Extract from phyllosphere bacteria with antibiofilm and quorum quenching activity to control several fish pathogenic bacteria

Olivia Nathalia and Diana Elizabeth Waturangi*

Abstract

Objective: The objective of this research were to screen quorum quenching activity compound from phyllosphere bacteria as well as antibiofilm activity against several fish pathogen bacteria such as *Aeromonas hydrophila*, *Streptococcus agalactiae*, and *Vibrio harveyi*.

Results: We found eight phyllosphere bacteria isolates with potential quorum quenching activity to inhibit *Chromobacterium violaceum* as indicator bacteria. Crude extracts (20 mg/mL) showed various antibiofilm activity against fish pathogenic bacteria used in this study. Isolate JB 17B showed the highest activity to inhibit biofilm formation of *A. hydrophila* and *V. harveyi*, meanwhile isolate JB 3B showed the highest activity to inhibit biofilm of *S. agalactiae*. From destruction assay, isolate JB 8F showed the highest activity to disrupt biofilm of *A. hydrophila* isolate JB 20B showed the highest activity to disrupt biofilm of *V. harveyi*, isolate JB 17B also showed the highest activity to disrupt biofilm of *S. agalactiae*.

Keywords: Antibiofilm, Phyllosphere bacteria, Quorum sensing, Quorum quenching, Fish pathogen

Introduction

The high production of aquaculture has contributed to the development of the economy of Indonesia when there was a significantly increased demand for marine products, such as fish. However there are problems in this field need to be handled including infectious diseases caused by several pathogenic bacteria. Antibiotic therapy is a major treatment for these diseases, but incidence of antibiotic resistance in pathogenic bacteria is increasing, on other hand the use of antibiotics can disrupt the presence of intestinal microbiota communities and trigger a population of bacteria becoming resistant and effects on public health [1, 2]. A lot of research reported several pathogenic bacteria capable form biofilm to protect their cell from environmental stresses and antibiotics exposure. Therefore exploration of antibiofilm agents is important. The most studied regulatory mechanism that has been found to control biofilm formation is quorum sensing regulation [3]. Quorum sensing is a process in which bacteria can secrete specific extracellular signalling molecules called autoinducer [4].

Medicinal plants have been studied possess therapeutic properties in infectious disease treatments caused by pathogenic bacteria. Microbiota on the leaves surface contribute important role in these properties. There are various kinds of microbial communities including bacteria, fungi, as well as algae on the surface of leaves, these microbes live in interaction with plants by using plant’s exudate as for their nutritional resources, while in return microbe also contribute to support the growth and health of plants. Bacteria are known as the most abundant inhabitants of the phyllosphere. Compound from phyllosphere bacteria have been used as biocontrol to prevent plant from infectious diseases causing...
by pathogenic bacteria, these compound degrade molecular signal (AHL) of pathogenic bacteria therefore inhibit their infection [5]. Most of the compound isolated from phyllosphere bacteria with anti quorum sensing activities are lactonase. Another study showed the first enzyme was identified from Bacillus sp. Strain 24B01, and the gene encoding the enzyme was named aiiA for AI inactivation and its protein showed capability in inactivation of AHL signals through hydrolysis of the lactone ring [6]. Similarly, aiiD gene in P. aeruginosa also showed activity decreased the concentration of AHL, this bacteria produce extracellular proteolytic enzymes such as lactonase and acylase and breakdown the AHL molecules [7]. In fact, phyllosphere bacteria can act as phytopathogenic or inhibit phytopathogen colonization and infection.

**Main text**

**Methods**

**Bacterial cultivation**

In this study, fifty-three phyllosphere bacteria isolates were recovered from Guava (Psidium guajava), Starfruit (Averrhoa carambola), Madeira Vine (Anredera cordifolia) leaves from previous study [8, 9]. It were collected from Pondok Bambu and Karanganyar, Indonesia. For isolation of phyllosphere bacteria, the leaves were put into the tubes containing 10 mL of phosphate buffer 10 mM pH 8. The tubes then put into sonicator for 5 min to release the bacteria from the leaves and homogenized into the buffer. Then, the bacterial suspensions were diluted using phosphate buffer with serial dilution started from 10⁻¹ to 10⁻⁴. As much as 100 µL of the suspension were spread onto BHIA (Oxoid, Basingstoke, United Kingdom) and incubated at 28 °C for 48 h. All of the phyllosphere bacteria isolates were streaked onto King’s B 10% Agar and incubated at 28 °C for 48 h [10].

**Screening for anti-quorum sensing activity**

All of the phyllosphere bacteria isolates were streaked onto King’s B 10% Agar and incubated at 28 °C for 48 h. While for C. violaceum as indicator strain were grown separately in 5 mL of Luria Broth and incubated at 28 °C overnight with an orbital shaker at 120 rpm. The monitor strain (100 µL) from a culture with OD₆₀₀ = 0.132 was added to semisolid agar (0.75% agar) of 7 mL volume for an overlay on top of the isolates. These plates were incubated at 28 °C for 24 h [10].

**Production of crude extract**

Phyllosphere bacteria isolates were inoculated in 100 mL of Luria Broth and incubated at 28 °C for 48 h using orbital shaker incubator. After that, the suspension of bacteria was centrifuged at 13,888 × g for 15 min and then the supernatant was mixed with an equal volume of ethyl acetate (SmartLab) and it were kept in a rotary shaker 120 rpm overnight. The solvent layer was harvested and evaporated by a rotary evaporator, while the remaining solvent was evaporated using a vacuum oven overnight. After the extract were free from solvent, it was weighed and dissolved with 1% of DMSO (Smart Lab) with the final concentration reached at 20 mg/mL (w/v), then kept in the freezer −20 °C [11].

**Antibacterial activity assay**

As much as 100 µL of pathogenic bacteria with OD₆₀₀ = 0.132 were streaked continuously onto Brain Heart Infusion Agar (BHIA) (Oxoid, Basingstoke, United Kingdom) using sterile cotton buds. Then, the sterile cork borer was used to make a hole and filled by 100 µL of extract. In this study as positive control we used 10 mg/mL of streptomycin antibiotic (Merck), while 1% of DMSO was used as negative control. These plates were then incubated at 28 °C (A. hydrophila and V. harveyi) and 37 °C (S. agalactiae) for 24 h, this assay was performed in triplicate. Determination of Antibacterial activity were done through the clear zone performed as growth inhibition of fish pathogenic bacteria [12].

**Detection of quorum quenching activity**

Chromobacterium violaceum as indicator strain was added to BHIA and streaked continuously using sterile cotton buds. After that, similar with antibacterial activity assay, a hole were made using sterile cork borer and then filled with 100 µL of phyllosphere bacterial extract. Streptomycin (10 mg/mL) also used as positive control, for negative control we used 1% of DMSO. Incubation were done at 28 °C for 24 h. This assay was performed in triplicate. Determination of quorum sensing inhibition were done through formation of
clear zone around the extract which indicated inhibition of violacein pigment production [10].

**Quantification of anti-biofilm activity**

In this assay we conducted two analysis for each extract which were inhibition or destruction of biofilm formed by pathogenic bacteria. For the inhibition assay, 100 µL of pathogen culture and 100 µL of the extract were transferred into 96 wells microplate. For positive control we used only pathogen. While for negative control we used BHIB (Oxoid, Basingstoke, United Kingdom) medium without pathogens. Microplates were then incubated at 28 °C and 37 °C overnight. For the destruction assay, 100 µL of pathogen culture were transferred into 96 wells microplate and incubated overnight. After incubation, we added 100 µL of the phyllosphere extracts into the microplate followed by incubated overnight. Planktonic cells and media were discarded after incubation, and adherent cells were rinsed with deionized water two times and then kept for 30 min for air-dried. Staining were done by using 200 µL of 0.4% crystal violet for 30 min to stain the biofilm adhere on microplates. After that the dye was discarded and rinsed five times with deionized water, and kept it air dried for 30 min. As much as 200 µL of 96% of ethanol was added into each well and resuspended. Then, 150 µL of 96% of ethanol from each well was transferred into a new microplate. The optical density was determined at 595 nm with microplate reader (TECAN M200 PRO) [13]. The percentage of inhibition is calculated using the following formula [13]:

\[
\%\text{inhibition/destruction} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \times 100\%
\]

**Results**

**Screening for anti-quorum sensing activity**

Screening of anti quorum quenching activity were conducted for all of 53 isolates. We recovered 34 out of the 53 phyllosphere bacteria isolates performed inhibition of violacein pigment production through determination of indicator strain. From these results we found that that phyllosphere bacteria isolates have quorum quenching activity against *Chromobacterium violaceum* (Fig. 1).

**Antibacterial activity assay**

Antibacterial Activity assay performed that isolates JB 14B and JB 20B showed antibacterial activity against *A. hydrophila*. Isolate JB 14B, JB 16B, and JB 17B have antibacterial activity against *V. harveyi*. Isolate JB 20B, JB24B, and EJB 5F have antibacterial activity against *S. agalactiae* (Fig. 2).

**Detection of quorum quenching activity**

From this assay, we found that 8 isolates from *P. guajava* form clear zone around the extract which indicate inhibition of pigment production. This result showed that the crude extracts have quorum quenching activity against *Chromobacterium violaceum*.

**Quantification of anti-biofilm activity**

The result of inhibition activity assay showed that crude extracts from isolate JB 17B showed the highest
activity to inhibit biofilm formation of *A. hydrophila* with the result up to 86%. While isolate EJB 5F showed the highest inhibition activity in inhibiting biofilm formation of *V. harveyi* with the result up to 72%. Isolate JB 3B showed the highest activity to inhibit biofilm formation of *S. agalactiae* with the result up to 81% (Table 1).

From destruction assay, there were several isolates that performed high activity to destruct biofilm of one specific fish pathogenic bacteria. Extract from isolate JB 8F showed the highest activity to disrupt biofilm of *A. hydrophila* (81% of destruction). Isolate JB 20B performed the highest activity in disrupting biofilm formed by *V. harveyi* with the percentage destruction of 84%. While isolate JB 17B also showed the highest activity to disrupt biofilm of *S. agalactiae* with the result up to 73% (Table 1).

**Discussion**

Thirty-four isolates performed quorum quenching activity to inhibit the production of violacein pigment. Mechanism of quorum quenching were defined as inhibition of cell-to-cell communication through signal molecule interference, by using *C. violaceum*, these activity were determined in inhibition of violacein production [10].

Antibacterial activity assay showed that two out of fifty-three phyllosphere bacterial extracts inhibited the growth of *A. hydrophila*, while three out of fifty-three phyllosphere bacterial extracts performed antibacterial activity to *V. cholerae* and *S. agalactiae*. Those extracts with antibacterial activity were not continued for further assays, since it might cause false positive results in assessment of antibiofilm activity [12].

Several extracts showed quorum quenching activity due to inhibition of violacein pigment production, further studies were required to confirmed and determined the mechanism of bacterial communication inhibition.

**Table 1** Biofilm inhibition and destruction activity against fish pathogen bacteria

| Crude Extract | *A. hydrophila* | | % inhibition | % destruction | | % inhibition | % destruction | | % inhibition | % destruction |
|---------------|----------------|---|----------------|----------------|---|----------------|----------------|---|----------------|----------------|
| JB 3B         | 29             | 39 | 23             | X              | X             | 81             | 55             |
| JB 16B        | 37             | 56 | X              | X              | X             | 73             | 51             |
| JB 17B        | 86             | 72 | X              | X              | X             | 70             | 73             |
| JB 20B        | X              | X  | X              | 55             | 84             | X              | X              |
| JB 26B        | 24             | 28 | 41             | 32             | 52             | X              | 39             |
| JB 8F         | 78             | 81 | 58             | 75             | 62             | 52             | 52             |
| JB 12F        | -              | 39 | 65             | 39             | 79             | 60             | 60             |
| EJB 5F        | 42             | 29 | 72             | X              | X              | X              | X              |

NB: X = contains anti-bacterial activity, — = no activity
there are some possibilities might occurred which are inhibition of signal molecule production or interference of signal receptor [10].

The antibiofilm activity of phyllosphere bacterial extracts might come from their competencies to produce extracellular polymeric substance (EPS)-degrading enzyme. Both EPS production and biofilm formation are regulated by quorum sensing mechanism, which include production, release, as well as detection of signalling molecules. When population density are saturated it start induction of genes related with biofilm differentiation and maturation [14].

Inhibition of biofilm formation activity of phyllosphere bacteria might reflect quorum quenching activity of the phyllosphere bacteria extract to impede cell-to-cell communication of fish pathogenic bacteria used in this study. On the leaves surface there are several pressure which is need to be confronted by the microbe live in these habitats including nutrient limitation and environmental stress and physical condition. Hence, it is important for the bacteria in these habitat to develop adaptation and competitiveness to compete with other bacteria, one of them is capability to inhibit communication of other bacteria as well as production of extracellular polysaccharide and pigment to support adhesion on leaves surface also protection from other bacteria [15, 16].

There are several steps in the process of biofilm production in A. hydrophila. On the first step it were include production of lipopolysaccharides (LPS), and other surface polysaccharides (α-glucan), Mg2+ transporters, cytoskeletons as well as flagella and chemotaxis. This bacteria used.

AI-1 system to induce biofilm production and virulence-associated factors. The virulence factors regulated through this system were cover several virulence factors such as expression of hemolysin, metalloproteases, amylose, serine proteases, production of S-layer DNAase expression, including production of pigment.

There are two types of flagella (polar and lateral) is harbored by A. hydrophila, these two flagella are important for swimming and swarming and also play role in pathogenesis and biofilm formation of this bacteria [17, 18, 19]. According to Rasch et al. [20], the 3-oxo-C10-HSL produced by Vibrio anguillarum inhibits protease activities of A. hydrophila. Similarity, Ponnusamy et al. [21] showed that the synthetic 2(5H)-furanone derived from inhibitor AHL produced by Delisea pulcha, exhibited quorum quenching activity against C4-HSL and C6-HSL.

Biofilm formation of V. harveyi is under control of AHL-mediated QS mechanism. So far there are three molecules signals involved in quorum sensing mechanism of this bacteria, such as Harveyi autoinducer (HAI-1), Autoinducer 2 (AI-2), and Cholerae inducer 1 (CAI-1) [22]. There are positive correlation of biofilm formation with increasing in EPS production.

It was reported that the extract of Synechococcus sp interfere production of EPS. The GC–MS assays performed fatty acid and bioactive compound hexadecenoic acid [23]. Similarly, based on Kannan et al. [24], biosurfactant from Vibrio natriegens which identified as glycolipid showed aptitude to reduce biofilm formation, bioluminescence, EPS, as well as quorum sensing of V. harveyi.

According to Rosini et al. [25], pili formed by S. agalactiae are important in biofilm formation of this bacteria and the gene encode GBS pilus machinery are clustered in three related genomic islands (Islands PI-1, -2a, and -2b. These gene encode protein which is contribute for arrangement of pilus while other two gene encode enzyme called sortase which is important for make covalent linkage of the pilus protein into the polymers.

Based on Rinaudo et al. [26] pilus type 2a are contribute in biofilm formation, in the absence or low expression of pilus 2a condition conduce decrease of biofilm production. On the other hand, sortase enzyme also hinder biofilm formation.

Destruction activity of extract from phyllosphere bacteria might come from the production of matrix-degrading enzymes. EPS-degrading enzymes promote degradation of extracellular matrix as one of the major component in biofilm. Compare with other enzymes, protease known potential to destruct biofilm, since the major component of extracellular polymeric substance are proteins [27].

Based on antibiofilm activity results, both inhibition and destruction showed that not all of the extracts have both activities. V. harveyi and S. agalactiae biofilm could be inhibited by all extracts of isolates, meanwhile biofilm of A. hydrophila could not be inhibited by isolate JB 12F. This might be caused by the lack of enzyme production to inhibit the biofilm component of A. hydrophila. Furthermore, the variety of the result might come by several factors, including different component of biofilm formed by pathogenic bacteria, different compound in the extracts itself both for biofilm disruption and inhibition, environmental factors (pH and temperature) which affect the biofilm formation [28].

Conclusion
In this study we found eight extracts from phyllosphere bacteria with quorum quenching activity. All of these extracts are also capable to inhibit and disrupt biofilm formed by fish pathogenic bacteria. It is potential to be applied for prevention and treatment of infectious diseases caused by pathogenic bacteria used in this study.

Further research is important to be conducted to explore application of phyllosphere bacteria extract in the
aquaculture ecosystem. However, other assays are needed to be carried out such as validation of quorum quenching molecules, molecular identification of quorum quenching, biofilm destruction molecules, and identification of potential bacteria to support this research. In addition, a combination assay of different extracts also required to be explored which might vary in the effectiveness.

**Limitations**

The pathogen bacteria that we selected represents pathogens commonly found in fish. Activity of the extract with other fish pathogenic bacteria might be different and need to be explored for further study. The main component of the extract has not been identified although many studies describe the major component is protein. Quantitative assay for quorum quenching activity need to be done for further study as well as Scanning Electron Microscopy observation to get image on how the biofilm destruction process.

**Abbreviations**

EPS: Extracellular polymeric substances; AHL: Acyl homoserine lactone.

**Acknowledgements**

The authors acknowledge research funding support by Indonesian Ministry of education and culture through competitive national research grant 2019-Fundamental research.

**Authors’ contributions**

ON: conduct research, data analysis, manuscript preparation under the advisory of DEW. DEW: personal investigator and design proposal and advisory the research. All authors read and approved the final manuscript.

**Funding**

This study was funded by DIKTI 2019. The funder has no contribution in design, collection, writing, and interpreting data in this study.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 3 February 2021 **Accepted:** 13 May 2021 **Published online:** 25 May 2021

**References**

1. Defoirdt T, Boon N, Bossier P, Verstraete W. Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. Curr Opin Microbiol. 2004;7(4):69–88. https://doi.org/10.1016/j.copm.2004.06.031.

2. Dong YH, Wang LH, Zhang LH. Quorum-quenching microbial infections: mechanisms and implications. Philos Trans R Soc Lond B Biol Sci. 2007;362(1483):1201–11. https://doi.org/10.1098/rstb.2007.2045.

3. Nithya C, Begum MF, Pandian SK. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of Pseudomonas aeruginosa PDK1. Appl Microbiol Biotechnol. 2010;88:341–58. https://doi.org/10.1007/s00253-010-2777-y.

4. Dong YH, Zhang LH. Quorum sensing and quorum quenching enzymes. J of Microbiol. 2005;43(1):101–9.

5. Ma A, Lv D, Zhuang X, Zhuang G. Quorum quenching in cultivable phyllosphere bacteria from tobacco. Int J Mol Sci. 2013;14(7):14607–19. https://doi.org/10.3390/ijms140714607.

6. Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature. 2001;411(6839):813–7. https://doi.org/10.1038/35081101.

7. Holden MT, Ram Chhabra S, de Nys R, Stead P, Bainton NJ, Hill PJ, Manefield M, Kumar N, Labatte M, England D, Rice S, Givskov M, Salmond GP, Stewart GS, Bycroft BW, Kjelleberg S, Williams P. Quorum sensing cross talk: isolation and chemical characterization of cyclic dipeptides from Pseudomonas aeruginosa and other gram-negative bacteria. Mol Microbiol. 1999;33(6):1254–66. https://doi.org/10.1046/j.1365-2958.1999.01577.x.

8. Juliana. Screening of phyllosphere and endophytic microbes producing antibacterial or anti quorum sensing activity from Agaratum conyzoides, Coleus amboinicus, and Psidium guajava [skripsi]. Jakarta (ID): Universitas Katolik Indonesia Atma Jaya; 2011.

9. Listarieni H. Screening of endophytic and phyllosphere microbes with antibacterial activity from Centella asiatica, Mirabilis jalapa, and Averrhoa bilimbi [skripsi]. Jakarta: Universitas Katolik Indonesia Atma Jaya; 2011.

10. Abudoleh SM, Mahasneh AM. Anti-quorum sensing activity of substances isolated from wild berry associated bacteria. Avicenna J Med Biotech. 2017;9(1):2330. https://doi.org/10.1142/9789814400501_0114.

11. Younis KM, Usup G, Ahmad A. Secondary metabolites produced by marine streptomycetes as antibiofilm and quorum-sensing inhibitors of uropathogen Proteus mirabilis. Environ Sci Pollut Res. 2015;22(3):4756–67. https://doi.org/10.1007/s11356-015-5687-9.

12. Padma R, Yalavarthty PD. Screening of diclofenac for antibacterial activity against pathogenic microorganisms. Int J Adv Pharm Biol Chem. 2015;4(3):5554558.

13. Waturangi DE, Rahayu BS, Lalu KY, Michael MN. Characterization of bioactive compound from Actinomyces from antibiofilm activity against Gram-negative and Gram-positive bacteria. Malays J Microbiol. 2016;12(4):291–9. https://doi.org/10.21161/mjm.809115.

14. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Soper J. Control of biofilm growth and quorum sensing systems. Front Microbiol. 2017;8:389. https://doi.org/10.3389/fmicb.2017.0037.

15. Lindow SE, Brandt MT. Microbiology of the phyllosphere. Appl Environ Microbiol. 2003;69(4):1875–83. https://doi.org/10.1128/aem.69.4.1875-1883.2003.

16. Whipp JM, Hand P, Pink D, Bending GD. Phyllosphere microbiology with special reference to diversity and plant genotype. J Appl Microbiol. 2008;105(6):1744–55. https://doi.org/10.1111/j.1365-2672.2008.03906.x.

17. Simoes M, Simoes LC, Vieira MJ. A review of current and emergent biofilm control strategies. LWT Food Sci Technol. 2010;43:573–83.

18. Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, Chhabra SR, Williams P, Macintyre S, Stewart GS. Quorum sensing in Aeromonas hydrophila and Aeromonas salmonicida: identification of the LuxRI homologs AhyRI and their cognate -acylhomoserine lactone signal molecules. J Bacteriol. 1997;179:5271–81.

19. Talagrand-Reboul E, Jumas-Bilak E, Lamy B. The social life of Aeromonas salmonicida. J Bacteriol. 1999;181(14):4320–30. https://doi.org/10.1128/JB.181.14.4320-4330.1999.

20. Rasch M, Kastbjerg VG, Bruhn JB, Dalgaard I, Givskov M, Gram M. Quorum sensing signals are produced by Aeromonas salmonicida and quorum sensing inhibitors can reduce production of a potential virulence factor. Dis Aquat Organ. 2007;78:105–13. https://doi.org/10.3354/dao01865.

21. Ponnusamy K, Paul D, Sam Kim Y, Kweon JH. 2S(5H)-furanone: a prospective strategy for biofouling-control in membrane biofilm bacteria by...
22. Henke JM, Basler BL. Quorum sensing regulates type III secretion in Vibrio harveyi and Vibrio parahaemolyticus. J Bacteriol. 2004;186(20):3794–805. https://doi.org/10.1128/JB.186.20.6902-6914.2004.

23. Santhakumari S, Kannappan A, Pandian SK, Thaikudin N, Rajendran RB, Ravi AV. Inhibitory effect of marine cyanobacterial extract on biofilm formation and virulence factor production of bacterial pathogens causing vibriosis in aquaculture. J Appl Phycol. 2015;28(1):313–24. https://doi.org/10.1007/s10811-015-0554-0.

24. Kannan S, Krishnamoorthy G, Kulanthaiyesu A, Marudhamuthu M. Effect of biosurfactant derived from Vibrio natriegens MK3 against Vibrio harveyi biofilm and virulence. J Basic Microbiol. 2019;59(9):859–960. https://doi.org/10.1002/jobm.201800706.

25. Rosini R, Rinaudo CD, Sorani M, Lauer P, Mora M. Identification of novel genomic islands coding for antigenic pilus-like structures in Streptococcus agalactiae. Mol Microbiol. 2006;61(1):126–41. https://doi.org/10.1111/j.1365-2958.2006.05225.x.

26. Rinaudo CD, Rosini R, Galeotti CL, Berti F, Necchi F, Reguzzi V, Ghezzo C, Telford JL, Grandi G, Maione D. Specific involvement of pilus type 2a in biofilm formation in group B Streptococcus. PLoS ONE. 2010;5(2):1–11. https://doi.org/10.1371/journal.pone.0009216.

27. Saggu SK, Jha G, Mishra PC. Enzymatic degradation of biofilm by metalloprotease from Microbacterium sp. SKS10. Front Bioeng Biotechnol. 2019;7(192):1–13.

28. Mizan FR, Jahid IK, Park SY, Ha SD. Effects of temperature on biofilm formation and quorum sensing of Aeromonas hydrophila. Italian J of Food Sci. 2018;30(3):456–66. https://doi.org/10.14674/ijfs-1004.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.