International Journal of Environmental Science and Technology
https://doi.org/10.1007/s13762-018-1930-5

ORIGINAL PAPER

Screening of quorum quenching activity of the bacteria isolated from dairy industry waste activated sludge

J. Kaur[1] · **K. N. Yogalakshmi**[1]

Received: 2 July 2017 / Revised: 26 June 2018 / Accepted: 21 July 2018

Abstract

Intercellular bacterial communication process via exchange of signalling molecules acyl homoserine lactone results in various group activities like bioluminescence, antibiotic production, biofilm formation, sporulation, and virulence. The signalling molecules are targeted, and the communication is interrupted by a group of bacteria termed quorum quenching bacteria. The present study aims to isolate the quorum quenching bacteria from the waste activated sludge collected from the dairy industry effluent treatment plant and explore for its quorum quenching potential. The bacteria were cultured in the KG medium containing *n*-hexanoyl homoserine lactone as a sole source of carbon and nitrogen. The isolates were identified by the 16S ribosomal deoxyribonucleic acid analysis and subsequently were evaluated for its quorum quenching activity through *Chromobacterium violaceum* CV026 biosensor assay. The n-hexanoyl homoserine lactone degradation was quantified by GC–MS analysis. The 16S ribosomal deoxyribonucleic acid analysis revealed the isolated bacteria as *Klebsiella pneumoniae* (JYQ1 and JYQ5), *Acinetobacter baumannii* JYQ2, *Pseudomonas nitroreducens* JYQ3, and *Pseudomonas* JYQ4. The biosensor strain assay and GC–MS analysis indicated that all the isolates possessed an inherent ability to degrade *N*-hexanoyl homoserine lactone. The strain *Pseudomonas* JYQ4 exhibited the highest quorum quenching activity of 84 ± 3.3% within 6 h of incubation. The strain *A. baumannii* JYQ2 acted both as quorum sensing and as quorum quenching bacteria as evidenced by the decrease in quorum quenching from 79 ± 3.1 to 76.8 ± 2.5%.

Keywords *Acinetobacter baumannii* · Intercellular communication · *Klebsiella pneumoniae* · N-hexanoyl homoserine lactone · *Pseudomonas nitroreducens* · Quorum quenching

Introduction

Unicellular prokaryotic organisms, especially the bacteria, communicate and co-ordinate group activities using chemical signals through a process termed quorum sensing (QS). Acyl homoserine lactone (AHL), furanosyl borate diester, 4,5-dihydroxy-2,3-pentanedione, methyl dodecenoic acid, and diketopiperazines are few of the signalling molecules reported so far by various researchers (Parsek et al. 1999; Chen et al. 2002; Zhu et al. 2003; Li et al. 2009; Gu et al. 2013). Gram-negative bacteria mainly use AHL as a signalling molecule which is synthesized within the bacterial cell by a family of LUX I protein and are released into their surrounding environment. At threshold population density, these signalling molecules bind to the receptor LUX R and stimulate expression of genes. This allows the bacteria to co-ordinate group behaviour. Various gram-negative bacteria utilize these QS communication circuits for regulating diverse physiological activities including bioluminescence in *Vibrio* species, antibiotic production in *Erwinia carotovora*, conjugation in *Agrobacterium tumefaciens,* and biofilm formation in *Pseudomonas aeruginosa* (Miller and Bassler 2001; Williams et al. 2007).

Microbial degradation of the signalling molecules became vital to counter measure the negative effects of QS such as virulence and biofilm formation. Bacteria co-existing and sharing the habitat with the QS bacteria can disrupt these AHL-mediated signalling molecules to have a competitive advantage over the QS bacteria (Chan et al. 2007). The QS signal interruption is done by enzymes such as lactonases and acylases secreted by quorum quenching (QQ) bacteria (Dong et al. 2000; Sio et al.

Editorial responsibility: M. Abbaspour.

✉ K. N. Yogalakshmi
yogalakshmi25@gmail.com

[1] Centre for Environmental Sciences and Technology, School of Environment and Earth Sciences, Central University of Punjab, Bathinda 151001, India

2006). The pioneer study in enzymatic degradation of AHL molecules has been given by Dong et al. (2000) in gram-positive bacteria *Bacillus* which hydrolyses the homoserine lactone ring of AHL signals via lactonase activity. Further hydrolysis of the amide bond of AHLs via AHL acylase secreted by *Varivorax paradoxus, P. aeruginosa* PAO1, and *Streptomyces* sp. has also been reported by Leadbetter and Greenberg (2000); Huang et al. (2003); Park et al. (2005). So far, sources such as soil, sewage, *Zingiber officinale* rhizosphere, and Chinese soya sauce brine have been explored to isolate QQ bacteria (Chan et al. 2007, 2009, 2011; Chong et al. 2012 Yin et al. 2012). Dairy waste activated sludge (WAS) as a source of QQ bacteria remains unexplored. In view of this, the present study attempted to isolate indigenous QQ bacteria from dairy industry WAS and study its AHL-degrading efficiency. The study was conducted during April 2013 to June 2015 at Environmental Science and Technology Laboratory of Central University of Punjab, Bathinda, India.

Materials and methods

Biosensor and bacterial strains

Chromobacterium violaceum CV026 was used as the biosensor strain. The CV026 is a gram-negative proteobacteria which produces a purple pigment called violacein in the presence of signalling molecules such as *n*-hexanoyl homoserine lactone (Mani et al. 2012). Hence, this strain was selected as a biosensor strain with the aim of isolating bacteria with an inherent ability to consume *N*-hexanoyl homoserine lactone (C6-HSL). All bacteria were regularly grown in Luria–Bertani (LB) medium and buffered with 3-[*N*-morpholino] propanesulfonic acid (50 mM) to pH 6.8 to prevent alkaline degradation of C6-HSL.

Growth medium

KG medium formulated by Kok-Gan was used for the enrichment and isolation of AHL-degrading bacteria (Chan et al. 2009). The composition of the KG medium is as follows g/L: NaCl, 1.0; KCl, 0.5; $MgCl_2$, 0.4; $CaCl_2$, 0.1; Na_2SO_4, 0.15; KH_2PO_4, 5.0; and 2-(*N*-morpholino)-ethanesulfonic acid (MES), 1.0. The pH of the basal medium was adjusted to 5.5 with 1 M NaOH and autoclaved at 121 °C for 15 min. To the cooled medium, trace elements, 1 mg $FeCl_3$, 0.1 g of $MnCl_2$, and 0.46 g of $ZnCl_2$, were added aseptically to 1 litre of basal medium.

QS signalling molecules

AHL molecule C6-HSL was procured from Sigma-Aldrich and utilized as the QS signal molecules. Fifty millilitre stock solution of C6-HSL prepared in acetonitrile was dispensed into a sterile tube and evaporated in a rotary evaporator to remove acetonitrile. The remaining C6-HSL was rehydrated with sterile KG medium to a final concentration of 500 µg/mL. C6-HSL is the solitary source of carbon and nitrogen for liquid KG medium.

Enrichment and isolation of AHL-degrading bacteria from activated sludge sample

The activated sludge for isolating QQ bacteria was collected from Verka Milk Plant, Bathinda, India (N30°11″086′ and E74°56″356′). The sludge sample was collected in cans of capacity 1 L and transported to the laboratory. The QQ bacteria were isolated from the sludge sample according to the enrichment method given by Chan et al. (2009). For the isolation of QQ bacteria, 1 g of dairy sludge was suspended in 10 mL of KG medium and vortexed vigorously for 10 min. Two millilitres of this suspension was taken and spun at 7000 ×*g* to remove any particles. The supernatant of the suspension was further centrifuged at 13,000×*g*. The pellet obtained after centrifugation was washed with KG medium and resuspended in 2 mL of KG medium. About 100 µL of the suspension was inoculated into 3 mL of C6-HSL-containing KG medium (500 µg/mL) and incubated at 28 °C and 220 rpm. After 48 h, 10% (v/v) of the cultured broth was transferred to fresh KG medium containing C6-HSL. The transfer procedure was repeated six times to enrich for C6-HSL-metabolizing bacteria. After the sixth enrichment cycle, a diluted suspension of the culture broth was plated onto the LB agar and incubated at 28 °C. Each single colony was picked and streaked repeatedly on LB agar to obtain a pure culture.

Identification of AHL-degrading bacteria

The AHL-degrading bacteria isolated from dairy WAS were identified using molecular analysis. The bacterial colonies were picked up with a sterilized toothpick and suspended in sterilized saline water (0.5 mL) in a centrifuge tube. The tubes were centrifuged at 10,000 rpm for 10 min. The pellet was collected and suspended in 0.5 mL of InstaGene Matrix (Bio-Rad, USA). The mixture was incubated at 56 °C for 30 min and then heated at 100 °C for 10 min. The supernatant obtained after heating was used in polymerase chain reaction (PCR) for DNA amplification.

Approximately 1 µL of template DNA was added to 20 µL of PCR solution. The reaction mixture was amplified using primers 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1525R (5′ AAGGAGGTGWTCCARCC 3′). About 35 amplification cycles were performed at 94 °C for 45 s, 55 °C for 60 s and 72 °C for 60 s. Unincorporated PCR primers and dNTPs were removed from PCR products by using Montage

PCR Clean up kit (Millipore). The purified PCR products of approximately 1400 bp were sequenced using BigDye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an automated DNA sequencing system (Applied BioSystems, model 3730XL, USA) with M13 forward and reverse primers.

The sequences were aligned and trimmed to exclude low-quality sequences from beginning and end using software CodonCode Aligner (version 5.1.5). The DNA sequences were identified through nucleotide Basic Local Alignment Search Tool (BLAST) run at NCBI.

QQ activity

The QQ activity of the isolates was evaluated both qualitatively and quantitatively through purple pigment decolourization and GC–MS analysis, respectively. Qualitative confirmation employing degradation efficiency of all the five isolated strains was performed using CV026.

Resting cells preparation for isolated colonies

Freshly grown bacterial isolates in LB broth (28 °C, 220 rpm for 24 h) were centrifuged at 9400 rpm at 4 °C for 10 min. The pellets obtained were washed twice and suspended in 10 mL of 100 mM (pH 6.5) phosphate buffer solution (PBS). The cell suspension with an OD_{600} of 1.0 was used as a source of resting cells for in vitro AHL inactivation assay.

Whole cell inactivation assay

The whole cell inactivation assay was performed as per the protocol suggested by Chan et al. (2011). C6-HSL mixed in acetonitrile was evaporated to dryness in sterile tubes and rehydrated with resting cell suspension to the final concentration of 0.025 µg/µL. The reaction mixtures were incubated at 28 °C for 24 h at 220 rpm. Samples of 10 µL were withdrawn at time intervals of 0, 6, 12, 18, and 24 h and heated to stop the sample reaction. The reaction mixture was then cooled at room temperature for 15 min.

After cooling, the reaction mixture was spotted onto the CV026 lawn, followed by an overnight incubation at 28 °C. After 24-h incubation, the degradation of C6-HSL molecules was assessed based on the decolourization or loss of purple pigment produced by CV026. The zone of decolourization was determined through diameter measurements.

Gas chromatography–mass spectrometry (GC–MS) analysis

The sample preparation was similar to whole cell inactivation except for the concentration of the C6-HSL compound. The concentration of C6-HSL after rehydration with cell suspension was around 25 ppm. After every 6-h interval up to 24 h, about 1 mL of sample was withdrawn and extracted with 1 mL ethyl acetate. The extraction procedure was repeated three times. The top layer of the extract was collected and analysed for C6-HSL compounds using GC–MS (Shimadzu GC–MS Model QP 2010 Ultra) as described by Rani et al. (2011). RTxi-1ms capillary column having dimensions 30 m × 1 mm × 0.1 µm was used for the separation of C6-HSL molecules. Helium gas at the flow rate of 1 mL/min was used as a carrier gas. Mass spectrometry conditions were as follows: electron ionization source was set at 70 eV, MS ion source temperature was at 200 °C, and solvent cut time was 3.5 min. The mass spectrometer was run in full-scan mode (m/z 30–500) and in selected ion monitoring (SIM) mode at 143 m/z. Samples quantification was done by using SIM mode. The GC–MS spectra obtained were compared with the standard C6-HSL spectra, and their degradation percentages were calculated by the formula given in the following equation:

$$\text{Rate of C6-HSL degradation}\,(\%) = \frac{A_\mathrm{i} - A_\mathrm{f}}{A_\mathrm{i}} \times 100$$

where A_i = initial C6-HSL concentration and A_f = C6-HSL concentration after degradation.

Statistical analysis

All the experiments were performed in triplicate. Data were analysed statistically, and results were expressed as mean ± S.E. One way analysis of variance (ANOVA) was performed using software package SPSS Statistics 20 to evaluate the significant difference between different time intervals for C6-HSL degradation. Differences were considered significant at $p < 0.05$. The relative abundance of the individual QQ bacteria in the dairy WAS was calculated for determining the percentage of single QQ bacteria with respect to the total number of bacterial colonies obtained after enrichment by using the following formula:

$$\text{Relative abundance}\,(\%) = \frac{\text{Number of individual QQ bacteria}}{\text{Total number of QQ bacteria after enrichment}} \times 100$$

Results and discussion

Isolation of QQ bacteria from dairy sludge

The WAS inoculated in KG medium containing C6-HSL as a sole source of nitrogen and carbon exhibited turbidity within 48 h. However, no growth was observed in control KG medium deficient in C6-HSL. Further streaking of the consortium on LB agar resulted in five bacterial colonies JYQ1, JYQ2, JYQ3, JYQ4, and JYQ5. The colony morphology of all five bacterial isolates is summarized in Table 1. Almost all the isolates except JYQ2 showed similar morphology. The colonies were smooth, circular, and creamish white in colour with the entire margin. JYQ2 differed from other isolates by its off-white, irregular, contoured, and lobate colonies.

Identification of QQ bacteria

All the five QQ isolates from dairy sludge were identified by 16S ribosomal deoxyribonucleic acid (rDNA) sequencing. The aligned sequences were analysed by web-based similarity searches against GenBank database. The isolates were identified as *K. pneumoniae* JYQ1, *A. baumannii* JYQ2, *P. nitroreducens* JYQ3, *Pseudomonas* JYQ4, and *K. pneumoniae* JYQ5. All the isolates showed 99% similarity in NCBI BLAST search. The 16S rDNA sequence of isolate JYQ1, JYQ2, JYQ3, JYQ4, and JYQ5 was deposited in GenBank and Assigned Accession Nos. KP189202, KP340458, KP340459, KU555415, and KP780263, respectively. The isolates belonged to class gamma proteobacteria and families Enterobacteriaceae (JYQ1 and JYQ5), Moraxellaceae (JYQ2), and Pseudomonadaceae (JYQ3 and JYQ4). Uroz et al. (2009) reported that the QQ bacteria isolated so far belonged to the taxonomic groups comprising alpha, beta, and gamma proteobacteria and Firmicutes. The present study also confirms the observations of Uroz et al. (2009). All the isolates showed JYQ1–JYQ5 shared 40% relative abundance except the isolate JYQ2 which shared 20% of relative abundance in dairy activated sludge with respect to the family to which they belonged. The bacteria mentioned in the present study showed similarity with the isolates of the sewage sludge (AY540111.1, AB0768575.1, GQ916515.1), hospital effluent (LN624808.2), soil (GQ289378.1, JN093012.1), and rhizosphere (KJ767371.1) bacteria (Wu et al. 2008; Adav et al. 2010; Li et al. 2013).

QQ activity of isolates

Results of C6-HSL degradation study showed that all the five isolates JYQ1, JYQ2, JYQ3, JYQ4, and JYQ5 were able to quench the C6-HSL signalling molecule. However, the period of degradation varied among isolates as determined from the diameter of decolourization as shown in Table 2. *Pseudomonas* JYQ4 exhibited a greater zone of decolourization with the diameter of 1.8 ± 0.03 cm after 24 h of incubation (Fig. 1). Other isolates showed a slight difference in the size of the zone of decolourization with the lowest diameter of 1.1 ± 0.06 cm observed in *K. pneumoniae* JYQ5. The isolate *A. baumannii* JYQ2, however, showed the diameter of 1.25 ± 0.04 cm after 24 h when compared to 18 h (1.15 ± 0.05).

The GC–MS analysis further confirmed the amount of C6-HSL consumed by the isolated strains. The results revealed that all the five isolates exhibited a significant ($p < 0.05$) degradation of C6-HSL with the increase in incubation time. The results confirmed that the isolate *Pseudomonas* JYQ4 showed strong QQ activity through degradation of C6-HSL within 6 h followed by *P. nitroreducens* JYQ3 within 12 h. Similarly, *A. baumannii* JYQ2 and *K. pneumoniae* JYQ1 and JYQ5 showed a significant ($p < 0.05$) QQ activity in 18 and 24 h, respectively. The QQ activity in *Acinetobacter* has been studied previously by Kang et al. (2004). It was interesting to note that isolate *A. baumannii* JYQ2 showed purple colour after 24 h of incubation. It might be due to the production of C6-HSL molecules by

Table 1 Colony morphological characteristics of isolated QQ bacteria

Characteristics	JYQ1	JYQ2	JYQ3	JYQ4	JYQ5
Colour	Creamish white	Off-white	Creamish white	Creamish white	Creamish white
Colony surface	Smooth	Contoured	Smooth	Smooth	Smooth
Colony form	Circular	Irregular	Circular	Circular	Circular
Elevation	Convex	Convex	Convex	Convex	Convex
Margin	Entire	Lobate	Entire	Entire	Entire

Table 2 Diameter studies showing purple pigment decolourization by different bacterial isolates

Isolates	Diameter ± S.E. (cm)				
	0 h	6 h	12 h	18 h	24 h
Klebsiella pneumoniae JYQ1	No decolourization	No decolourization	0.7 ± 0.06	0.85 ± 0.005	1.3 ± 0.1
Acinetobacter baumannii JYQ2	No decolourization	No decolourization	0.81 ± 0.04	1.15 ± 0.05	1.25 ± 0.04
Pseudomonas nitroreducens JYQ3	No decolourization	1.35 ± 0.05	1.61 ± 0.03	–	–
Pseudomonas JYQ4	No decolourization	1.84 ± 0.03	–	–	–
Klebsiella pneumoniae JYQ5	No decolourization	No decolourization	0.55 ± 0.05	0.8 ± 0.02	1.1 ± 0.06

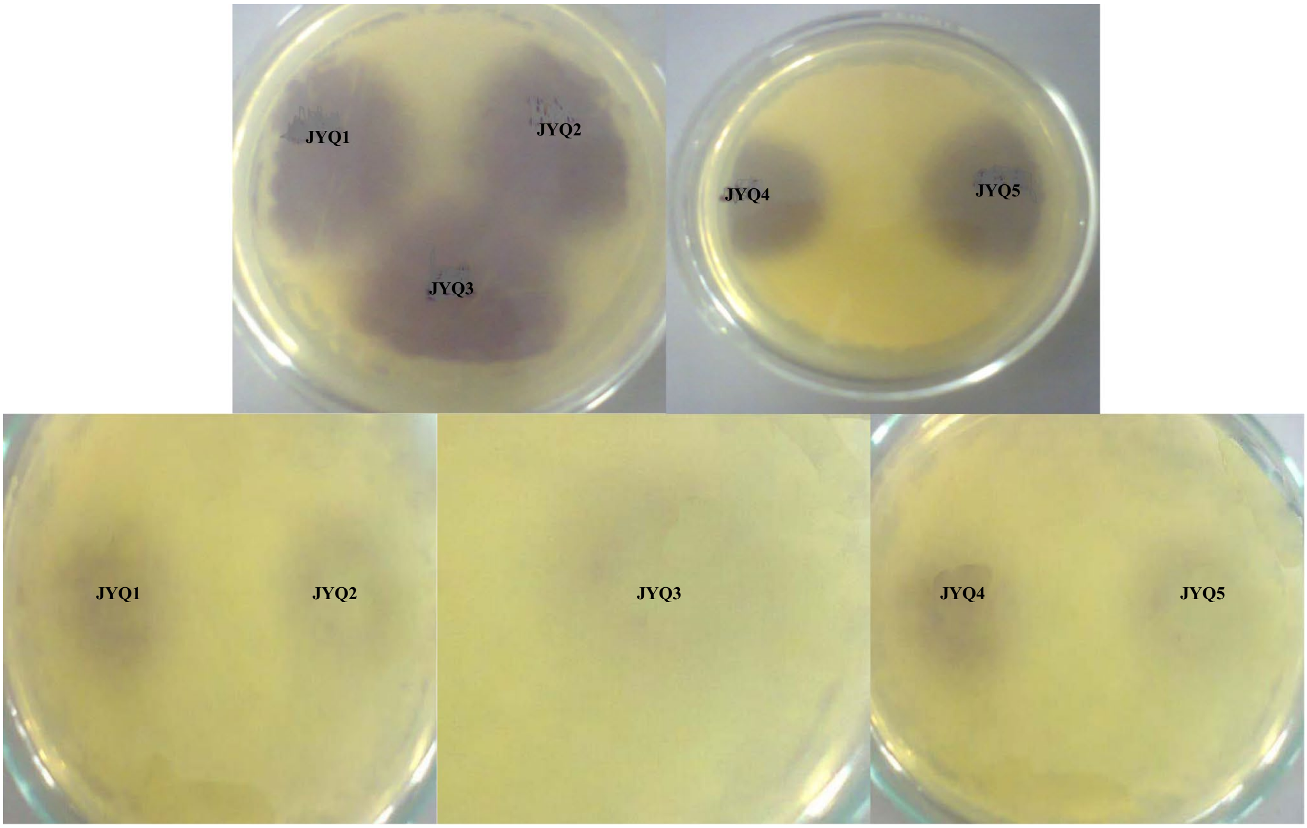

Fig. 1 C6-HSL degradation by *Klebsiella pneumoniae* (JYQ1 and JYQ5), *Acinetobacter baumannii* JYQ2, *Pseudomonas nitroreducens* JYQ3, and *Pseudomonas* JYQ4. Each dairy WAS was incubated with C6-HSL at different intervals. Pigment formation in the top row indicates the presence of C6-HSL in 0-h incubation with QQ bacteria, whereas the bottom row indicates the absence of pigment formation, indicating QQ activity by *Klebsiella pneumoniae* (JYQ1 and JYQ5), *Acinetobacter baumannii* JYQ2, *Pseudomonas nitroreducens* JYQ3, and *Pseudomonas* JYQ4 in 24, 18, 12, and 6 h, respectively

the isolate. The results of GC–MS analysis also confirm the production of C6-HSL molecules by isolate JYQ2 at 24th hour. An increase of about 1.13 times in C6-HSL concentration was observed at 24th hour with respect to the 18th hour. Earlier studies by Chan et al. (2014) also reported the production of C6-HSL molecules by *A. baumannii*. Thus, the reversion of purple colour at the 24th hour of incubation might be due to the expression of the C6-HSL molecules.

Figure 2 depicts the C6-HSL degradation efficiency of QQ isolates. It was evident from the figure that C6-HSL degradation efficiency was observed to increase from 6 to 24 h. While comparing the degradation efficiency, it was observed that *A. baumannii* JYQ2 isolate exhibited degradation of C6-HSL within 18 h showing a better degrading efficiency of 79 ± 3.1%. The *A. baumannii* JYQ2 strain isolated in the present study showed much faster degradation when compared to *Acinetobacter* GG2 (24 h) isolated from the rhizosphere of *Z. officinale*. The isolate *Pseudomonas* JYQ4 exhibited highest degradation efficiency of 84 ± 3.3% at 6 h, whereas *P. nitroreducens* (JYQ3) showed 68.4 ± 0.025% degradation at 12 h of incubation. With further increase in incubation time, *Pseudomonas* (JYQ4) and *P. nitroreducens* (JYQ3) showed no trace of C6-HSL at 12 and 18 h, respectively, indicating complete degradation. Similarly, *K. pneumoniae* JYQ1 and JYQ5 showed maximum degradation in the range of 81–82% at 24 h.

According to the previous study by Sio et al. (2006), it was observed that the isolate *P. aeruginosa* PAO1 has PA2385 protein which is capable of hydrolyzing long-chain AHL (acyl chain length ranging from 11 to 14 carbon atoms) such as 3-oxo-C12-HSL within 6 h of incubation. The short-chain AHLs such as *N*-butanoyl homoserine lactone (BHL) and C6-HSL degradation were resistant to *P. aeruginosa* isolate. Unlike *P. aeruginosa,* the *P. nitroreducens* (JYQ3) bacteria isolated in the present study showed complete degradation of short-chain HSL molecules within 24 h of incubation. Chan et al. (2011) reported the reduction in 3-oxo-C6-D-HSL by *Klebsiella* after 3-h incubation. Continued incubation resulted in complete absence of the C6-HSL peak after 24 h of incubation.

Furthermore, the metabolites of C6-HSL degradation were identified by the GC–MS analysis. Figure 3 illustrates the degradation of C6-HSL degradation into the *n*-hexadecanoic acid. It was found that all the four isolates degraded the C6-HSL molecule into fatty acids such as hexadecanoic acid, pentadecanoic acid, octadecanoic acid, and tetradecanoic acid as shown in Table 3. The mass of degradation products was confirmed by NIST library. The presence of fatty acid degradation products indicated that the isolates possessed the enzyme acylase that degraded the C6-HSL into corresponding fatty acids and HSL (Chen et al. 2013). The degradation of C6-HSL by acylase activity in the strains *Acinetobacter* isolated from activated sludge and *P. aeruginosa* PAO1 genome sequence has been studied by Ochiai et al. (2014) and Sio et al. (2006), respectively. According to Ochiai et al. (2014), the *Acinetobacter* sp. strain Ooi24

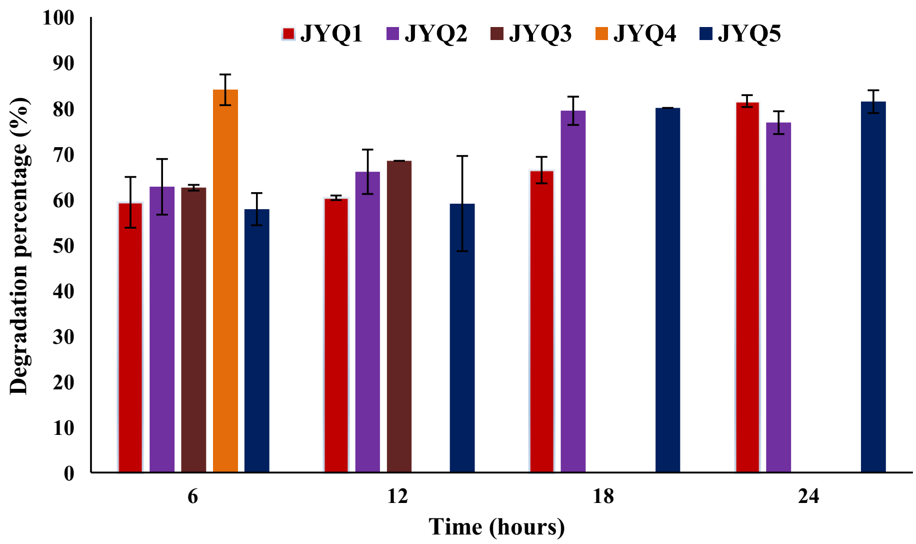

Fig. 2 Comparison of C6-HSL degradation efficiency of five strains

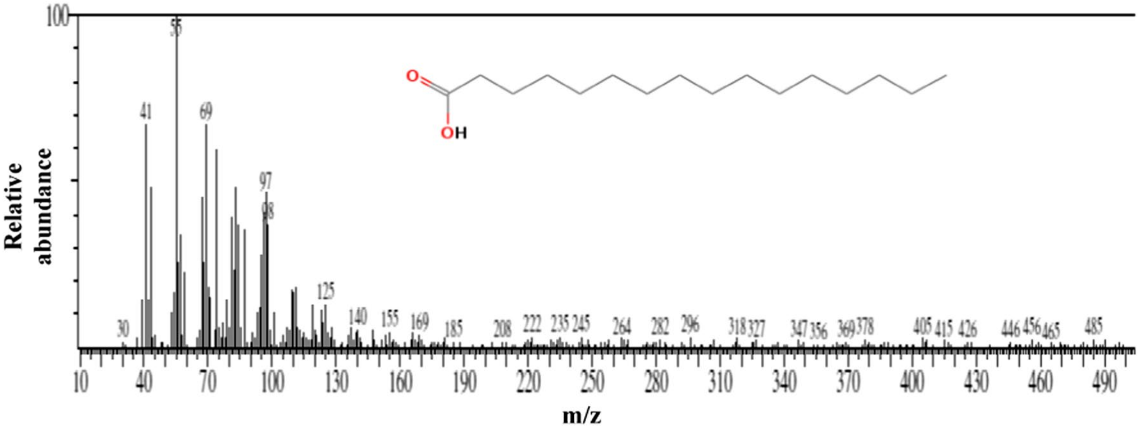

Fig. 3 Mass spectra of n-hexadecanoic acid product generated by the degradation of C6-HSL by *Pseudomonas* JYQ4

Table 3 Degradation of C6-HSL into fatty acids by different QQ bacteria

Isolates	Degradation products
Klebsiella pneumoniae JYQ1	Pentadecanoic acid, octadecanoic acid
Acinetobacter baumannii JYQ2	*n*-hexadecanoic acid
Pseudomonas nitroreducens JYQ3	*n*-hexadecanoic acid, octadecanoic acid
Pseudomonas JYQ4	Hexadecanoic acid
Klebsiella pneumoniae JYQ5	*n*-hexadecanoic acid, tetradecanoic acid, octadecanoic acid

isolated from the activated sludge has AmiE that functions as an AHL acylase, hydrolyzing the amide bond of AHL. It was also reported that *Acinetobacter* sp. GG2 isolated from the rhizosphere of *Z. officinale* possessed an enzyme lactonase that carried out the QQ activity via the hydrolysis of the lactone ring and formed *n*-acyl homoserine compound (Chan et al. 2011).

Conclusion

From the current study, it can be concluded that all the five QQ bacteria, namely *K. pneumoniae* (JYQ1 and JYQ5), *A. baumannii* JYQ2, *P. nitroreducens* JYQ3, and *Pseudomonas* JYQ4, showed significant C6-HSL degradation and QQ activity. *Pseudomonas* JYQ4 strain showed maximum degradation ability (84 ± 3.3%) within the 6 h of the incubation period. *A. baumannii* JYQ2 showed the generation of short-chain AHL molecules indicating the QS activity. To the best of the author's knowledge, this study is the first of its kind to isolate QQ bacteria from dairy WAS.

Acknowledgements The authors gratefully acknowledge University Grant Commission, India, for providing Maulana Azad Senior Research Fellowship (F1-17.1/2010/MANF-SIK-PUN-5668) under the scheme of Maulana Azad National Fellowship for Minority Students. We also thank Verka Milk Plant, Bathinda, Punjab, for the waste activated sludge sample.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Adav SS, Lee DJ, Lai JY (2010) Potential cause of aerobic granular sludge breakdown at high organic loading rates. Appl Microbiol Biotechnol 85:1601–1610. https://doi.org/10.1007/s00253-009-2317-9

Chan KG, Tiew SZ, Ng CC (2007) Rapid isolation method of soil bacilli and screening of their quorum quenching activity. Asia Pac J Mol Biotechnol 15:153–156

Chan KG, Yin W, Sam CK, Koh CL (2009) A novel medium for the isolation of *N*-acyl homoserine lactone-degrading bacteria. J Ind Microbiol Biotechnol 36:247–251. https://doi.org/10.1007/s10295-008-0491-x

Chan KG, Atkinson S, Mathee K, Sam CK, Chhabra SR, Camara M, Koh CL, Williams P (2011) Characterization of n-acyl homoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. BMC Microbiol 11:1–13. https://doi.org/10.1186/1471-2180-11-51

Chan KG, Cheng HJ, Chen JW, Ying WF, Ngeow YF (2014) Tandem mass spectrometry detection of quorum sensing activity in multidrug resistant clinical isolate *Acinetobacter baumannii*. Sci World J 2:1–6. https://doi.org/10.1155/2014/891041

Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415:545–549. https://doi.org/10.1038/415545a

Chen F, Gao Y, Chen X, Yu Z, Li X (2013) Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. Int J Mol Sci 14:17477–17500. https://doi.org/10.3390/ijms140917477

Chong TM, Koh CL, Sam CK, Choo YM, Yin WF, Chan KG (2012) Characterization of quorum sensing and quorum quenching soil bacteria isolated from Malaysian Tropical Montane Forest. Sensors 12:4846–4859. https://doi.org/10.3390/s120404846

Dong YH, Xu JL, Li XZ, Zhang LH (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci USA 97:3526–3531. https://doi.org/10.1073/pnas.060023897

Gu Q, Fu L, Wang Y, Lin J (2013) Identification and characterization of extracellular cyclic dipeptides as quorum-sensing signal molecules from Shewanella baltica, the specific spoilage organism of Pseudosciaena crocea during 4 °C storage. J Agric Food Chem 61:11645–11652. https://doi.org/10.1021/jf403918x

Huang JJ, Han JI, Zhang LH, Leadbetter JR (2003) Utilization of acyl- homoserine lactone quorum signals for growth by a soil pseudomonad and *Pseudomonas aeruginosa* PAO1. Appl Environ Microbiol 69:5941–5949. https://doi.org/10.1128/AEM.69.10.5941-5949.2003

Kang BR, Lee JH, Ko SJ, Lee YH, Cha JS, Cho BH, Kim YC (2004) Degradation of acyl-homoserine lactone molecules by *Acinetobacter* sp. strain C1010. Can J Microbiol 50:935–941. https://doi.org/10.1139/w04-083

Leadbetter JR, Greenberg EP (2000) Metabolism of acyl-homoserine lactone quorum sensing signals by *Varivorax paradoxus*. J Bacteriol 182:6921–6926. https://doi.org/10.1128/JB.182.24.6921-6926.2000

Li MY, Zhang J, Lu P, Xu JL, Li SP (2009) Evaluation of biological characteristics of bacteria contributing to biofilm formation. Pedosphere 19:554–561. https://doi.org/10.1016/S1002-0160(09)60149-1

Li Q, Wang M, Feng J, Zhang W, Wang Y, Gu Y, Song C, Wang S (2013) Treatment of high-salinity chemical wastewater by indigenous bacteria-bioaugmented contact oxidation. Bioresour Technol 144:380–386. https://doi.org/10.1016/j.biortech.2013.07.004

Mani A, Hameed SS, Ramalingam S, Narayanan M (2012) Assessment of quorum quenching activity of *Bacillus* species against *Pseudomonas aeruginosa* MTCC2297. Glob J Pharmacol 6:118–125. https://doi.org/10.5829/idosi.gjp.2012.6.2.6571

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Ann Rev Microbiol 55:165–199. https://doi.org/10.1146/annurev.micro.55.1.165

Ochiai S, Yasumoto S, Morohoshi T, Ikeda T (2014) AmiE, a novel *N*-Acylhomoserine lactone acylase belonging to the amidase family, from the activated sludge isolate *Acinetobacter* sp. Ooi24. Appl Environ Microbiol 80:6919–6925. https://doi.org/10.1128/AEM.02190-14

Park SY, Kang HO, Jang HS, Lee JK, Koo BT, Yum DY (2005) Identification of extracellular *N*-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. Appl Environ Microbiol 71:2632–2641. https://doi.org/10.1128/AEM.71.5.2632-2641.2005

Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP (1999) Acyl homoserine-lactone quorum-sensing signal generation.

Proc Natl Acad Sci USA 96:4360–4365. https://doi.org/10.1073/pnas.96.8.4360

Rani S, Kumar A, Malik AK, Kopplin PS (2011) Occurrence of *N*-acyl homoserine lactones in extracts of bacterial strain of *Pseudomonas aeruginosa* and in sputum sample evaluated by gas chromatography-mass spectrometry. Am J Anal Chem 2:294–302. https://doi.org/10.4236/ajac.2011.22037

Sio CF, Otten LG, Cool RH, Diggle SP, Braun PG, Bos R, Daykin M, Camara M, Williams P, Quax WJ (2006) Quorum quenching by an *N*-acyl-homoserine lactone acylase from *Pseudomonas aeruginosa* PAO1. Infect Immun 74:1673–1682. https://doi.org/10.1128/IAI.74.3.1673-1682.2006

Uroz S, Dessux Y, Oger P (2009) Quorum sensing and quorum quenching: the yin and yang of bacterial communication. ChemBioChem 10:205–216. https://doi.org/10.1002/cbic.200800521

Williams P, Winzer K, Chan WC, Camara M (2007) Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond B Biol Sci 362:1119–1134. https://doi.org/10.1098/rstb.2007.2039

Wu KJ, Saratale GD, Lo YC, Chen WM, Tseng ZJ, Chang MC, Tsai BC, Su A, Chang JS (2008) Simultaneous production of 2,3-butanediol, ethanol and hydrogen with a *Klebsiella* sp. Strain isolated from sewage sludge. Bioresour Technol 99:7966–7970. https://doi.org/10.1016/j.biortech.2008.03.062

Yin WF, Tung HJ, Sam CK, Koh CL, Chan KG (2012) Quorum quenching *Bacillus sonorensis* isolated from Soya Sauce Fermentation Brine. Sensors 2:4065–4073. https://doi.org/10.3390/s120404065

Zhu J, Dizin E, Hu X, Wavreille AS, Park J, Pei D (2003) S-Ribosyl homocysteine (LuxS) is a mononuclear iron protein. Biochemistry 42:4717–4726. https://doi.org/10.1021/bi034289j