INTRODUCTION
Success in nature depends upon a trend to perceive and to adapt the contiguous environment. Bacteria are not an exception; they recognize and persistently adjust to various circumstances by self-producing and sensing signals and accordingly alter their own gene expressions (Pereira et al., 2013). These modifications are uttered by the interaction and communication between complex networks of signalling pathways. To communicate efficiently, it is necessary to understand the language being spoken. This process of communication is common to individual of every biological community or a population. Bacteria are also capable to coordinate themselves by a phenomenon called quorum sensing (QS). QS is a cell density depended gene expression mechanism in bacteria through synthesis and release of small diffusible signal molecules termed as autoinducers (AIs). The concentration of AIs increases as a function of increasing cell density. This event is referred as “autoinduction” (Nealson, 1977). Bacteria sense the accumulation of a minimal threshold stimulatory concentration of these AIs and alter their own gene expression. This proves that the mode of communication is not restricted only to eukaryotes.

The nature of signals, receptors, mechanisms and target outputs of each QS system reveals the unique biology of a particular bacterial species. So far, three different kinds of QS system have been described in bacteria, which utilize distinct classes of secreted AIs. (1) Gram negative bacteria utilize acyl homoserine lactone (AHL) signals to communicate with each other (Fuqua and Greenberg, 2002). In the AHL dependent QS system, LuxI/R system is the base for the complex hierarchical regulatory circuit. In which, LuxI protein is the AHL synthase and LuxR protein is the response regulator. The AHL signal molecules either diffuse or transport across the
plasma membrane. As the bacterial cell density increases, the AHL concentration also increases. Once AHL reaches a critical concentration, it binds to the LuxR protein. The AHL-LuxR protein complex triggers the transcription of target virulent genes. (2) Gram positive bacteria utilize small post translational modified peptides for the cell to cell signalling system (Miller and Bassler, 2001). Peptide mediated QS system is found in Gram positive bacteria such as *Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumoniae* etc., (Dunny and Leonard, 1997). In this system, cleavage of peptide signal precursor protein results in the release of signal molecules. Once the extracellular peptide signal reaches a critical concentration, the response regulator gets phosphorylated by a sensor kinase protein. The phosphorylated response regulator activates transcription of the target genes. (3) The last class of QS system shared by both Gram negative and Gram positive bacterial species by utilizing autoinducer-2 (AI-2) as signal molecule. LuxS is the protein responsible for the AI-2 activity. Thus, the LuxS/Lsr transporter existing species applies AI-2 for their communication across the species barrier (Fig. 1) (Bassler, 1999; Surette *et al*., 1999).

![Fig. 1 Schematic representation of quorum sensing signalling pathways. A. LuxI/LuxR in a Gram negative bacteria, B. Autoinducing peptide in a Gram positive bacteria and C. LuxS/Lsr transporter in both Gram negative and Gram positive bacteria](image)
**QS in Gram negative bacteria**

Gram negative bacteria employ a diverse of QS signalling systems in which LuxI/R is a well characterized QS system. This QS circuit has been recognized in more than 70 Gram negative bacterial species (Fuqua et al., 2001; Whitehead et al., 2001). The AHLs are biosynthesised by AHL synthase, LuxI type enzyme by utilizing the S-adenosyl methionine (SAM) and the acyl-carrier proteins (ACPs) as substrates (Parsek et al., 1999). The synthesized AHLs vary in their fatty acid side chain length (Fig. 2), degree of saturation and acyl side chain substitutions. List of various AHL molecules present in the Gram negative bacteria are described in the Table. 1. A significant increase in the concentration of AHL indicates high cell density; diffusion of AHLs into the cell overwhelms export and as a result they bind to the cytoplasmic LuxR type receptors. AHLs with short acyl side chains are thought to require passive export and long acyl side chains need active export to cross the bacterial cell membrane (Pearson et al., 1999). LuxR proteins possess specific AHL binding pockets which allow AHLs to bind and activate LuxR receptor (Vannini et al., 2002; Zhang et al., 2002). This AHL-LuxR complex binds to promoter sequences and activates the transcription of QS genes responsible for several characteristics including bioluminescence, biofilm formation, toxin secretion and extracellular virulence enzymes production. At low cell density, less amount of AHL molecules were found in the surrounding environment. In the absence of AHLs, LuxR proteins degrade quickly. In contrast, at high cell density AHL molecules reaches the threshold concentrations. In the presence of AHL at threshold concentration, LuxR proteins are stabilized by binding with the AHL signal molecules and initiate the QS cascade (Fig. 3). (Koch et al., 2005). This type of AHL mediated QS systems are found in
many Gram negative bacterial genera such as *Agrobacterium*, *Aeromonas*, *Burkholderia*, *Chromobacterium*, *Citrobacter*, *Escherichia*, *Enterobacter*, *Erwinia*, *Hafnia*, *Nitrosomonas*, *Obesumbacterium*, *Pantoea*, *Proteus*, *Pseudomonas*, *Rahnella*, *Klebsiella*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Serratia*, *Vibrio*, *Xenorhabdus* and *Yersinia* (Eberl, 1999; Bosgelmez and Ulusoy, 2008).

![Structure of acyl-homoserine lactone molecule](image)

**Fig. 2** Structure of acyl-homoserine lactone molecule

**Table 1.** Types of acyl-homoserine lactone molecules in Gram negative bacteria

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
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</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-butanoyl-L-homoserine lactone (C4-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-(3-hydroxybutanoyl)-L-homoserine lactone (3-hydroxy-C4-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-hexanoyl-L-homoserine lactone (C6-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-octanoyl-L-homoserine lactone (C8-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-(3-oxooctanoyl)-L-homoserine lactone (3-oxo-C8-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-decanoyl-L-homoserine lactone (C10-HSL)</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>N-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C10-HSL)</td>
</tr>
</tbody>
</table>
Genus *Serratia*

Earlier *Serratia* was believed as a harmless saprophyte, but now it has been recognized as one of the opportunistic pathogens responsible for healthcare-associated infections and antibiotic resistance. The genus *Serratia* belongs to Enterobacteriaceae family; motile with peritrichous flagella, non-endospore forming, Gram negative and facultative anaerobic bacilli. *Serratia* is a ubiquitous inhabitant of water, soil, plants,
insects and humans. Phenotypically, *Serratia* is one of the easiest genera to
differentiate within the Enterobacteriaceae family. Some of the promising pathogenic
factors found in *Serratia* strains are formation of fimbriae, production of potent
siderophores, presence of cell wall antigens and resistance to the bactericidal action of
serum (Grimont and Grimont, 1978). QS mediated production of extracellular
enzymes have also been reported in strains of the genus *Serratia*, which includes
production of prodigiosin, gelatinase, protease, hemolysin, lipase and DNase (Slater *et
al.*, 2003). Besides the regulation of prodigiosin production and extracellular enzyme
secretion, QS is crucial for biofilm formation (Houdt *et al.*, 2007). Recently, 14
species were recognized in the genus *Serratia*; of all species *Serratia marcescens*, *S.
liquefaciens* and *S. odorifera* are associated with human infections (Mahlen, 2011). Out of
which, *S. marcescens* is the best known human pathogen.

**S. marcescens**

Earlier, bacteriologists considered *S. marcescens* as non-pathogenic organism
and used as a tracer organism. It has also been used as a biological warfare test agent
because of the prodigiosin pigment production. Later, medical literatures implicated
that this organism could cause opportunistic infections in humans. *S. marcescens* is
now globally accepted as a clinical pathogen and recognised as multi-drug resistant
organism (Hejazi and Falkiner, 1997). This Gram negative bacterium inhabits in
various ecological niches and causes disease in a wide range of vertebrate as well as
invertebrate hosts (Grimont and Grimont, 1978). It has also been emerged as a
frequent source of outbreaks in nosocomial infections (Merkier *et al.*, 2013) and is
responsible for an increased number of serious health issues (Hejazi and Falkiner,
1997). *S. marcescens* is hardly ever causes primary invasive infections. It act as a true
opportunistic pathogen, whenever it gains access of compromised host, it causes a serious of infections which includes lower respiratory tract infections (van der Vorm and Woldring-Zwaan, 2002), urinary tract infections (UTIs) (Kawecki et al., 2011), pneumonia (Jones, 2010), bloodstream infections, conjunctivitis, septicaemia, cellulitis, wound infections, meningitis, arthritis, endocarditis and rare reports on skin, ocular, soft tissue infections etc., (Donnenberg, 2005, Mlynarczyk et al., 2007; Giraldez et al, 2011; Merkier et al., 2013). The diseases caused by *S. marcescens* are hard to treat due to their resistance towards a wide variety of antibiotics, which often lead to specific and prolonged treatment. The ability of biofilm formation and virulence factors production are the basic rationale to recognize the *S. marcescens* as a successful opportunistic pathogen. In *S. marcescens*, the biofilm formation and most of the virulence factors production are regulated by QS system (Fig. 4).

**QS in *S. marcescens***

Genetic studies have revealed that, *S. marcescens* has QS system similar to LuxI/R type AHL dependent QS system of *Vibrio fischeri*. In addition, the presence of number of other QS systems in various strains of *S. marcescens* has also been reported (Houdt et al., 2007). Some of the examples are SmaI/R, SwrI/R and SpnI/R QS systems. These QS systems secrete and utilize AHLs as same as *V. fischeri*. The *S. marcescens* QS system involves two major proteins namely I protein homologous to LuxI of *V. fischeri*, which catalyses the synthesis of AHL signalling molecules, and the R protein homologous to LuxR of *V. fischeri*, acts as a transcriptional activator, that upon activation triggers the expression of phenotypic factors. Different strains of *S. marcescens* produce multiple AHLs which includes C4-HSL, C6-HSL, 3-oxo-C6-HSL, C7-HSL and C8-HSL (Williams, 2007). These AHL signalling molecules
regulate the phenotypic characteristics of *S. marcescens* including prodigiosin, antibiotic (carbapenem), biosurfactant production, biofilm formation, swarming and sliding motility (Fig. 4).

**Fig. 4** Quorum sensing mechanism in *S. marcescens*

**Genus Aeromonas**

The genus *Aeromonas* is not only a disease causing pathogen of fish, amphibians, reptiles, birds and other cold-blooded organisms; but also responsible for a variety of infectious complications in immunocompromised patients (Martin-Carnahan and Joseph, 2005; Figueras, 2005). Initially, *Aeromonas* was positioned in the Vibrionaceae family, later the results of phylogenetic analyses pointed out that genus *Aeromonas* is not closely related to vibrios. Subsequently, *Aeromonas* was
relocated from the family Vibrionaceae to the family Aeromonadaceae (Abbott et al., 1998; Abbott et al., 2003; Villari et al., 2003). Generally, the genus *Aeromonas* are Gram negative, facultative anaerobic, oxidase positive bacteria and which is found in natural habitats such as aquatic environment, soil, fish, invertebrate species, domesticated pets, birds, insects and food (Martin-Carnahan and Joseph, 2005). This vast panorama of environmental sources of aeromonads and the constant exposure leads to the interactions between the genus *Aeromonas* and humans. The QS system in *Aeromonas* spp. plays an important role in causing infections in humans. However, the available literatures provide only a limited data about the role of QS mechanism in this genus. The suspected outcomes of *Aeromonas* QS system are formation of biofilm and regulation of virulence factors production. To the surprise, QS molecules have been detected in *Aeromonas* species such as *Aeromonas hydrophila* and *A. salmonicida* (Swift et al., 1997). In the genus *Aeromonas*, three genomospecies namely *A. hydrophila*, *A. caviae*, and *A. veronii* bv. sobria are accountable for 85 % of human infections (Janda and Abbott, 1998). Among the mesophilic *Aeromonas* species, *A. hydrophila* is notably linked with major fish diseases and act as an opportunistic human pathogen.

*A. hydrophila*

*A. hydrophila* is a facultative anaerobic short rod shaped bacteria present in all aquatic environments. This bacterium has been recognized as an etiological agent in causing illness in fish and amphibians as well as in humans. It causes septicaemia diseases like motile aeromonas septicaemia (MAS), hemorrhagic septicaemia, ulcer diseases, red-sore diseases, abscess, exophthalmia and abdominal distension in fish (Paniagua et al., 1990; Arulvasu et al., 2013). Motile aeromonads primarily affects
freshwater fish such as catfish, carp, eel, goldfish, rainbow trout, salmon and tilapia. Thus, the *A. hydrophila* infection is one of the most important problems and challenges facing in commercial freshwater aquaculture. This in turn contributes a substantial economic loss in fish farming industry (Harikrishnan and Balasundaram, 2005; Citarasu *et al.*, 2011). *A. hydrophila* also causes opportunistic infections in humans through open wounds or by ingestion of contaminated food or water with this pathogen. The *Aeromonas* clinical infections fall into four different categories, including gastrointestinal tract, wound and soft tissue infections, blood borne dyscrasias, seldom encountered ailments and infectious processes (Janda and Abbott, 2010).

**QS in *A. hydrophila***

The pathogenicity of *A. hydrophila* infection is complex and multifactorial. Only a limited data is available on the role of QS system in *A. hydrophila*. At high cell density, the QS system in *A. hydrophila* regulates the formation of biofilm and secretion of several virulence factors in response to environmental triggers (Chopra *et al.*, 2000; Lynch *et al.*, 2002). *A. hydrophila* possess AhyI/R QS signalling pathway (Fig. 5) and produces AHLs such as C4-HSL and C6-HSL, of which C4-HSL is the most predominant type (Swift *et al.*, 1999). Factor’s contributing to the virulence of *A. hydrophila* includes exotoxin, enterotoxin, protease, aerolysin, hemolysin, lipase, lactamase, adhesin, siderophore and agglutinin (Janda and Abbott, 1996; Janda and Abbott, 2010).
Biofilms

Microorganisms are frequently live in dense and diverse communities, termed as biofilms (Hall-Stoodley et al., 2004). Biofilm is an aggregation of bacteria embedded in a self secreted extracellular polymeric substances (EPS) adherent to biotic or abiotic surfaces (Vert et al., 2012). The development of a biofilm involves four different stages (Stoodley et al., 2002) as follows, (1) reversible attachment of bacterial cells to a surface, (2) irreversible attachment mediated by the formation of microcolonies, (3) maturation of biofilm and (4) detachment and dispersion of cells from the matured biofilm (Fig. 6). It has also been well stated that biofilm dwelling bacteria communicate through QS mechanism and results in the enhancement of antibiotic resistance (Smith and Iglewski, 2003; Bjarnsholt and Givskov, 2007; Harraghy et al., 2007). The biofilm dwelling bacteria are differs from their planktonic counterparts by EPS production, retard growth and differential expression of biofilm associated genes. EPS acts as a filter and channel for transportation of nutrients and minerals to interior cells and protects the dwelling bacteria from potentially harmful
agents including antibiotics, biocides and host defense mechanisms (Donlan, 2002). Thus makes the treatment measures difficult and its eradication is almost impossible. The advantages of biofilm over the dwelling bacteria are protection from the inhibitory effects of antibiotics, chemical and physical stresses like pH, oxygen, pressure, heat and freezing. (Davey and O’Toole, 2000; Donlan, 2002; Trachoo, 2003).

The Center for Disease Control (CDC) has estimated as 65 % and the National Institute of Health (NIH) has estimated as almost 80 % of the world’s microbial biomass exists in form of biofilm, this allocate biofilms to spread all over the world (Richards and Melander, 2009). Formation of biofilms on every industries, medicines and environmental settings lead to heavy economic loss worldwide (Pulcini, 2001; Davies, 2003). In the clinical perspectives, biofilm is an intrinsic factor responsible for a variety of intractable infections in humans. The infections caused by bacterial biofilms includes UTIs, catheter or medical implant infections, lung infections of cystic fibrosis patients, ear infections, bacterial endocarditis, wound infections and tooth decay (Singh et al., 2000; Davies, 2003). Next to the medical sector, biofilms have several negative impacts on aquaculture industries worldwide. Fish culture is one of the powerful income generating industries, however huge economic losses are brought by bacterial infections in this sector. These infectious diseases are the major limiting factors in aquaculture and control of these diseases have become a key to successful fish farming management. The bacterial infections increase the mortality rate of fish populations and subsequently cause several diseases to humans through consumption of contaminated or uncooked seafood (Kaysner and DePaola, 2001). In order to prevent or control the impacts of biofilms, development of a new generation
QS inhibitors (QSIs) is essential for targeting the bacterial QS system (Pulcini, 2001). Such QSIs does not inhibit the growth of the pathogenic bacterium and would hinder the induction of selective pressure to develop resistance.

**Fig. 6** Stages involved in the formation of biofilm

**Bacterial pathogenesis and virulence factors**

Virulence factors are often involved in direct interactions with host tissues and conceal the bacterial surface from host’s immune system. A disease caused by pathogenic bacteria is being determined by the multiple virulence factors acting individually or together at different stages of infection. QS mechanism regulates the expression of bacterial virulence factors, but typically stimulation occurs at high cell density. The QS mediated virulence factors production at high cell density is collectively more difficult for the host to eradicate than individual planktonic bacteria (Fuqua et al., 1994). In spite of their abundance, most of the virulence factors fall into one of a few categories when classified based on their functions (Fig. 7). These includes (1) membrane proteins like adhesins, (2) polysaccharide capsules such as anti-phagocytic factors, (3) secretory proteins like toxins, (4) cell wall and outer membrane components such as lipopolysaccharides (LPS) and lipoteichoic acids, (5)
other virulence factors such as biofilm forming proteins and siderophores (Wu et al., 2008).

The virulence factors produced by pathogenic bacteria are biosurfactant (serrawettin), pigments (prodigiosin), adhesions, toxins (aerolysin, hemolysin) and extracellular enzymes such as protease, lipase, elastase, phospholipase, gelatinase, etc., which plays an important role in pathogenesis (Janda and Kokka, 1991; Williams, 2007). In S. marcescens, surfactant serrawettin helps in colonization on surfaces (Matsuyama et al., 1992). Protease promotes keratitis by cleaving IgG, IgA and lysozyme in host cells (Kurz et al., 2003). The production of hemolysin causes hemolysis in human or animal erythrocytes and releases inflammatory mediators (Kurz et al., 2003). In A. hydrophila, hemolytic toxins are a diverse group of multifunctional enzymes, involved in membrane pore formation of the target cell (Wadstrom et al., 1976; Asao et al., 1984). Lipase has been found to damage the host cell plasma membrane and elastase secreted by A. hydrophila cause diseases in fish and humans (Stehr et al., 2003; Nam and Joh, 2007). These virulence factors play an important role in pathogenesis of bacteria associated with health effects in humans. The exact relationship between the presence of virulence factors and the ability of bacteria to cause human disease has not been recognized. This indicates the necessity of novel preservation approaches to complement conventional therapy (Persson et al., 2005).

**Antibiotic resistance**

Generally, free living bacteria are susceptible to antibiotic treatment and to host defence mechanisms. Traditionally antibiotics are being used to treat or prevent bacterial infections. Consequently, the continuous demand of antibiotics increased
their production to millions of metric tons and employed them for a wide variety of purposes. These less expensive compounds reduced the burden of diseases imposed by bacterial infections and parallely encouraged non-prescription and off-label uses. Unfortunately, this mode of optimism has not been succeeded and ends in emergence of resistance. This leads to constant selection pressure over human on the application of antibiotics.

Inappropriate and unnecessary use of antibiotics has resulted in the increasing rate of antibiotic resistance among bacterial pathogens. Conventionally, planktonic bacteria show antibiotic resistance by inactivation or exclusion of the antibiotics and modification of drug targets (Fig. 8) (Patel, 2005). Biofilms inhabitant bacterial cells are 100-1000 fold resistant to antibiotics than their planktonic counterpart (Patel, 2005). It has also been believed that biofilm mode of growth promote antibiotic resistance by three different mechanisms: 1) reduction of antibiotic penetration across the EPS, 2) a favourable (e.g., anaerobic) environment within the inner layers, 3) bacterial cell differentiation and specialization of function that provides increased protection (Stewart and Costerton, 2001). The world health organisation (WHO) has declared antibiotic resistance as one of the greatest threats to human health. Hence, to overcome the current situation, it is believed that inhibition of bacterial QS system would be an alternative approach to prevent the QS mediated virulence genes expression in pathogenic bacteria. This could be achieved by application of antipathogenic agents, as they would not exert any selective pressure for the development of antibiotic resistance (Hentzer and Givskov, 2003). Hence, it is valuable to use natural antipathogenic drugs rather than conventional antibiotics.
Emergence of antibiotic resistance among pathogenic bacteria, limited the antibiotic treatment options against bacterial infections. Thus, there is an urgent need for novel antibacterial therapy against the bacterial infection. Interfering with the microbial QS system also called as QSI mechanism has been suggested as a potential strategy for microbial disease control (Dong et al., 2000). This shuts down the expression of virulence in pathogenic bacteria rather than controlling the growth of bacteria (Rasmussen and Givskov, 2006). All QSI strategies that have been characterized to date found to target at least one of these three ways, they are (1) enzymatic degradation of the signal molecules, (2) blocking signal generation and (3) blocking signal receptor (Fig. 9) (Hentzer and Givskov, 2003; Roche et al., 2004; Kjelleberg et al., 2008).
Fig. 9 Quorum sensing inhibitory strategies against Gram negative bacteria

Degradation of signal molecule

The inactivation or complete degradation of signal molecules can be achieved either by chemical or enzymatic destruction (Fig. 10). The action of lactonases and decarboxylases in AHLs leads to lactonolysis, the degradation of lactone ring without affecting the rest of the signal molecule structure (Dong et al., 2000; Leadbetter, 2001; Yates et al., 2002). Acylases and deaminases cause cleavage of AHL molecule into homoserine lactone and free fatty acid moiety. Oxidoreductase does not degrade the AHL but rather modify it to an inactive form by oxidizing or reducing the acyl side chain (Uroz et al., 2005). The first AHL degrading enzyme, lactonase (AiiA) was purified from Bacillus sp. strain 240B (Dong et al., 2000). Later, lactonase was discovered in many other bacteria such as Bacillus thuringiensis, Geobacillus kaustophilus HTA426, G. caldoxylislyticus YS-8, Microbacterium testaceum StLB037, Agrobacterium tumefaciens, and Rhodococcus sp. (Dong et al., 2002; Lee et al., 2002; Zhang et al., 2002; Carlier et al., 2003; Park et al., 2006; Chow et al.,
Acylases are reported from *Rhodococcus eutropha*, *V. paradoxus* and *Streptomyces* sp. (Zhang *et al*., 2002; Park *et al*., 2005; Uroz *et al*., 2008). Comparative genomics has publicized the presence of both AHL-acylases and -lactonases encoding genes in the same organisms, such as 1) *Deinococcus radiodurans* R1, 2) *Hyphomonas neptunium* ATCC 15444 and 3) *Photorhabdus luminescens* subsp. *laumondii* TTO1 (Kalia *et al*., 2011).

**Fig. 10** Degradation of acyl-homoserine lactones by lactonase, acylase and oxidoreductase

### Blocking signal generation

Prevention of signal generation is one of the most instinctive and viable approaches for the disruption of QS mechanism. As mentioned earlier, LuxI type synthases mediated AHL production occurs by utilizing SAM as the amino donor for lactone ring formation and ACP as precursor for acyl side chain (Schaefer *et al*., 2008). Notionally, inhibition of AHL production could be accomplished during suppression of SAM biosynthesis, interruption of acyl-ACP generation, or inactivation of the synthase enzyme. So far, very few reports were found for the inhibition of AHL production by these strategies. However, substrate analogues such as holo-ACP, butyryl-SAM and L/D-S-adenosylhomocysteine, sinefungin inhibited the *in vitro* production of AHLs (Parsek *et al*., 1999). However, these strategies were
not yet proved through *in vivo* studies. These substrate analogues bind to the synthase gene as an alternative to SAM and thus inhibit the synthesis of AHLs. To date, inhibition of QS signal biosynthesis remains as the least investigated QSI strategy.

**Blocking signal receptor**

Blocking the AHL receptor site with a QSI is the most intensively investigated strategy for the inhibition of bacterial QS system. QSI and AHL analogues can recognize and competitively bind to LuxR receptor elements and subsequently prevent signalling through an inactive ligand-receptor complex or degrade the receptor protein by making extreme changes in protein folding (Schaefer *et al.*, 2008). The list of compounds proven to be potential QSI’s includes 4-nitropyridine-N-oxide (4-NPO) (Rasmussen *et al.*, 2005), phenyl acetic acid (Musthafa *et al.*, 2012), methyl eugenol (Packiavathy *et al.*, 2012a), phytol (Srinivasan *et al.*, 2016), palmitic acid (PA) (Santhakumari *et al.*, 2017) etc., against various bacterial pathogens.

**Sources for QSI agents**

Natural ecosystems co-exist with large number of organisms which perform as a sources for QSIIs. Prokaryotes have the abilities to produce AHL degrading enzymes; which show efficient antibiofilm activity against Gram negative bacteria (Kalia, 2013). Different types of AHL degrading enzymes such as AHL-lactonases, AHL-acylases and decarboxylases are reported from various sources. These enzymes are produced by Actinobacteria - *Rhodococcus* and *Streptomyces*, Firmicutes - *Arthrobacter, Bacillus* and *Oceanobacillus*, Cyanobacteria - *Anabaena*, Bacteroidetes - *Tenacibaculum* and Proteobacteria - *Klebsiella pneumoniae* (Dong and Zhang, 2005; Park *et al.*, 2005; Romero *et al.*, 2010). AHL degrading enzymes have also been
found in animals such as mice, rats and zebrafish (Joint et al., 2002; Williams, 2007). Porcine kidney acylase I was shown to inactivate QS signals (Paul et al., 2009); mammalian paraoxonases (PONs) are known to perform hydrolytic activity and these PONs differ from prokaryotic lactonases (Billecke et al., 2000). Plant extracts and their phytochemicals also act as QSI due to their structural similarity with AHL signal molecule (Teplitski et al., 2010; Vattem et al., 2007). Extracted pyrogallol from medicinal plant Emblica officinalis, seed exudate L-canavanine from Medicago sativa are known to degrade signal molecules and thus inhibits the expression of virulence genes in pathogenic bacteria (Keshavan et al., 2005).

Plant extracts and their secondary metabolites are also known to possess antagonism properties. Naturally occurring grapefruit juice, sour orange seeds and green tea contains QSIIs such as furocoumarins, limonoids and catechins, respectively (Zhao et al., 2001; Vikram et al., 2011). Aqueous extracts of Ananas comosus, Musa paradiciaca, Manilkara zapota and Ocimum sanctum have been proved to be as potential QSIIs (Musthafa et al., 2010). Certain plant parts of Imperata cylindrica (underground stem), Nelumbo nucifera (leaf), Prunella vulagris (whole plant), Vetiveria zizanioides (root) and Punica granatum (bark) also shows effective QSI activities against various pathogenic bacteria (Karamanoli and Lindow, 2006; Koh and Tham, 2011, Kannappan et al., 2017). More recently, various plant extracts including chilli, tomato, crown vetch, soybean, water lily, Daucus carota subsp. sativu, M. sativa, Pisum sativum seedling, Allium cepa, A. sativum, Lycopersicium esculentu, M. truncatula, vanilla, Piper betle, Cuminum cuminum and some medicinal plants were found to possess QSI activities (Teplitski et al., 2000; Rasmussen et al., 2005; Sanchez-Contreras et al., 2007; Packiavathy et al., 2012a; Srinivasan et al., 2016).
The very first QSI compound halogenated furanone was reported from Australian marine macroalgae *Delisea pulchra*, secreted, which is (Bauer and Robinson, 2002). Similarly, *Chlamydomonas reinhardtii*, unicellular green alga, *Laminaria digitata* (Brown alga), and *Ahnfeltiopsis flabelliformis* (Korean red alga) also revealed the QSI potential against Gram negative bacteria (Borchardt et al., 2001; Teplitski et al., 2004; Kim et al., 2007). Marine sponge *Laffariella variabilis*, cyanobacteria *Lyngbya majuscula* secondary metabolite and marine bacteria also have ability to interrupt the QS system (Rasmussen et al., 2005; Skindersoe et al., 2008).

Secondary metabolites produced by fungus such as *Penicillium* spp. and *Auricularia auricular* also act as QSIs (Rasmussen et al., 2005). All the natural QSIs are available in less concentration; to overcome this limitation synthetic analogues have also been reported (Hentzer and Givskov, 2003). Synthetic compounds like 5-hydroxy-3-[(1R)-1-hydroxy-2,2-dimethylpropyl]-4-methylfuran-2(5H) (Martinelli et al., 2004), synthetic signal analogues of halogenated furanone such as Fimbrolide (F1) (Kim et al., 2005), and (ii) N-(sulfanyl acetyl)-L-HSL blocked QS system (Koch et al., 2005).

Commercial compounds such as embelin and piperine inhibit *S. mutans* biofilm, piericidin A and glucopiericidin A suppresses the expression of the virulence genes of *Erwinia carotovora* subsp. atroseptica, naringenin and quercetin attenuated the *Pseudomonas aeruginosa* PA01 (Vandeputte et al., 2011, Ouyang et al., 2016; Dwivedi and Singh, 2016; Kang et al., 2016). Although, several studies have already been reported a number of QSIs for the disruption of bacterial QS system, the necessity for new classes of QSIs is still in demand.

With these background informations, the present study has been designed to explore the QSI potential of rhizosphere soil bacterium against *S. marcescens*. Rhizosphere soil bacterium *B. subtilis* R-18 was isolated from the rhizosphere of
**INTRODUCTION**

*Curcuma longa*. The AHL degrading enzyme and active principles present in the cell-free culture supernatant (CFCS) of R-18 with QSI potential were partially purified using chromatographic techniques and successive solvent extraction along with GC-MS analysis, respectively. Further, the QSI potentials of identified active principles were assessed through various physiological assays and microscopic analysis. All the obtained results were validated through FT-IR and transcriptomic analysis.

In this study, different virulent strains of *A. hydrophila* were isolated from diseased zebrafish. Selected polyphenols were tested for its QSI activity against these *A. hydrophila* isolates through biochemical and microscopic analyses. The differentially expressed intracellular proteins of untreated and polyphenols treated *A. hydrophila* were also determined using gel based proteomic approach. Further, the *in vivo* efficacy of polyphenols was investigated *in vivo* using zebrafish as an animal model.

This thesis was structured into four different chapters,

**Chapter I:** Screening, purification and characterization of extracellular protein with QSI potential from bacteria associated with *Curcuma longa* rhizosphere soil

**Chapter II:** Antibiofilm and anti-virulent potential of bioactive compounds from rhizosphere soil bacterium against uropathogen *S. marcescens*

**Chapter III:** *In vitro* efficacy of polyphenols on QS mediated virulence factors production in *A. hydrophila* through transcriptomic and proteomic approaches

**Chapter IV:** *In vivo* anti-infective potential of polyphenols in *A. hydrophila* infected *Danio rerio* (Zebrafish)