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Research article

Green tea (*Camellia sinensis*) extract inhibits biofilm formation in acyl homoserine lactone-producing, antibiotic-resistant *Morganella morganii* isolated from Pasig River, Philippines

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ABSTRACT

The drastic development of urban districts around the world has caused changes in the environment, specifically on metropolitan waterways such as the Pasig River in the Philippines. These significant changes resulted in diversity of microorganisms and their mechanisms employed such as antibiotic resistance and their communication system or quorum sensing (QS). In this study, four bacterial isolates from Pasig River, identified as *Aeromonas salmonicida*, *Acinetobacter* sp., *Morganella morganii*, and *Citrobacter freundii*, were observed to employ short-chain acyl homoserine lactone (AHL) as their signalling molecule based on *in vitro* assays using the biosensor strain *Chromobacterium violaceum* CV026. Furthermore, *M. morganii* isolate was shown to be resistant to chloramphenicol. This poses a significant threat not just to public health but also to the aquatic life present in the river. Thus, green tea (*Camellia sinensis*) extract was tested for its capability to inhibit *in vitro* biofilm formation in *M. morganii*, as well as the short-chain acyl homoserine lactone QS system using *C. violaceum* ATCC 12472. Results showed that the extract significantly ($p < 0.05$) inhibited biofilm formation in *M. morganii* at as low as 62.5 μg/mL (31.55%). Increasing the concentration (500 μg/mL) did not significantly ($p > 0.05$) enhance the activity (41.21%). Furthermore, the extract also inhibited pigmentation in *C. violaceum* ATCC 12472, suggesting QS inhibition. This study adds into record the production of short-chain AHLs by *Aeromonas salmonicida*, *Acinetobacter* sp., *Morganella morganii*, and *Citrobacter freundii*, as well as the potential of green tea extract as inhibitor of biofilm formation in antibiotic-resistant *M. morganii* possibly through QS inhibition.

1. Introduction

The continuous economic and infrastructure developments in Metro Manila pose threats to the existing ecological systems in the area including rivers. The Pasig River, one of the main waterways in the capital, has continuously deteriorated as a result of increasing industrial and human activities [1]. Microbial diversity in highly-urbanized waterways are exposed to various environmental stressors linked to urban infrastructure and land use. Approximately 12% of intolerant species in streams are lost due to these developments [2, 3]. In turn, the changes in the water quality of urban waterways resulted in changes in microbial diversity and the resistance profiles of these microorganisms [4, 5]. These anthropogenic activities also contributed to the prevalence of specific taxa such as *Aeromonas, Acinetobacter, Bacteroidetes, Proteobacteria* and *Trichococcus* [6].

One of the significant concerns on water quality is the contamination of various pathogens which cause water-borne diseases such as diarrhea and gastrointestinal illnesses. Although water bodies are not favorable environments for most human pathogens, there are still manifolds of microorganisms that can survive and multiply in these communities [7]. Their presence in water sources can result in disease outbreaks, further spread of infection, and even loss of aquatic biodiversity, which are threats to public health [8]. These pathogens, which include bacteria,

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viruses, and protozoa, have caused several outbreaks which infected millions worldwide [9].

Therefore, it is important to know the mechanisms behind the pathogenicity and virulence of these microorganisms in order to formulate strategies as to how to control these pathogens. One of the novel strategies in combating bacterial infection is through the inhibition of quorum sensing (QS) [10]. Quorum sensing is the cell-to-cell communication system mediated by signalling molecules and receptors to trigger the expression of several bacterial mechanisms such as biofilm formation, pigmentation, and production of virulence factors [11]. In Gram-negative bacteria, acyl homoserine lactones (AHLs) are usually employed as their signalling molecules [12]. Previous studies show that several aquatic pathogens including *Aeromonas* sp. [13], *Acinetobacter* sp. [14], *Citrobacter* sp. [15], and *Morganella morganii* [16] use AHLs as their signalling molecules.

In the aquatic ecosystem, biofilm has been known as the principal form of microbial life since bacterial connections within this matrix supply a number of benefits to the microorganisms [17]. Biofilm formation was also observed to be an important mechanism involved in pathogenesis in aquatic life such as in shrimp [18] and fish [19]. Furthermore, it was shown that biofilm helps associated bacteria obtain virulence properties [20]. Additionally, biofilm formation in aquatic environments also causes the release of chemical compounds that affect odor, taste, and color [21]. In humans, infections associated with seafoods caused by biofilm-forming bacteria were also reported [22, 23]. Development of resistance to antibiotics also occurs in biofilms [24]. An increase in antibiotic resistance is observed on biofilms compared with their planktonic counterparts due to the cooperation between cells in biofilms which are mediated by quorum sensing [25].

Since biofilm formation is controlled by quorum sensing, pathogenic and opportunistic microorganisms are able to survive and adapt in different environments by regulating their genes which are responsible for virulence, biofilm production and genetic transfer [26]. Inhibitors of both QS and biofilm formation are currently being studied as potential alternatives to antibiotics because of their low to zero possibility of developing resistance [10]. Information regarding their QS systems, particularly on the signaling molecules they employ, allows the discovery of novel inhibitors especially from natural products. Hence, it is important to know the QS pathways of these pathogens in order to understand the modes of action of inhibitors of this bacterial mechanism.

The use of natural products, such as those from plant extracts, against biofilm formation and QS are extensively being studied due to their phytochemical diversity [27]. Green tea, a popular drink and a food supplement based on the extract of *Camellia sinensis* leaves, has a wide array of phytochemicals which are responsible for its broad applications [28]. Around 30% of its total composition are polyphenols, mainly catechins, of which 65% of the total catechin content is Epigallocatechin-3-gallate (EGCG) [29]. Previous studies showed that polyphenols from tea were able to inhibit AHL-mediated QS and biofilm formation in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, respectively [30, 31].

Therefore, in this study, short-chain acyl homoserine lactone-producing isolates from Pasig River, Philippines were collected and their antibiotic susceptibility profiles were noted. Isolates which were observed to be resistant to antibiotics were then used in the investigation of the potential biofilm and QS inhibitory activities of green tea extract.

2. Materials and methods

2.1. Sample collection

Approximately 1 L of water sample from Pasig River were collected using a sterile glass bottle. The sample was stored in an ice chest during transport to the laboratory and was immediately used for the isolation.

2.2. Isolation and purification of Gram-negative bacteria

Ten milliliters of the water sample were first diluted with 90 mL sterile Alkaline Peptone Water (APW). Thereafter, ten-fold serial dilutions were made up to 10^{-4} dilutions. One hundred microliters of the sample were spread evenly on MacConkey Agar (MCA) and then incubated for 24 h at 35 °C. Colonies of different morphologies were isolated on Nutrient Agar (NA) then incubated for 24 h at 35 °C. The isolates were streaked onto fresh NA plates until a pure culture was obtained as confirmed through Gram staining.

2.3. Detection of short-chain AHL production

Twenty four-h old cultures of the isolates were screened for their short-chain AHL production by cross-streaking them on NA plates with *Chromobacterium violaceum* CV026, a mini-Tn5 mutant which is incapable of producing its own autoinducer but is able to produce pigment when supplemented with external AHL. Violet pigmentation will be observed on *C. violaceum* CV026 if the isolates were able to produce short-chain AHL [32]. The type strain *C. violaceum* ATCC 12472 served as the positive control [33] and a local isolate *Bacillus cereus CRK1-1* served as the negative control [34].

2.4. Identification of short-chain AHL-producing isolates

Colonial and cellular morphologies, as well as biochemical profile (API 20E, catalase, oxidase) of the isolates were noted and compared with literature. For molecular identification, the genomic DNA of the isolates was extracted using Vivantis GF-1 Bacterial DNA Extraction Kit (Vivantis, Malaysia) following the manufacturer's protocol. The 16S rRNA gene was amplified for 40 cycles through 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) primers using MyTaq Red PCR Mix (Bioline, USA) and the following conditions were set: Initial denaturation at 98 °C for 5 min; denaturation at 95 °C for 5 s; annealing at 58.6 °C for 5 s, extension at 72 °C for 20 s, and final extension at 72 °C for 1 min. Gel electrophoresis was done to check for the amplified genes. The amplicons were sent to Macrogen, Inc., South Korea for sequencing. The sequences were analyzed using BioEdit software prior to BLASTn search to look for the possible identification of the isolates. Phylogenetic analyses were performed using TrEase software (http://www.thines-lab.senckenberg.de/trease/). The sequences were aligned using Mafft (G–INS–I model) and a phylogenetic tree was constructed through FastTree using the gtr algorithm.

2.5. Antibiotic susceptibility profiling

Twenty four-h old cultures of the isolates were prepared in Tryptone Soya Broth (TSB) and standardized using 0.5 McFarland Standard to obtain a cell density of 1.5×10^8 CFU/mL. Bacterial lawns of the isolates were made on Tryptone Soya Agar (TSA) plates. Antibiotic disks (Oxoid, USA) were used and placed on the surface of the TSA plates. The selected antibiotics (30 μg) were: Amoxicillin clavulanate (AMC), Chloramphenicol (C), Gentamicin (GM), Tobramycin (NN), and Cefotaxime (CTX).

2.6. Preparation of green tea extract (GTE)

Green tea extract (GTE) from Healthy Options®, Philippines was used. According to the manufacturer, it contains 90% total polyphenols wherein 70% was total catechins (with 50% epigallocatechin (EGCG)). A starting concentration of 5 mg/mL was prepared by dissolving 250 mg GTE in 50 mL sterile distilled water. Two-fold serial dilution was performed to obtain different stock concentrations of GTE (2.5 mg/mL, 1.25 mg/mL, 0.6 mg/mL, and 0.3 mg/mL).

2.7. Antibacterial activity

2.7.1. Disk diffusion assay

Bacterial lawns of the test organism were made on TSA plates using 24-h old standardized inocula (1.5×10^6 CFU/mL). Paper disks containing different concentrations of GTE were placed on the surface of the agar. The plates were then incubated at 35 °C for 24 h. The zones of inhibition (ZOI), indicated by clear halos around the disks, were measured using a Vernier caliper [35]. Tryptone Soya Broth (TSB) was used as the negative control.

2.7.2. Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations

Twenty microliters of the different concentrations of GTE were placed on the wells of a 96-well microtitre plate. Thereafter, 180 μL of standardized 24-h old inocula (1.5×10^6 CFU/mL) of the test organism were placed on each well. The wells were incubated at 35 °C for 24 h. The absence of pellets at the bottom of the wells indicates growth inhibition. The lowest concentration that showed growth inhibition was the MIC. To determine the MBC, the wells used in the determination of the MIC were streaked onto freshly-prepared TSA plates. The lowest concentration that yielded no growth was the MBC [35]. Tryptone Soya Broth (TSB) was used as the negative control.

2.8. Biofilm inhibition assay

Different concentrations of GTE (20 μL), along with TSB as the negative control, were placed on the wells of a 96-well microtitre plate. Afterwards, 180 μL of standardized inocula (1.5×10^6 CFU/mL) of the test organism were inoculated on each well. The wells were incubated at 35 °C for 24 h. The contents of the wells were discarded and washed with sterile distilled water to remove the non-adhering cells. The biofilms formed on the sides of the wells were stained by adding 125 μL of 0.1% crystal violet for 15 min and then washed again with sterile distilled water to remove excess stain. The stained biofilms were then solubilized using 33% acetic acid for 15 min. The solubilized biofilms were transferred onto new 96-well microtitre plate and then quantified using a microplate reader (PowerWave 340, Bio-tek Instruments, USA) at 550 nm [36].

2.9. Quorum sensing inhibition assay

Standardized 24-h old inoculum (1.5×10^6 CFU/mL) of *C. violaceum* ATCC 12472 was used to create bacterial lawns on TSA plates. Disks containing different concentrations of GTE were placed on the surface of the agar. Sterile TSB was used as the negative control. The plates were then incubated at 35 °C for 24 h. Zones of QS inhibition were indicated by opaque halos with growth around the disks [37].

2.10. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 2.0 software. One-Way Analysis of Variance (ANOVA) was performed to determine if a significant difference exists among the zones of inhibition and absorbance readings of the different concentrations of GTE and the control. To determine where the significant difference lies, Tukey's *post hoc* test was performed.

3. Results and discussion

Since QS is involved in biofilm formation as well as production of virulence factors, it is important to understand this bacterial communication system, particularly the signaling being employed [11]. This will serve as a guide in designing drugs used in controlling bacterial pathogens through the inhibition of their communication systems as well as the mechanisms dependent on it.

3.1. Isolation of short-chain acyl homoserine lactone-producing Gram-negative bacteria from Pasig River, Philippines

A total of 15 randomly-selected colonies from the Gram-negative-selective MCA plates were transferred onto TSA plates for purification. The isolates were then cross-streaked with the QS biosensor strain *C. violaceum* CV026. Four isolates (T1, T2, T3, and T4) were shown to produce AHL due to the pigmentation observed on the biosensor strain (Figure 1). This indicates that the isolates were able to produce significant amounts of AHLs resulting in the induction of violacein production in *C. violaceum* CV026 [32].

Gram-staining was done to confirm that the isolates were indeed Gram-negative since bacterial species under this group typically employ AHLs as their autoinducers [12]. Phenotypic characterization of the short-chain AHL-producing isolates showed that they were indeed Gram-negative (Figure 2) and are potentially enteric bacteria due to their capability to grow on MacConkey agar. Previous studies show that aquatic enteric pathogens such as *Aeromonas* spp. [38], *Acinetobacter baumannii* [14], and *Citrobacter freundii* [15] usually employ AHL-mediated quorum sensing systems.

To further identify the short-chain AHL-producing isolates, molecular identification was performed. Figure 3 shows the identification of the isolates based on the sequencing analysis of their 16S rRNA gene. Isolate T1 was identified as *Aeromonas salmonicida*. It is a pathogen causing furunculosis in salmonid fishes previously isolated in the effluent of a hatchery tank of Atlantic salmons [39]. In a previous study, *A. salmonicida* produced diffusible AHLs as observed with the violet pigmentation when cross-streaked with *C. violaceum* CV0blu [13]. Furthermore, they were able to discern that QS in *A. salmonicida* employed AhyI and AsaI which are both LuxI homologues. They were also able to identify the autoinducers involved in its QS system as N-(butanoyl)-L-homoserine lactone, its primary autoinducer, and N-hexanoyl-L-homoserine lactone as its other signalling molecule. Similarly, *Aeromonas* spp. isolated from clinical samples were able to induce violacein production in *C. violaceum* CV026. Their results also showed that *Aeromonas* spp. synthesize C4-HSL and C6-HSL as its autoinducers [38].

Furthermore, previous reports of screening for AHL production of *Acinetobacter* spp. showed that these species synthesize long-chain AHLs 3-oxo-C12-HSL and 3-hydroxy-C12-HSL [40]. Similarly, a clinical isolate of *Acinetobacter* sp. was not able to induce pigmentation in *C. violaceum* CV026 which is an indication that it did not produce short-chain AHLs. However, LC-MS/MS analysis showed that this isolate did produce short-chain AHLS such as C6-HSL and C8-HSL. They stated that this may be due to the medium used as well as with the growth phase as to which the analysis was done [14]. The results of this study are rather similar to the latter since T2, identified as *Acinetobacter* sp., was able to synthesize short-chain AHLs as observed with the pigmentation produced by *C. violaceum* CV026 in the cross-streaking assay.

After 24 h of incubation, T3, later identified as *Morganella morganii*, produced a weakly-positive reaction evident with the faint pigmentation produced by *C. violaceum* CV026. This agrees with a previous study wherein *M. morganii* isolated from catheters produced a similar weakly-positive reaction when cross-streaked with *Agrobacterium tumefaciens* A136 - a QS biosensor strain used to detect production of C6- to C12-HSLs. Further incubation of this clinical isolate resulted in a more intense reaction with the biosensor strain [16]. Interestingly, an isolate of *M. morganii* from lettuce leaves was observed not to produce short-chain AHLs using the same biosensor strain used in this study [41]. Specifically, the type of substrate, electron acceptors, sludge concentration, pH, and temperature were found to be affecting AHL production by microbial communities found in activated sludge [42, 43]. Hence, the variations between the production of AHL of the same species isolated from different sources may be due to the conditions of their environment which may affect signalling [44]. Interestingly, AHL quenching was also found to be more stable in varying environmental conditions [43]. This

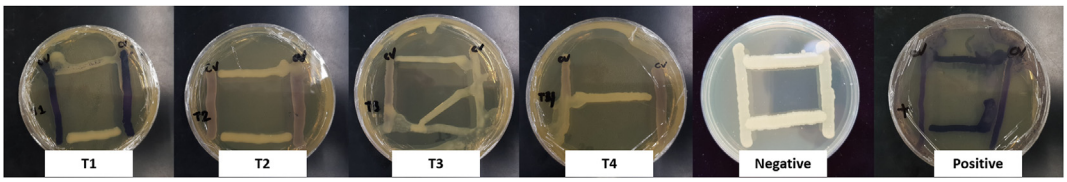

Figure 1. Screening for short-chain acyl homoserine lactone production through cross-streaking with quorum sensing biosensor strain *Chromobacterium violaceum* CV026. Four isolates (T1, T2, T3, and T4) were able to induce pigmentation in the biosensor strain *C. violaceum* CV026 indicating their capability to produce short-chained AHLs. Negative control *Bacillus cereus* CRK1-1 did not yield pigmentation whereas *C. violaceum* ATCC 12472, which served as positive control, was able to induce pigmentation.

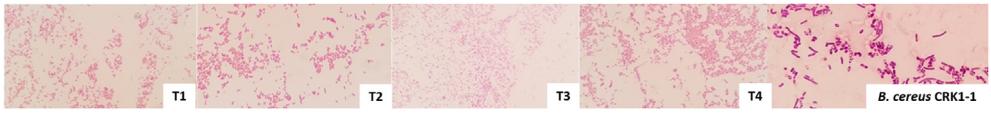

Figure 2. Cellular morphology of the short-chain acyl homoserine lactone-producing isolates from Pasig River, Philippines (10000x). All four AHL-producing isolates were observed to be Gram-negative.

therefore shows that AHL quenching may be an effective tool in combating QS even on varying environmental conditions.

Lastly, isolate T4, which was able to induce pigmentation in the *C. violaceum* CV026 biosensor strain, was identified as *Citrobacter freundii*. Previous characterization of the QS genes and autoinducers by *Citrobacter* spp. revealed their production of AHLs as their signalling molecules. *Citrobacter amalonaticus* isolated from dental plaque showed that it was able to produce several AHLs such as C8-HSL, C14-HSL and C16-HSL [45]. Similarly, a *C. amalonaticus* isolated from clams showed that this species was able to produce multiple types of AHLs which include short-chain AHLs such as C4-HSL, C6-HSL, C8-HSL, as well as long-chain C14-HSL and C-16 HSL [46]. *Citrobacter freundii* isolated from marine dinoflagellates were also observed to produce C4-HSL, C8-HSL, and C10-HSL using *Agrobacterium tumefaciens* biosensor strain A136 [15].

These results show that water samples from metropolitan waterways such as the Pasig River harbour different species of bacteria that employ

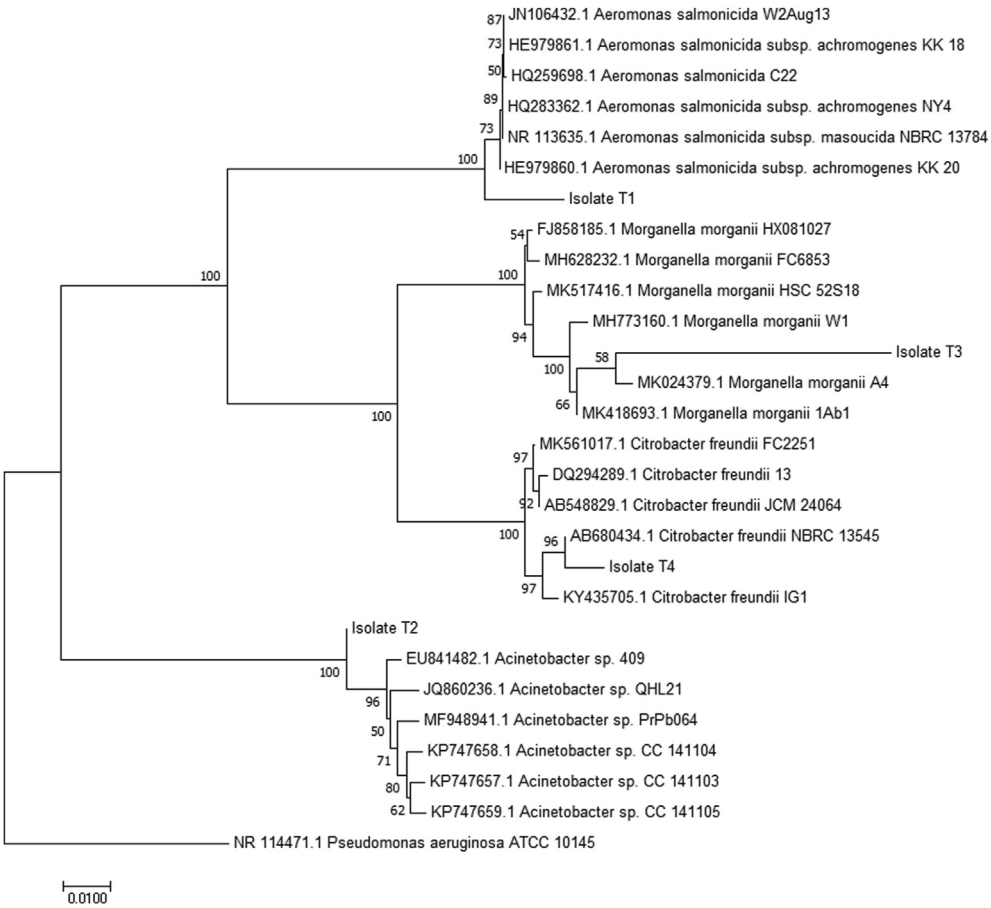

Figure 3. Phylogenetic analysis of the short-chain acyl homoserine lactone-producing isolates from Pasig River, Philippines. Molecular sequencing and phylogenetic analysis of the 16S rRNA gene of the isolates identified T1 as *Aeromonas salmonicida*, T2 as *Acinetobacter* sp., T3 as *Morganella morganii* and T4 as *Citrobacter freundii*.

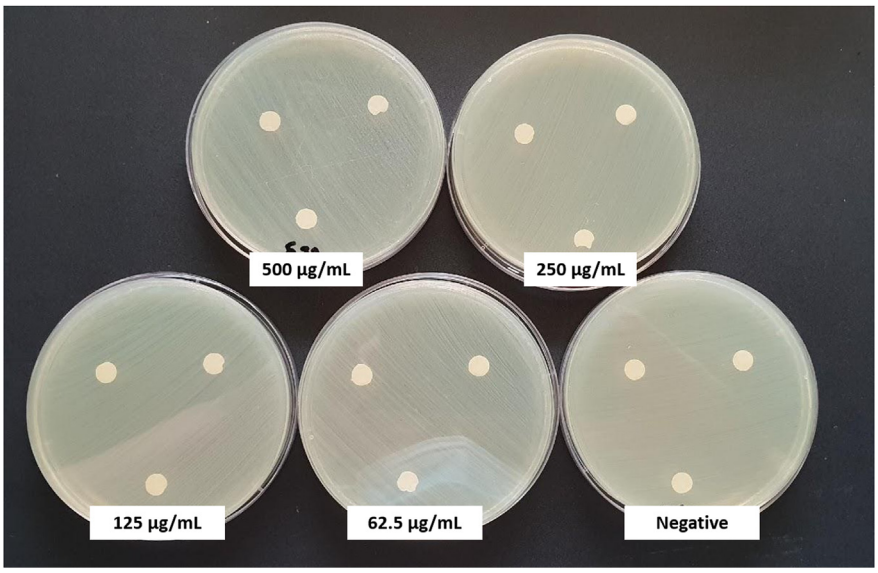

Figure 4. All concentrations of green tea extract tested (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL) did not inhibit the growth of *Morganella morganii* based on disk diffusion assay.

Table 1. Antibiotic susceptibility profile of AHL-producing fish pathogens.*

Antibiotic (30 µg)	T1	T2	T3	T4
Amoxicillin clavulanate	S	S	R†	S
Chloramphenicol	S	S	R	S
Gentamicin	S	S	S	S
Tobramycin	S	S	S	S
Cefotaxime	S	I	S	S

* *based on Clinical Laboratory Standards Institute (CLSI) 2015 standards for zones of inhibition using disk diffusion assay* [52].
† *intrinsic resistance* [50].

AHL-mediated QS systems. These also suggest that potentially-pathogenic bacterial species capable of expressing virulence properties and forming biofilms through QS thrive in aquatic environments as it was previously shown that QS circuits regulate the production of virulence factors and biofilm formation [11, 27]. The QS network, described in most bacteria including drug-resistant biofilm-forming pathogens, is composed of synthase proteins that produce extracellular signaling molecules or autoinducers. *N*-acyl homoserine lactones (AHLs) are the most common QS signals in Gram-negative bacteria [47]. The detection of these signaling molecules regulate gene expression [48], as a function in the formation, detachment, and development of biofilm [49]. The involvement of QS in the expression of genes attributes to the virulence and pathogenicity of bacteria [11]. Since these signaling molecules play an important role in the QS system, these information on AHLs employed by the isolates are therefore vital in the understanding of controlling these pathogens.

3.2. *Antibiotic susceptibility profiling revealed resistance of* Morganella morganii *against chloramphenicol*

Table 1 shows the antibiotic susceptibility profile of the isolates. Isolate T2, identified as *Acinetobacter* sp., showed intermediate susceptibility to cefotaxime, whereas among the isolates, T3, identified as *M. morganii* was the only isolate resistant to antibiotics (amoxicillin clavulanate and chloramphenicol). Related literature states that *M. morganii* is intrinsically resistant to cephalosporins which include amoxicillin clavulanate but is naturally susceptible to chloramphenicol [50]. Thus, the resistance to chloramphenicol of T3 was only acquired by the isolate. The development of resistance may be due to the extensive use of antibiotics, such as chloramphenicol, as evident on the detection of residues on surface waters [5]. Lastly, both isolates T1 and T4 were susceptible to the tested antibiotics.

Several physiological mechanisms dependent on QS pathways confer antibiotic resistance to bacterial cells. These include biofilm formation which is a complex community of microbial cells characterized by a

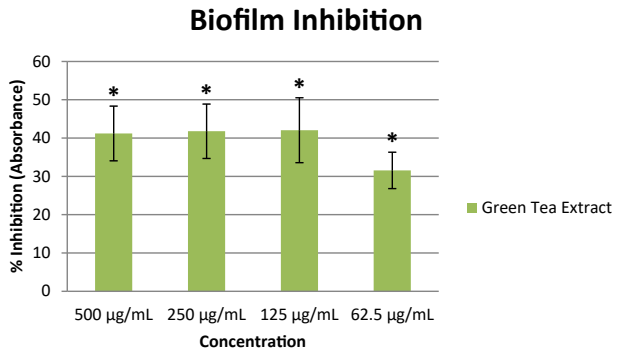

Figure 5. Relative biofilm inhibition of different concentrations of green tea extract (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL) compared against the negative control. Green tea extract significantly inhibited biofilm formation in *Morganella morganii* at a concentration as low as 62.5 µg/mL. However, increasing the concentration did not result in an increase in activity. *indicates significant difference against the negative control.

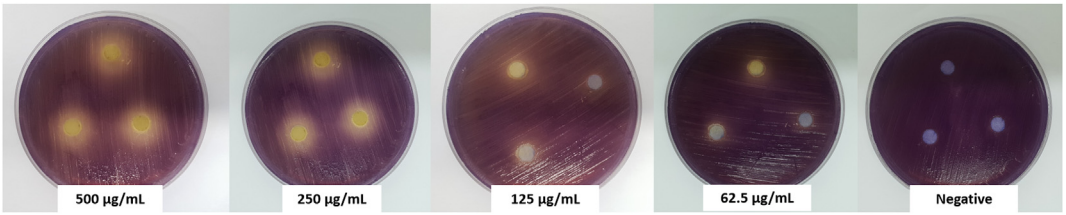

Figure 6. Disk diffusion assay of the different concentrations of green tea extract (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL) against short-chain acyl homoserine lactone-producing biosensor strain *Chromobacterium violaceum* ATCC 12472. Green tea extract inhibited pigmentation in the biosensor strain - indicative of its inhibitory activity against the short-chain AHL-mediated quorum sensing in *C. violaceum* ATCC 12472.

protective "coating" against foreign substances and from the conditions of the "external" environment; enzymes such as peroxidases which protects cells from β-lactam antibiotics; and inducement of programmed cell death which promotes tolerance to antibiotics on other cells. Thus, the expression of virulence factors mediated by QS may enhance the capability of microbial cells to resist the effects of antibiotics [51]. Due to the biofilm and QS inhibitory activities against *C. violaceum* and *Pseudomonas aeruginosa* of green tea (*Camellia sinensis*) polyphenols previously observed [30, 31], as well as the popularity of green tea as a drink and food supplement, this study further investigated the capability of green tea extract in inhibiting biofilm formation of the antibiotic-resistant *Morganella morganii* T3 isolated in this study.

3.3. Green tea extract (GTE) inhibits biofilm formation of M. morganii T3 without growth inhibition

Figure 4 shows that GTE did not exhibit antimicrobial activity against *M. morganii* at all concentrations used. Furthermore, results obtained through the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays showed that the concentrations tested do not inhibit the growth of *M. morganii* (data not shown). Although this might be the first report available on the activity of GTE against *M. morganii*, the results are similar with a previous study wherein green tea polyphenols were not able to inhibit the growth of *Pseudomonas aeruginosa* and *C. violaceum* at concentrations lower than ~6 mg/mL [31]. Thus, this suggests that the concentrations used in the antimicrobial assay will not affect the results of the succeeding assays.

The results presented in Figure 5 shows that a concentration as low as 62.5 µg/mL (31.55%) was able to significantly inhibit biofilm formation when compared with the control ($p < 0.05$). Increasing the concentration up to 500 µg/mL (41.21%) did not yield a significant increase in the activity. It is hypothesized that the absence of a concentration-dependent activity might be due to the fact that the extract used is a crude extract, hence, it is possible that there are several compounds that may be acting either in synergy or in antagonism with one another which affects the overall bioactivity. Moreover, the concentration of each compound present in the crude extract may also vary. Further study on the isolation of the bioactive compounds as well as of their activities might shed more light on the bioactivity of the green tea extract. Nonetheless, a similar study on the biofilm inhibitory activity of green tea against *P. aeruginosa* showed that the extract was able to reduce biofilm formation by half at concentration as low as 159 µg/mL with a significant reduction observed until 49 µg/mL, possibly through QS modulation [31]. It was previously observed that compounds from green tea extract inhibited biofilm formation in *P. aeruginosa* through interference with the QS system [53].

3.4. Short-chain acyl homoserine lactone-mediated quorum sensing is inhibited by green tea extract (GTE)

Since biofilm formation is a QS-dependent mechanism [11, 27], the effects of GTE against QS mediated by short-chain AHLs were also determined through the QS biosensor strain *C. violaceum* ATCC 12472. Figure 6 shows that the concentrations of GTE used in the preceding biofilm inhibition assay (62.5–500 µg/mL) were also able to inhibit QS. Thus, hinting that the inhibition of biofilm formation may have been due to the inhibition of QS. Previously, it was observed that tea polyphenols such as EGCG may modulate pigmentation and biofilm formation through the interference of QS [54]. Similar results were also obtained in the commercial green tea extract claiming to have 94–99% EGCG wherein QS inhibition was observed starting at 250 µg/mL concentration [30]. Similarly, previous study on the anti-QS properties of polyphenols of green tea showed that these compounds were able to inhibit QS in *C. violaceum* by approximately 50% at concentration as low as 0.098 mg/mL. Furthermore, previous study on green tea polyphenols concluded that the observed inhibition of QS in *C. violaceum* may be through the antagonism with the AHL receptors since no effects on both AHL synthesis and activity were observed [31]. Recently, it was shown that compounds from green tea extract modulate QS and QS-dependent mechanisms such as biofilm formation via the interference with the receptors in both *P. aeruginosa* and *C. violaceum* [53].

Knowing the potential mechanism of action of the green tea extract in modulating both QS and QS-dependent biofilm formation of the test pathogen (*M. morganii*) was made possible through the help of the information on the signaling molecules being employed in the QS systems of these isolates. It is possible that the green tea extract used in this study may have inhibited biofilm formation through the interference with the AHL-mediated QS system. However, further investigation is needed in order to elaborate how these inhibitory activities were yielded by the extract. Nonetheless, these results show the potential of green tea extract in controlling bacterial pathogens employing the said QS system.

4. Conclusions

In this study, four isolates which employ short-chain acyl homoserine lactone in their quorum sensing system were identified. Antibiotic susceptibility profiling showed that an isolate, *Morganella morganii* has developed antibiotic resistance. Thus, alternative strategies to combat pathogens without the use of antibiotics are needed. Therefore, extract from green tea (*Camellia sinensis*), a popular food supplement, was tested in this study and was observed to be capable of inhibiting both biofilm formation of the antibiotic-resistant *M. morganii* isolate, as well as the short-chain AHL-mediated QS system using *Chromobacterium violaceum* biosensor strains. This study also puts into record the use of short-chain AHLs by *Aeromonas salmonicida, Acinetobacter* sp., *Morganella morganii*, and *Citrobacter freundii* in their QS system. Furthermore, the results suggest the potential of green tea extract in the inhibition of biofilm formation and QS of an antibiotic-resistant *M. morganii* isolate.

Declarations

Author contribution statement

John Paul Matthew D. Guzman, Christine Eden C. Sevilla: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Trisha Pamela L. De las Alas, Margie C. Lucban: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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