

Anti-quorum sensing activity from marine bacteria

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ABSTRACT: Unregulated consumption and overexploitation of antibiotics have paved the way for antibiotic-resistant strains and ‘superbugs’ emergence, posing a severe challenge in combating infectious diseases. Finding new effective antibiotic compounds is costly and time-consuming, and the possibility of developing resistance is high. In the last few decades, researchers have concluded that Quorum sensing (QS) genes firmly control the virulence artillery of the pathogen, and their expression drives the aggressiveness of the infection. Any organism's antibiotic resistance (AR) mechanism strengthens with the biofilm formation ability of microorganisms, which is mainly regulated by quorum sensing (QS). Quorum sensing (QS) is a global gene regulatory mechanism in bacterial pathogens expressing virulence factors by producing and secretion of small signalling molecules. QS is well studied at *Pseudomonas aeruginosa*. Turning off the QS system with an anti-infective agent is a sustainable and potential strategy to tackle bacterial pathogens. QS inhibitors do not kill pathogens but disrupt their communication. Samples were collected from undisturbed areas along Gujarat's coast of Gujarat like Mandvi, Dwarka and Diu. A total of 72 marine isolates were obtained, out of which 18 were associated with various marine macro-organisms like algae, whereas 54 were free living. The ability of quorum-sensing inhibition of all the isolates was tested against *Serratia marcescens* by co-culture technique to simultaneously detect signal-degrading and non-degrading quorum-sensing inhibitors. From primary co-culture screening total 44 bacterial isolates, including 12 macro-organism-associated bacteria and 32 living bacteria, were potentially found to have quorum sensing inhibitory potential against *S. marcescens* without affecting its growth. The present study describes the experimental results of selected isolates MB2 and DG5. Crude extract of both isolates was extracted with ethyl acetate to obtain the anti-QS compounds. Pigment inhibition in *S. marcescens* treated with crude extract was demonstrated by standard well diffusion assay and was found to have quorum sensing inhibitory activity without affecting its growth. Based on the above-obtained results, marine isolates were found to be a good candidate for the production of anti-quorum sensing molecules, which may serve as alternatives to conventional inhibitory molecules and can be a good candidate in future for the treatment of antimicrobial resistance disease.

1. INTRODUCTION

Quorum sensing (QS) is a global gene regulatory phenomenon in bacterial pathogens expressing virulence factors by cell–cell communication. QS was first discovered as a fundamental principle behind the bioluminance of luciferase in the marine bacterium *Vibrio fischeri* from the light organ of its symbiotic Hawaiian squid partner *Euprymna scolopes* (Engebrecht *et al.*, 1983). Since then, there has been a significant discovery of the QS signals, their molecular mechanisms, gene regulons, and QS-regulated responses in diverse bacteria, especially which cause life-threatening infections. Through QS, bacteria can communicate to regulate multiple phenotypes, including virulence, biofilm formation, secondary metabolite production, sporulation, AMR development, horizontal gene transfer, antibiotic synthesis and so forth (Subhadra *et al.*, 2018). QS systems produce and sense extracellular signals known as Autoinducers (AIs). Bacteria continually secrete QS signal molecules in a fresh culture. As cellular density increases, QS signals accumulate in the local environment. Once a bacterial population reached its threshold level (quorum level), the QS receptor sensed the QS signal. It will induce changes in QS-dependent target gene expression, facilitating multicellular

behaviour patterns in the population. QS bioluminescence in *V. fischeri* is observed when its cellular density reaches 10^{10} - 10^{11} cells/ml. In bioluminescence expression by *V. fischeri*, autoinducer LuxI protein interact with transcriptional activator protein LuxR, which further activates luciferase gene expression (Kalia V. 2013).

The QS process is common in Gram-negative and Gram-positive bacteria, although it differs significantly in terms of inducer molecules, response circuits, and mechanisms. This communication system operates through various signals in Gram-Positive and Gram-Negative bacteria. Hundreds of Gram-negative bacterial species contain homologues of LuxI–LuxR circuits employing N-acyl-homo-serine lactones (HSLs), the most common class of autoinducer (AIs). Other than that, various classes of diffusible signal factors, autoinducer 2 (AI-2), 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS), Dialkylresorcinols (DARs) and Photopyrones (PpyS) are some of QS signalling molecules used by Gram-negative microbes (Papenfort *et al.*, 2016; Kalia V. 2013). In quorum sensing, Gram-positive bacteria typically use secreted oligopeptides as QS signalling molecules or autoinducers. In this case, a posttranslational unmodified/modified peptide was secreted by a committed ATP-binding cassette (ABC) transporter. These peptide signals interact with the sensory element of a two-component histidine kinase signalling system. QS is mediated by two-component adaptive response pathways that enable bacteria to adapt to alterations in environmental conditions and relay signals by phosphorylation/dephosphorylation cascades. Such as (1) Oligopeptides (5–10 amino acid cyclic thiolactone), (2) N-acyl homoserine lactones (AHLs), (3) Furanosyl borate (Autoinducer-2, AI-2), (4) Hydroxyl-palmitic acid methylester, and (5) Methyl dodecanoic acid (Kalia V. 2013).

The phenomenon of virulence and its associated gene expression in several animal and plant pathogens are also regulated by quorum sensing. Some life-threatening human pathogens that regulate virulence phenotypes by QS include *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio cholera*, *Staphylococcus aureus*, *Streptococcus pneumonia*. The severity of these pathogens is under serious consideration in AR Threats Report 2019 prepared and published by The Center for Disease Control and Prevention (CDC, 2019). QS also play a vital role in causing plant disease, including crown gall and soft rots caused by plant pathogen *Agrobacterium tumefaciens* and *Pectobacterium carotovorum*, respectively (Singh *et al.*, 2021).

The traditional treatment of infectious diseases with antibiotics and their inappropriate use has led to the emergence of resistant pathogens. The severity and mortality rate of multidrug resistance (MDR) pathogenic infections has increased noticeably and cannot be cured by advanced antibiotic therapies. Modern antibiotic treatments are no longer treatable (Hutchings *et al.*, 2019). Hence, there is a need for the hour to develop strategies that provide sustainable and long-term effectiveness against resistant pathogenic bacteria. Simultaneously, it's also necessary that a new antimicrobial strategy carries minimum chances of drug resistance in future. Quorum-sensing inhibitors (QSI) or quorum quenchers do not generally affect the growth of pathogens, thereby reducing the chance of resistance. Therefore, antivirulence prospects of Quorum sensing inhibitors (QSI) or quorum quenchers can be used to overcome the pathogenesis of bacteria.

In the last few decades, researchers found various quorum sensing inhibitors from marine macro and microorganisms, which indicate vast diversity and tremendous potential of marine ecosystems. The discovery of a new and powerful QSI molecule from the marine ecosystem is still being studied. This ability to interfere with intercellular communication is a frequent phenomenon in the marine ecosystem. It has been identified in many marine microorganisms, including free-living and symbiotic with marine sponges, corals, mussels, algae, cyanobacterial mats, seagrasses etc. (El-Kurdi *et al.*, 2021) since various identified QSI

could disrupt bacterial pathogenicity at very low concentrations without imposing selective pressure involved in antibacterial treatment, which has attracted the researcher's attention.

The present investigation draws from the isolation of QSI produce microorganisms from undisturbed areas along Gujarat's coast. Gujarat covers approximately 1600 km of coastline that has not yet been thoroughly explored. Besides that, anti-QS proficiency of marine bacteria and their metabolites will also be presented. There is a high probability of discovering novel QSI molecules to avoid pathogenicity through opportunistic diseases. Hence, it is necessary to investigate marine habitats for QSI investigations.

2. MATERIALS AND METHODS

2.1 Sample collection

Marine water and/or marine macroorganisms such as marine algae with sediment samples were collected from various undisturbed coastal sites of Gujarat, including Diu, Devbhoomi-Dwarka and Mandvi. The sample was collected in October 2021, January 2022 and March 2022, respectively. These samples were collected at a depth of 0.5 to 1 m. All the samples were collected in sterile plastic containers and processed within 24 h to avoid spoilage of macroorganisms. Samples were stored at 4°C until use.

2.2 Isolation of marine bacteria

Samples of pretreatment and isolation procedures were performed according to the protocol proposed by the previous researchers (Singh *et al.*, 2020). To isolate free-living marine bacterial, each marine water sample was diluted up to 10^{-3} dilutions in Luria Bertani Broth (LB; Himedia, India) and uniformly spread over Luria Bertani Agar plate (LB; Himedia, India) containing 2% NaCl, followed by incubation at 37°C for 1 to 7 days. To isolate macroorganisms associated marine bacteria, all macroorganisms were surface-sterilized with 70% ethanol and washed twice with sterile seawater, then macerated individually in sterile seawater, and the homogenate was diluted up to 10^{-3} dilutions in LB Broth. From each dilution tube, 100 µL of the sample was uniformly spread on an LB Agar plate containing 2% NaCl, followed by incubation at 37°C for 1 to 7 days. All morphologically diverse colonies were transferred into fresh LB plates for pure culture.

2.3 Co-culture screening of marine isolates for QS inhibition

To study quorum sensing inhibition ability of marine isolates, all actively growing pure marine isolates were co-cultured with *Serratia marcescens* (SM; ATCC 14756 Microbial Culture Collection, Pune, India) in LB Agar plate. Incubation was done at 28 °C for 1 to 4 days according to the nature of each isolate to observe inhibition of red pigment prodigiosin produced by SM in a shaking incubator. Prodigiosin is a pigment produced by *Serratia marcescens* as a product of cell-cell density-dependent quorum sensing phenomenon. Suppose marine isolates can produce an extracellular quorum sensing inhibitor or quorum quencher that will diffuse towards nearby growing SM culture. Under the influence of QS inhibitor, the absence of red/pink pigment production by SM colonies in co-culture screening was a qualitative indication of respective marine isolates' Quorum sensing inhibition/quorum quenching ability. Pure SM without any marine isolates was used as a negative control of co-culture screening.

2.4 Preparation of crude QSI extract

Pure single colonies of the positive screened isolates were cultured in LB medium at 37 °C and 150 rpm (1–7 days, according to the nature of each isolate) in a shaking incubator. Cell-free suspension of positively screened culture was prepared by centrifugation at 10,000 g. The extraction of the metabolites was carried out by the method of solvent-solvent extraction by using ethyl acetate based on the best solubility as described by (El-Kurdi *et al.*, 2021) and kept overnight in shaking condition. The aqueous and ethyl acetate phase was separated by using a separating funnel. The ethyl acetate was evaporated at 50°C using a rotatory evaporator

machine. The Remaining compounds were collected in methanol and stored at 4°C until further use.

2.5 Growth Inhibition study of crude extracts

The anti-QS testing of crude extract was carried out by Standard well diffusion assay of MB2 and DG5 as described by (Singh *et al.*, 2020) using biosensor strain *S. marcescens*. The plates were incubated for 24 hrs. at 28 °C. The anti-QS activity was recorded by measuring the inhibition in the pigment production around the well-known zone of clearance after incubation. To ensure anti-QS activity, swabs from the zone of pigment inhibition were subcultured into fresh LB agar. Pigmented growth indicates the anti-QS activity of the crude extract.

3. RESULTS

3.1 Isolation and screening of marine

Isolation of marine-derived quorum sensing inhibitor bacteria A total of 72 different isolates were recovered from marine samples with different macromorphology. Out of 72 isolates, 54 free-living isolates were isolated from seawater (Table 1), and 18 macroorganism-associated bacteria were isolated (Table 2).

3.2 Screening marine isolates of the QS inhibition activity

All marine isolates were screened against the bioreporter strain *Serratia marcescens* using the co-culture technique with necessary modification, as Chu *et al.* described. Out of 54 free-living isolates obtained from marine samples, 32 showed inhibition of prodigiosin (shown in Table 1). In contrast, out of 18 macroorganism associate marine isolates, 12 were found to exhibit degradation of pigment (shown in Table 2) on co-culturing with *S. marcescens* without affecting the cell growth shown in Figure 1. None of the positive screened isolated were found to inhibit the growth of *S. marcescens*.

TABLE 1 Anti-quorum sensing potential of free-living marine microorganisms.

Sr. no.	Sample site	Isolate	Prodigiosin Inhibition in co-culture screening
1	Mandvi	MA1	+
		MA2	++
		MA3	ND/-
		MA4	+
		MA5	++
		MA6	+
		MA7	-
		MA8	ND/-
		MA9	+
		MA10	ND/+
		MA11	-
		MB1	-
		MB2	+++
		MB3	+++
		MB4	-
		MB5	ND/-
		MB6	-
		MB7	-

		MB8	++
		MB9	+
		MB10	-
		MB11	ND/-
		MB12	+
		MB13	-
		MB14	-
		MB15	-
2	Devbhoomi-Dwarka	DWC1	ND/-
		DWC2	ND/-
		DWC3	ND/+
		DWC4	ND/-
		DWC5	ND/-
		DWC6	ND/+
		DWC7	ND/-
		DWC8	ND/-
3	DIU	DE1	+
		DE2	+++
		DE3	+
		DE4	-
		DE5	ND/+
		DE6	++
		DG1	+
		DG2	+
		DG3	++
		DG4	-
		DG5	+++
		DG6	+
		DG7	+
		DG8	ND/+
		DG9	+
		DG10	++
		DG11	+
		DG12	ND/+
		DG13	+
		DG14	++

TABLE 2 Anti-quorum sensing potential of macroorganisms associated with marine microorganisms.

Sr. no.	Sample site	Marine macroorganisms	Isolates	Prodigiosin inhibition in co-culture screening
1	Devbhoomi-Dwarka	Green (Valoniopsis pachynema) alga	DWD1	ND/-
			DWD2	-
			DWD3	-
			DWD4	+
			DWD5	++
			DWD6	+
			DWD7	+
			DWD8	++
			DWD9	-
2	Diu	Alga (Ulva sp.)	DF1	++
			DF2	-
			DF3	ND/+
			DF4	+
			DF5	-
			DF6	+
			DF7	+
			DF8	++
			DF9	+

3.3 Extraction and bioassay of crude extract from marine bacteria

The metabolic crude extracts obtained from the ethyl acetate extraction method of selected isolates MB2 and DG5 showed anti-quorum sensing as it could degrade the prodigiosin pigment of the bioreporter strain SM on treatment with both crude extracts. The zone of clearance surrounding the well containing crude extract of MB2 and DG5, shown in (Figure 1), was indicative of the anti-quorum sensing potential of MB2 and DG5 isolates. The measurement of the clearance zone was 1.0 mm and 1.7 mm, respectively.

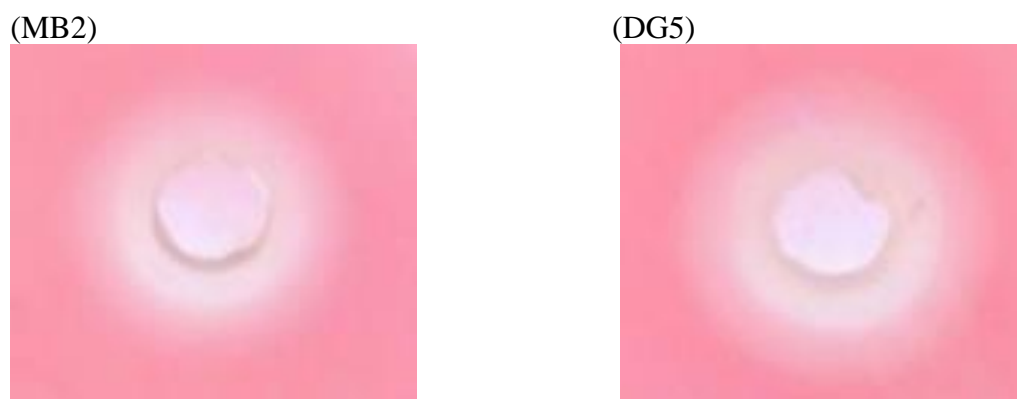


FIGURE 1 Effect of crude extract of MB2 and DG5 on prodigiosin pigment production by *S. marcesens*. Reduction in pigment production by *S. marcesens* surrounding the wells containing crude extract of isolates MB2 and DG5 indicates the anti-QS potential of respective marine isolates. The measurement of the clearance zone was 1.0 mm and 1.7 mm, respectively.

4. DISCUSSION

Marine microorganisms are a diverse bioactive compound source with therapeutic applications, including antimicrobial, antibiofilm, anticancer, and anti-quorum sensing activity. Worldwide research reports have indicated anti-QS activity in marine water, and marine macroorganism, including sponges, invertebrates, algae, and coral-associated bacteria (Singh *et al.*, 2020; El-Kurdi *et al.*, 2021). A QS inhibitor or anti-quorum sensing mechanism can manage the threat of multidrug resistance pathogen and associated diseases. In the present study, we are exploring the potential of marine bacteria to produce anti-quorum sensing compounds. QS is cell to cell communication mechanism. QS hold control of different multicellular behaviours and gene which regulate several functional activities such as AMR development including horizontal gene transfer, bioluminescence, Virulence regulatory genes including expression of secondary messengers, sigma factor, which affect gene expression, antibiotic synthesis, sporulation, secretion of enzymes generation of reactive oxygen species (ROS), biofilm formation and so forth (Defoirdt, T. 2018; Ivanova *et al.*, 2018). As antivirulence agents do not affect the growth of bacteria and have no pressure on microorganisms' survival, there will not induce bacteria to develop resistance. With this concept, numerous drugs have been introduced as antivirulence agents before their clinical use alone or in combination with traditional antibiotics (Defoirdt, T. 2018; Ivanova *et al.*, 2018).

Quorum sensing inhibition can be possible by the following strategies (Subhadra *et al.*, 2018):

- i. Inactivation or enzymatic degradation of QS signalling molecule
- ii. Inhibition of signal molecule synthesis
- iii. Application of inductor antagonists molecules
- iv. Inhibiting the biosynthesis of signalling molecules
- v. Inhibition of signal transport

Hence, there was a need to choose a suitable screening method that could efficiently detect anti-QS with the four abovementioned inhibition modes. To fulfill the need, a simple and qualitative plate screening method of co-cultivation technique was used in the present study; a similar co-streaking method in agar plate was performed by Chu *et al.* for AHL-degradation bioassay with the biosensor strain and AHL donor strain and with minor modification, broth assay using microtiter plate was developed and used by Singh *et al.* to study quorum-sensing antagonists from bacteria associated with marine macroorganisms. In the present screening technique, the marine isolates were co-cultured with *S. marcescens* in a petri-plate containing LB agar. If marine microorganisms could produce an anti-QS molecule, irrespective of its mode of action, it would diffuse in nearby areas, reach growing *S. marcescens*, and inhibit its QS. Areas covered by anti-QS molecule by diffusion were observed with non-pigmented growth or white colour colony of bioreporter strain *S. marcescens*; the remaining unreached part of *S. marcescens* in co-culture was remain to grow of *S. marcescens* with its normal pigmented phenotype. Thus it also gives the basic idea of the efficiency of anti-QS molecules produced by marine isolates. Change in QS-based pigment prodigiosin production was visible after incubation; hence, no further confirmatory test or assays were required. Moreover, confirmation of anti-QS activity against the antimicrobial activity of marine isolates could be interpreted as test marine isolates only inhibited QS-based pigment. In contrast, the non-pigmented growth of *S. marcescens* remains unaffected. The screening method was significant in the unbiased detection of potential anti-QS isolates with no possibility of missing out on any quorum-sensing inhibitor. Thereby, it predicted the chance of developing resistance against QS inhibitor remain low.

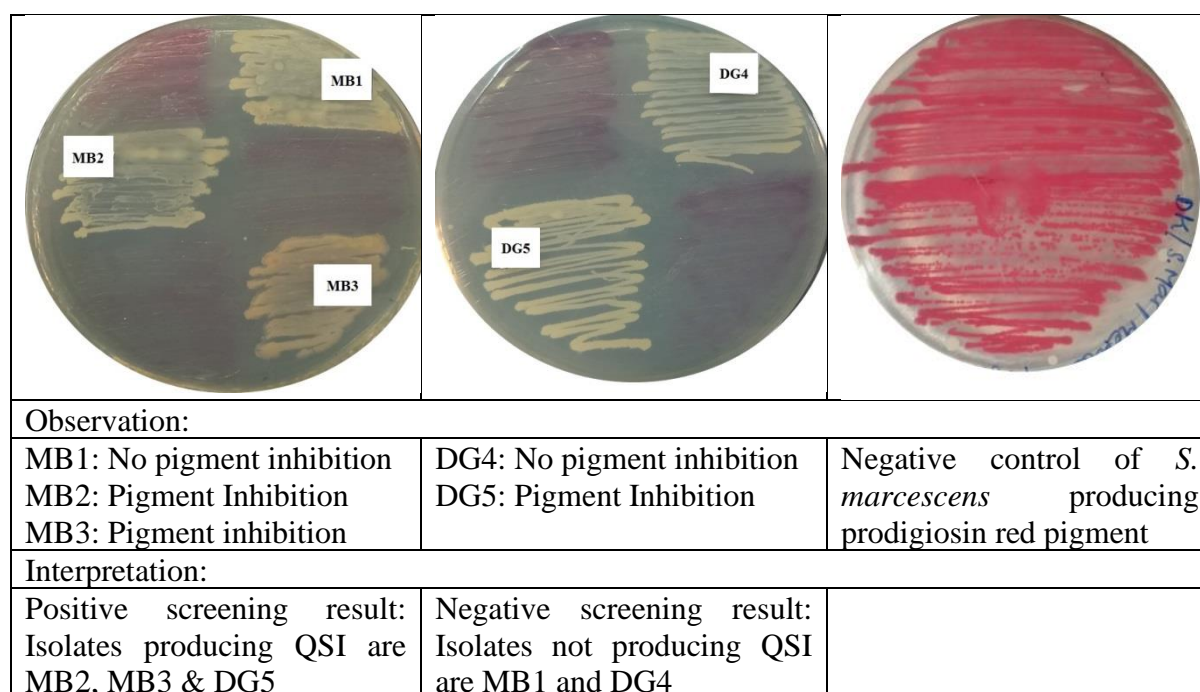


FIGURE 2 Co-culture studies of marine bacteria with *S. marcescens*. The absence of red colour prodigiosin pigment in co-culture screening indicates the anti-quorum sensing ability of marine isolates. *S. marcescens* without any co-culture was used as a negative control of quorum-sensing inhibition.

In the present study, approximately 59% of the free-living and 66% of the marine macroorganism associated marine bacteria with the potential of anti-QS were successfully isolated from the region of Gujarat coast. The highest anti-QS activity was found in isolate DG5, followed by MB2, MB3 and DE2. Out of four potent isolates, the present study describes the experimental results of MB2 and DG5. The mode of action of QSI from MB2 & DG5 is yet to be studied. Novel anti-quorum sensing molecule producers can be good candidates to overcome pathogens' virulence effects without hindering their growth. Such potential natural compounds can be most fitted in developing an alternative anti-virulent pharmaceutical agent replacing traditional antibiotics. Further studies to check the potential of anti-QS of MB3 and DE2 with advanced study of their structure and various parameter of quorum sensing are in progress.

5. CONCLUSION

Marine-derived bacteria are rich sources of diverse bioactive compounds with the main focus of potential anti-quorum sensing compounds. Advanced study of marine-based anti-QS compounds can give pharmaceutically important compounds that can combat the need for anti-virulence against multidrug resistance infection.

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Data Availability Statement

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. REFERENCES

- CDC. (2019). Antibiotic resistance threats in the United States, 2019, Atlanta, GA: US Department of Health and Human Services. Center for Disease Control and Prevention, 3,103-104. <https://ndc.services.cdc.gov/wp-content/uploads/Antibiotic-Resistance-Threats-in-the-United-States-2019.pdf>
- Chu, W., Lu, F., Zhu, W., & Kang, C. (2011). Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. *Journal of applied microbiology*, 110(1), 202-208. <https://doi.org/10.1111/j.1365-2672.2010.04872.x>
- Defoidt, T. (2018, April 1). Quorum-Sensing Systems as Targets for Antivirulence Therapy. *Trends in Microbiology*. Elsevier Ltd. <https://doi.org/10.1016/j.tim.2017.10.005>
- El-Kurdi, N., Abdulla, H. & Hanora, A. Anti-quorum sensing activity of some marine bacteria isolated from different marine resources in Egypt. *Biotechnol Lett* 43, 455–468 (2021). <https://doi.org/10.1007/s10529-020-03020-x>
- Engelbrecht, J., Nealson, K., & Silverman, M. (1983). Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell*, 32(3), 773-781. [https://doi.org/10.1016/0092-8674\(83\)90063-6](https://doi.org/10.1016/0092-8674(83)90063-6)
- Hutchings, M., Truman, A., & Wilkinson, B. (2019, October 1). Antibiotics: past, present and future. *Current Opinion in Microbiology*. Elsevier Ltd. <https://doi.org/10.1016/j.mib.2019.10.008>
- Ivanova, A., Ivanova, K., & Tzanov, T. (2018). Inhibition of quorum-sensing: A new paradigm in controlling bacterial virulence and biofilm formation. *Biotechnological Applications of Quorum Sensing Inhibitors*, 3-21. https://doi.org/10.1007/978-981-10-9026-4_1
- Kalia, V. C. (2013). Quorum sensing inhibitors: an overview. *Biotechnology advances*, 31(2), 224-245. <https://doi.org/10.1016/j.biotechadv.2012.10.004>
- Papenfort, K., & Bassler, B. L. (2016). Quorum sensing signal-response systems in Gram-negative bacteria. *Nature reviews. Microbiology*, 14(9), 576–588. <https://doi.org/10.1038/nrmicro.2016.89>
- Singh, A. A., Singh, A. K., & Nerurkar, A. (2020). Bacteria associated with marine macroorganisms as potential source of quorum-sensing antagonists. *Journal of basic microbiology*, 60(9), 799-808. <https://doi.org/10.1002/jobm.202000231>
- Singh, A. A., Singh, A. K., & Nerurkar, A. (2021). Disrupting the quorum sensing mediated virulence in soft rot causing *Pectobacterium carotovorum* by marine sponge associated *Bacillus* sp. OA10. *World Journal of Microbiology and Biotechnology*, 37, 1-11. <https://doi.org/10.1007/s11274-020-02982-4>
- Subhadra, B., Kim, D. H., Woo, K., Surendran, S., & Choi, C. H. (2018). Control of biofilm formation in healthcare: Recent advances exploiting quorum-sensing interference strategies and multidrug efflux pump inhibitors. *Materials*, 11(9), 1676. <https://doi.org/10.3390/ma11091676>

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