodgers CJ, Furones MD (2009) Antimicrobial agents in aquaculture: practice, needs and issues. In: Rodgers CJ, Basurco B (eds) The use of veterinary drugs and vaccines in Mediterranean aquaculture. CIHEAM, Zaragoza, pp 41–59

Roque A, Gómez-Gil B, Guerra FA (2001) Enfermedades infecciosas más comunes en la camaronicultura en México y el impacto del uso de antimicrobianos. In: Páez-Osuna F (ed) Camaronicultura y Medio Ambiente. Instituto de Ciencias del Mar y Limnología. Universidad Nacional Autónoma de México. Mazatlán, Sin, Unidad Académica Mazatlán, México, pp 315–346

Selvina J, Lipton AP (2004) Dendrilla nigra, a marine sponge, as potential source of antibacterial substances for managing shrimp diseases. Aquaculture 236(1–4):277–283

Sharma K, Shankar SR, Sathyanarayana KM, Sahoo ML, Rajreddy Patil AK, Narayanaswamy HD, Rao S (2010) Evaluation of immune response and resistance to diseases in tiger shrimp, *Penaeus monodon* fed with biofilm of Vibrio alginolyticus. Fish Shellfish Immunol 29:724–732

Soundarapandian P, Babu R (2010) Effect of probiotics on the hatchery seed production of Black Tiger Shrimp, Penaeus monodon (Fabricius). Int J Anim Vet Adv 2(1):9–15

Stevens JR, Newton RW, Tlusty M, Little DC (2018) The rise of aquaculture by-products: increasing food production, value, and sustainability through strategic utilisation. Mar Policy 90:115–124

Subramani R, Aalbersberg W (2012) Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res 167(10):571–580

Sung H-H, Hsu S-F, Chen C-K, Ting Y-Y, Chao W-L (2001) Relationships between disease outbreaks in cultured tiger shrimps (Penaeus monodon) and the composition of Vibrio communities in pond water and shrimp hepatopancreas during cultivation. Aquaculture 192:101–110

Taga ME, Bassler BL (2003) Chemical communication among bacteria. Proc Natl Acad Sci U S A 100(Suppl. 2):14 549–14 554

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population densitydependent behaviors in associated bacteria. Mol Plant-Microbe Interact 13:637–648

Tinh NTN, Asanka Gunasekara RAYS, Boon N, Dierckens K, Sorgeloos P, Bossier P (2007) N-acyl homoserine lactone-degrading microbial enrichment cultures isolated from Penaeus vannamei shrimp gut and their probiotic properties in Brachionus plicatilis cultures. FEMS Microbiol Ecol 62(1):45–53

Tinh NTN, Yen VHN, Dierckens K, Sorgeloos P, Bossier P (2008) An acylhomoserine lactone-degrading microbial community improves the survival of first-feeding turbot larvae (*Scophthalmus maximus* L.). Aquaculture 285:56–62

Tyagi A, Khushiramani R, Karunasagar I, Karunasagar I (2007) Antivibrio activity of recombinant lysozyme expressed from black tiger shrimp, *Penaeus monodon*. Aquaculture 272:246–253

Van Cam DT, Van Hao N, Dierckens K, Defoirdt T, Boon N, Sorgeloos P, Bossier P (2009) Novel approach of using homoserine lactone-degrading and poly-βhydroxybutyrate-accumulating bacteria to protect Artemia from the pathogenic effects of Vibrio harveyi. Aquaculture 291(1):23–30

Vaseeharan B, Sundararaj S, Murugan T, Chen JC (2007) Photobacterium damselae ssp. damselae associated with diseased black tiger shrimp Penaeus monodon Fabricius in India. Soc Appl Microbiol Lett Appl Microbiol 45(2007):82–86

Wang Y-B, Li J-R, Lin J (2008) Probiotics in aquaculture: challenges and outlook. Aquaculture 281:1–4

You YS, Marella H, Zentella R, Zhou Y, Ulmasov T, Ho TH et al (2006) Use of bacterial quorum-sensing components to regulate gene expression in plants. Plant Physiol 140:1205–1212

You J, Xue X, Cao L, Lu X, Wang J, Zhang L et al (2007) Inhibition of Vibrio biofilm formation by a marine actinomycete strain A66. Appl Microbiol Biotechnol 76:1137–1144

Zhang LH (2003) Quorum quenching and proactive host defense. Trends Plant Sci 8:238–244

Zhao Z, Eberhart LJ, Orfe LH, Lu SY, Besser TE, Call DR (2015) Genome-wide screening identifies six genes that are associated with susceptibility to Escherichia coli microcin PDI. Appl Environ Microbiol 81:6953–6963. https://doi.org/10.1128/AEM.01704-15

Strategies for Disruption of Biofilm Formation Ability and Intricate Quorum Sensing Networks in Aquaculture Vibrio Pathogens

19

Pallaval Veera Bramhachari, A. M. V. N. Prathyusha, Ganugula Mohana Sheela, Neelapu Nageswara Rao Reddy, Chanda Vikrant Berde, and N. M. Yugandhar

Abstract

Vibriosis is a serious disease problem in Indian aquaculture. In the past few years, disease problem particularly caused by *Vibrio harveyi* has been severe in hatcheries, and many units were shut down due to resistance against multiple antibiotics. Since two decades, antibiotics are widely used in aquaculture if bacterial infections are serious. Due to indiscriminate use of antibiotics, *Vibrio* sp. acquired resistance against multiple antibiotics. Moreover, recently, increasing literature clearly emphasized that antimicrobial-resistant (AMR) genes are transferred to human pathogens. Pertinantly, the quest for finding alternative methods to control multiple antibiotic-resistant *vibrios* is of utmost priority to have sustainable development in aquaculture. One such alternative and promising method is interruption of bacterial cell-to-cell communication, called quorum sensing (QS). The QS system of *V. harveyi* comprises of four parallel systems that con-

Pallaval Veera Bramhachari, A. M. V. N. Prathyusha, Ganugula Mohana Sheela, Neelapu Nageswara Rao Reddy, Chanda Vikrant Berde and N. M. Yugandhar have equally contributed to this chapter.

P. V. Bramhachari (✉) · A. M. V. N. Prathyusha · G. M. Sheela
Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
e-mail: veerabramha@gmail.com

N. N. Rao Reddy
Department of Biochemistry and Bioinformatics, Institute of Science, Gandhi Institute of Technology and Management (GITAM) (Deemed-to-be University), Visakhapatnam, Andhra Pradesh, India

C. V. Berde
Department of Microbiology, Gogate Jogalekar College, Ratnagiri, Maharashtra, India

N. M. Yugandhar
Department of Chemical Engineering, Andhra University, Visakhapatnam, Andhra Pradesh, India

P. V. Bramhachari (ed.), *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*,
https://doi.org/10.1007/978-981-32-9409-7_19

verge onto a single regulatory pathway, which in turn regulates a number of virulence genes required by the *V. harveyi* for its pathogenesis. Several studies reported that disruption of QS by enzymatic or chemical inactivation in *V. harveyi* is triumphant in controlling vibriosis, with some selective pressure for evolution of resistance.

Keywords
Aquaculture · Antibiotics · Antimicrobial-resistant (AMR) genes · Quorum sensing · Quorum quenching

19.1 Introduction

Aquaculture is an important food-producing sector to fulfill nutritional food demand for continuously growing population. With the abrupt increase in world population and the global decline of aquaculture supplies has constantly triggered the increase in fish; shrimp consumption along with improvement of aquatic animals' domestication has led to intense progression of aquaculture. Currently, aquaculture is one of the fastest developing food sectors, expanded to reach a global production of 90.4 million tons. With the intensification of aquaculture, animal and ecological health problems are deemed to emerge.

Bacterial infections are one of the serious threats to commercial aquaculture which cause huge economic losses. Vibriosis is considered to be one of the foremost diseases in the aquaculture ranging from mollusks to crustaceans to fish caused by a number of *Vibrio* species, viz., *V. vulnificus*, *V. harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. penaeicida* (Bramhachari and Dubey 2006; Haldar 2012). Interestingly, among these, the major pathogen reported in Asian countries is *V. harveyi*. Notably, the biofilms are preferred lifestyle of bacteria as they enhance virulence, stress tolerance, survival and aggregate cells together. Such biofilm-producing ability of *Vibrio* spp. is responsible for their growth, providing free entry to nutrients while preventing antimicrobial compounds. Nonetheless, development of mature biofilm demands secretion of exopolysaccharides (EPS) for the maintenance of biofilm architecture, and the significance was well recognized in various *Vibrio* spp. (Yildiz and Visick 2009). The condition is aggravated by the emergence of antibiotic resistance in *Vibrio* spp. and their ability to form biofilms. Multiple antibiotic-resistant (MAR) *Vibrio* isolates were earlier reported from India (Chari and Dubey 2006; Srinivasan and Ramasamy 2009), Thailand (Jiravanichpaisal 1994), the Philippines (Tendencia and de la Pena 2001), Ecuador (Austin and Zhang 2006), Venezuela (Alvarez, et al. 1998), Mexico (Molina-Aja 2002), and Iran (Raissy 2012). Interestingly, QS control the expression of virulence factors and biofilm formation (Antunes et al. 2010; Prathyusha et al. 2018). Therefore, hostility with *Vibrio* infections by meddling with AI molecules thus disrupts the consequent production of virulence factors and biofilm formation and can avert colossal economic loss in aquaculture industry. For this reason, the disruption of pathogen QS

may perhaps endow with a novel tactic for controlling pathogen infections. This chapter focuses on existing mechanisms that interfere with QS with possible applications in aquaculture as bacterial control (Bramhachari et al. 2018).

19.2 Significance of Quorum Sensing in Aquaculture

The QS mechanism is a synchronized gene expression that involves in production biofilms, virulence factors viz. exoproteases, exotoxins (Manefield et al. 2000), metalloprotease, phospholipase, caseinase, gelatinase (Natrah et al. 2011), siderophore (Lilley and Bassler 2000), type III secretion system in response to cell density (Fig. 19.1). Furthermore, involvement of QS in pathogen virulence regulation, metalloprotease expression, and several aquaculture infections in a number of *Vibrio* species was documented (Decker et al. 2013; Benitez and Silva 2016). The direct evidence for attenuation of QS and reduction of mortality in aquaculture hosts burbot (Natrah et al. 2012), larvae of brine shrimp, and giant freshwater prawn (Pande et al. 2013) was reported in pathogenic *Aeromonas* spp. and *V. campbellii*.

19.2.1 Novel Alternatives to Antibiotics Use: Quorum Sensing Inhibition

Currently keeping in view of the strict regulations and import/export enforecements with regard to ban of antibiotics (Europe and North America) in aquaculture and the emergence of antibiotic resistance are necessary for the development of new

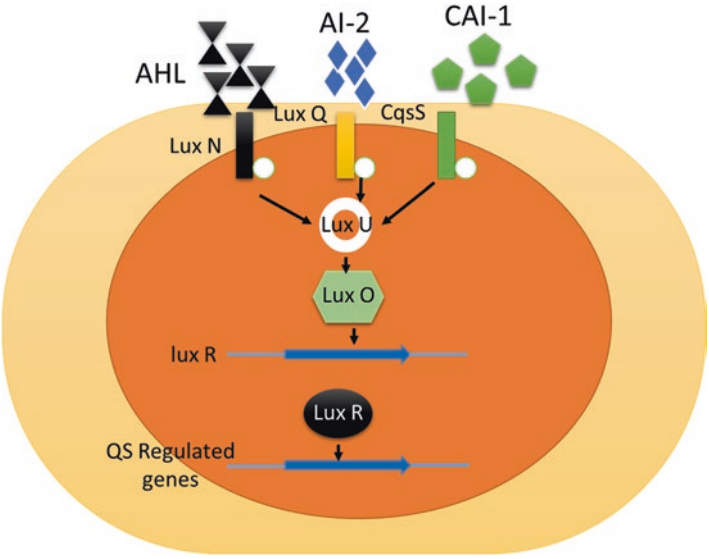


Fig. 19.1 Quorum sensing in *Vibrio harveyi*

strategies for disease control. One of the new emerging antivirulence strategies that was proposed to control bacterial infections in aquaculture is QS interruption. QS is a gene regulation mechanism through which bacteria synchronize target gene expression in response to concentration of small signal molecules often called autoinducers (AIs) (Nealson and Hastings 1979). Aquaculture pathogens like Gram-negative and Gram-positive bacteria employ QS to regulate the expression of important virulence phenotypes (Defoirdt et al. 2008).

19.3 Strategies for Quorum Sensing Inhibition in *Vibrio harveyi* Infections

Owing to close relation between QS and aquatic pathogen virulence, ecological strategies are the preferred alternatives to overcome difficulties of antibiotic resistance and spread of resistant genes in aquatic shrimps, fishes, and higher organisms (Homem and Santos 2011). Compounds that inhibit QS are quorum quenchers (QQs) or quorum sensing inhibitors (QSI), and they are antipathogenic molecules obtained from natural sources like bacteria, algae, plants, and fungi or prepared as synthetic compounds (Rasmussen and Givskov 2006). They function by either the following processes:

(a) Inhibition of autoinducers or signal molecule synthesis.
(b) Degradation of signal molecules.
(c) Inhibition of signal molecule/receptor interaction.

19.3.1 Interference with Signal Generation/Anti-Targets for AHL Synthesis

The blockage of AHL synthesis among the pathogenic aquaculture bacteria is conceptually a simple strategy for inhibition of QS pathways, therefore reducing the production of signal molecule and activating QS. AHL (Acyl Homoserine lactones) molecules are synthesized by AHL synthase from fatty acid biosynthesis pathway and S-adenosylmethionine (SAM). Compounds that inhibit fatty acid, SAM biosynthesis, and enzymatic activity of acyl synthase or efflux pumps would work at basal level in QS signal generation and act as QSI (Hirakawa and Tomita 2013) (Fig. 19.2).

19.3.2 Strategies Targeting QS Signal: Degradation of the Signal Molecule

Interference in bacterial communication is also accomplished by reducing active AHL molecules. Inactivation or complete degradation of AHL signal molecules can be achieved by chemical degradation and enzymatic destruction or metabolism of

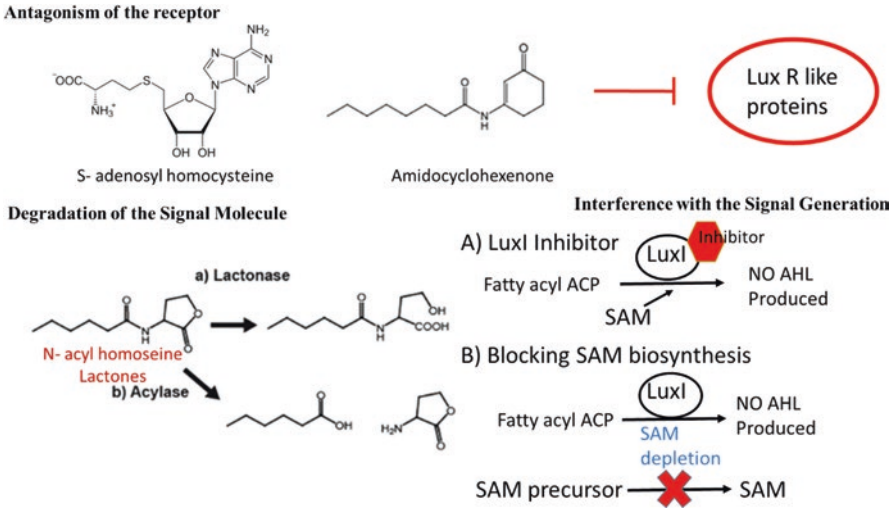


Fig. 19.2 Strategies for quorum sensing disruption

AHL molecules. In laboratory, AHL molecule degradation can be done by opening of the lactone ring either by increasing pH above 7.0 or temperature > 37 °C or by enzymatic degradation called lactonolysis (Yates et al. 2002). Another way of chemical inactivation is through oxidized halogen antimicrobials. The AHL signal molecules such as 3-oxo-C12 HSL (Homoserine lactone) get inactivated on reaction with oxidized halogen compounds like hypobromous and hypochlorous acids (Borchardt et al. 2001) (Fig. 19.2).

19.3.3 Inhibition of Signal Molecule/Receptor Interaction or Antagonism of the Receptor

The most significantly studied QSI strategy is analogs for signal molecules that block target receptors. Signal mimics bind receptor either by competitive binding or by displacing original AHL (Ni et al. 2009). The well-studied natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5 H)-furanone is reported to possess high inhibitory activities in different Gram-negative bacteria. Furanones were shown to block QS by AHL-controlled expression and also AI-2 signaling (Ren et al. 2001). QS is unique to each bacterial species; however, many QSI were developed for broad-spectrum effects (Fig. 19.2).

Other strategies, such as probiotics, prebiotics, symbiotics, phytobiotics, bacteriophages, quorum sensing interference, are reported. These biological control strategies were proposed to promote the health and welfare of farmed culture shrimps, fishes, etc. (Fig. 19.3) (Pérez-Sánchez et al. 2018).

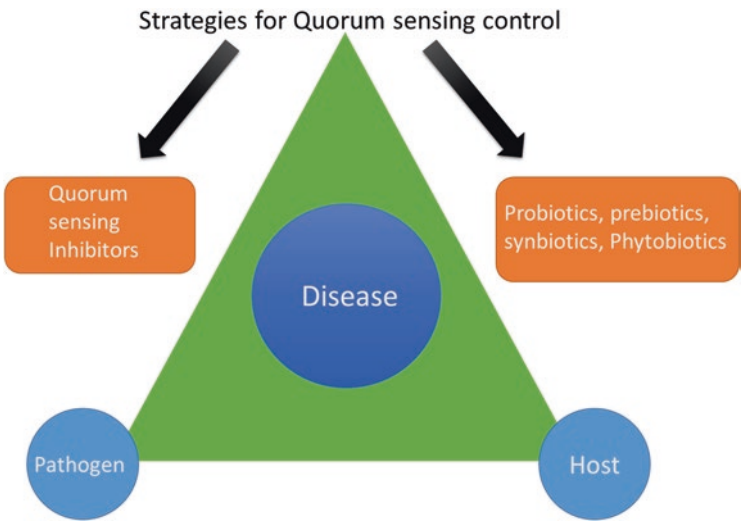


Fig. 19.3 Strategies for biocontrol of quorum sensing

19.4 Quorum Sensing Inhibitors

QSIs are mainly of two types: (a) extracted from natural sources such as natural furanones of (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone, halogenated furanones, furanones F2, brominated 3-alkyl-5-methylene-2(5H)-furanones, and alkylmaleic anhydrides and (b) obtained from chemical synthesis, viz., thiophenone (Z)-4-((5-(bromomethylene)-2-oxo-2,5-dihydrothiophen-3-yl)-4-oxobutanoic acid). These are effective in attenuating bacterial virulence. Owing to high toxicity of furanones to higher organisms, they are less likely to be used in aquaculture (Defoirdt et al. 2006; Pande et al. 2013). Coumarin was effective in reducing biofilm formation of *S. aureus*, *E. coli*, *V. anguillarum*, *and E. tarda*. These four strains use three AIs, viz., AHLs, AI-2, and agr, signifying the ability of coumarin to attenuate bacterial disease in aquaculture and could be universal QS inhibitor (Gutiérrez-Barranquero et al. 2015). DTBMP (2,6-di-tert-butyl-4-methylphenol) from *C. turgidus* is reported to effectively inhibit bioluminescence in *V. harveyi*. DTBMP also inhibit other biofilm-related virulence traits, viz., EPS production and swarming motility in three major aquatic pathogenic vibrios at its BIC concentration (Santhakumari et al. 2018).

19.5 Biocontrol Strategies for Disruption of QS in Aquaculture

The trend of using biological approaches to control aquaculture infections by disrupting QS systems of pathogens is encouraging. Probiotics can develop hostile conditions for pathogens by synthesis of antimicrobial compounds, generating

competition for space and nutrients, and disruption of quorum sensing. The positive effect of *Bacillus* sp. as probiotics organisms may be due to degradation of AIs along with the synthesis of growth-inhibiting substances (Kuebutornye et al. 2019). The microalgae *C. saccharophila* CCAP211/48, commonly employed in aquaculture, produces QS antagonistic metabolites that exhibited stable inhibitory activity on *V. harveyi* that wasn't reported previously (Natrah et al. 2011) (Fig. 19.3). Plant extracts reported with antiparasitic, anti-inflammatory, and antimicrobial activities were recently investigated in aquaculture. Essential oils (anethole, shogaol, thymol, and limonene) possessed antimicrobial activity against different Gram-negative and Gram-positive bacteria. Dietary administration of extracts from oregano (*Lippia berlandieri Schauer*), neem (*Azadirachta indica*), and ginger (shogaol) (Soowannayan et al. 2019) reported higher survival rates in white shrimp postlarvae exposed to *V. parahaemolyticus* infection compared to control group. Cinnamaldehyde, a QQ compound, protects burbot (*Lota lota* L.) larvae from *A. hydrophila* and *A. salmonicida*, giant freshwater prawn from *M. rosenbergii*, and brine shrimp larvae from *V. harveyi* (Natrah et al. 2012; Pande et al. 2013; Brackman et al. 2013). Synbiotics are combination of both probiotics with prebiotics and plant extracts, thereby improving the survival rate in commercial aquaculture.

19.6 Future Directions

In summary, the QS inhibition seems to be a promising strategy as antivirulence strategies to combat the antibiotic-resistant bacteria. However, there are a lot of difficulties that need to be overcome in the development of QS inhibitors. The mechanisms of some inhibitors are still unclear, and a few of inhibitors may possess potential toxicity toward host cells. Therefore, these aspects should be thoroughly investigated before any QS inhibitor is licensed and put on the market. In conclusion, a better comprehension of biofilm dynamics and chemical signals released by intricate biofilms will help develop novel antifouling alternatives. Nevertheless, an enhanced perception on the genetics with signal transduction pathways underlying settlement processes will be indispensable for advancements in new antifoulants where pathogens are barred from colonization.

Acknowledgments Dr. Bramhachari and colleagues are grateful to Krishna University, Machilipatnam for the extended support.

Conflict of Interest The authors declare that they have no competing interests.

References

Alvarez JD, Austin B et al (1998) Vibrio hurveyi: a pathogen of penaeid shrimps and fish in Venezuela. J Fish Dis 21(1):313–316

Antunes LC, Ferreira RB, Buckner MM, Finlay BB (2010) Quorum sensing in bacterial virulence. Microbiology 156:2271–2282

Austin B, Zhang XH (2006) Vibrio harveyi: a significant pathogen of marine vertebrates and invertebrates. Lett Appl Microbiol 43(1):119–124

Benitez JA, Silva AJ (2016) Vibrio cholerae hemagglutinin (HA)/protease: an extracellular metalloprotease with multiple pathogenic activities. Toxicon 115:55–62

Borchardt SA, Allain EJ et al (2001) Reaction of acylated homoserine lactone bacterial signaling molecules with oxidized halogen antimicrobials. Appl Environ Microbiol 67(7):3174–3179

Brackman G, Al Quntar AAA, Enk CD, Karalic I, Nelis HJ, Van Calenbergh S et al (2013) Synthesis and evaluation of thiazolidinedione and dioxazaborocane analogues as inhibitors of AI-2 quorum sensing in Vibrio harveyi. Bioorg Med Chem 21(3):660–667

Bramhachari PV, Dubey SK (2006) Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. Lett Appl Microbiol 43(5):571–577

Bramhachari PV, Yugandhar NM, Prathyusha AMVN, Sheela GM, Naravula J, Venkateswarlu N (2018) Quorum sensing regulated swarming motility and migratory behavior in Bacteria. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 49–66

Chari PB, Dubey SK (2006) Rapid and specific detection of luminous and non-luminous Vibrio harveyi isolates by PCR amplification. Curr Sci, 1105–1108

De Decker S, Reynaud Y, Saulnier D (2013) First molecular evidence of cross-species induction of metalloprotease gene expression in Vibrio strains pathogenic for Pacific oyster Crassostrea gigas involving a quorum sensing system. Aquaculture 392:1–7

Defoirdt T, Crab R, Wood TK, Sorgeloos P, Verstraete W, Bossier P (2006) Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp Artemia franciscana from pathogenic Vibrio harveyi, Vibrio campbellii, and Vibrio parahaemolyticus isolates. Appl Environ Microbiol 72(9):6419–6423

Defoirdt, T., N. Boon, et al. (2008). "Quorum sensing and quorum quenching in Vibrio harveyi: lessons learned from in vivo work." ISME J**2**(1): 19–26

Gutiérrez-Barranquero JA, Reen FJ, McCarthy RR, O'Gara F (2015) Deciphering the role of coumarin as a novel quorum sensing inhibitor suppressing virulence phenotypes in bacterial pathogens. Appl Microbiol Biotechnol 99(7):3303–3316

Haldar SC a S (2012) Vibrio related diseases in aquaculture and development of rapid and accurate identification methods. J Mar Sci Res Dev S1:002. https://doi.org/10.4172/2155-9910.S1-002

Hirakawa H, Tomita H (2013) Interference of bacterial cell-to-cell communication: a new concept of antimicrobial chemotherapy breaks antibiotic resistance. Front Microbiol 4:114

Homem V, Santos L (2011) Degradation and removal methods of antibiotics from aqueous matrices–a review. J Environ Manag 92(10):2304–2347

Jiravanichpaisal P, T M, Limsuwan C (1994) Histopathology, biochemistry, and pathogenicity of Vibrio harveyi infecting black tiger prawn Penaeus monodon. J Aquat Aniln Health 6:27–35

Kuebutornye FK, Abarike ED, Lu Y (2019) A review on the application of Bacillus as probiotics in aquaculture. Fish Shellfish Immunol 87:820

Lilley BN, Bassler BL (2000) Regulation of quorum sensing in Vibrio harveyi by LuxO and sigma-54. Mol Microbiol 36(4):940–954

Manefield M, Harris L, Rice SA, de Nys R, Kjelleberg S (2000) Inhibition of luminescence and virulence in the black tiger prawn (Penaeus monodon) pathogen Vibrio harveyi by intercellular signal antagonists. Appl Environ Microbiol 66(5):2079–2084

Molina-Aja A, García-Gasca A, Abreu-Grobois A, Bolan-Mejia C, Roque A, Gomez Gil B (2002) Plasmid profiling and antibiotic resistance of Vibrio strains isolated from cultured penaeid shrimp. FEMS Microbiol Letts 213:7–12

Natrah FMI, Kenmegne MM, Wiyoto W, Sorgeloos P, Bossier P, Defoirdt T (2011) Effects of micro-algae commonly used in aquaculture on acyl-homoserine lactone quorum sensing. Aquaculture 317(1–4):53–57

Natrah FMI, Alam MI, Pawar S, Harzevili AS, Nevejan N, Boon N et al (2012) The impact of quorum sensing on the virulence of Aeromonas hydrophila and Aeromonas salmonicida towards burbot (Lota lota L.) larvae. Vet Microbiol 159(1–2):77–82

Nealson KH, Hastings JW (1979) Bacterial bioluminescence: its control and ecological significance. Microbiol Rev 43(4):496–518

Ni N, Li M et al (2009) Inhibitors and antagonists of bacterial quorum sensing. Med Res Rev 29(1):65–124

Pande GSJ, Scheie AA, Benneche T, Wille M, Sorgeloos P, Bossier P, Defoirdt T (2013) Quorum sensing-disrupting compounds protect larvae of the giant freshwater prawn Macrobrachium rosenbergii from Vibrio harveyi infection. Aquaculture 406:121–124

Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL (2018) Biological approaches for disease control in aquaculture: advantages, limitations and challenges. Trends Microbiol 26(11):896–903

Prathyusha AMVN, Triveni G, Bramhachari PV (2018) Quorum sensing system regulates virulence and pathogenicity genes in Vibrio harveyi. In: Implication of quorum sensing system in biofilm formation and virulence. Springer, Singapore, pp 221–231

Raissy M, M M, Ansari M, Rahimi E (2012) Antibiotic resistance pattern of some Vibrio strains isolated from seafood. Iran J Fish Sci 11:618–626

Rasmussen TB, Givskov M (2006) Quorum-sensing inhibitors as anti-pathogenic drugs. Int J Med Microbiol 296(2–3):149–161

Ren D, Sims JJ et al (2001) Inhibition of biofilm formation and swarming of Escherichia coli by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. Environ Microbiol 3(11):731–736

Santhakumari S, Jayakumar R, Logalakshmi R, Prabhu NM, Nazar AKA, Pandian SK, Ravi AV (2018) In vitro and in vivo effect of 2, 6-Di-tert-butyl-4-methylphenol as an antibiofilm agent against quorum sensing mediated biofilm formation of Vibrio spp. Int J Food Microbiol 281:60–71

Soowannayan C, Boonmee S, Puckcharoen S, Anatamsombat T, Yatip P, Ng WK et al (2019) Ginger and its component shogaol inhibit Vibrio biofilm formation in vitro and orally protect shrimp against acute hepatopancreatic necrosis disease (AHPND). Aquaculture 504:139–147

Srinivasan P, Ramasamy P (2009) Occurrence, distribution and antibiotic resistance patterns of Vibrio species associated with viral diseased shrimps of south Indian aquaculture environment. Int J Agri Sci 1(2):1–10

Tendencia EA, de la Pena LD (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193–204

Yates EA, Philipp B et al (2002) N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of Yersinia pseudotuberculosis and Pseudomonas aeruginosa. Infect Immun 70(10):5635–5646

Yildiz FH, Visick KL (2009) Vibrio biofilms: so much the same yet so different. Trends Microbiol 17:109–118