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ORIGINAL PAPER

The first report on *Listeria monocytogenes* producing siderophores and responds positively to *N*-acyl homoserine lactone (AHL) molecules by enhanced biofilm formation

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Abstract *Listeria monocytogenes* are Gram-positive well-known emerging food-borne pathogens causing listeriosis in humans. In the present study, we have isolated biofilm-forming *Listeria* sp. from utensils used by a local milk collection dairy society at Usgao Goa, which collects milk for Goa dairy. Through biochemical tests and 16S rRNA sequence analysis, the bacterium was confirmed to be *L. monocytogenes* and designated as strain BN3, having GenBank accession number MF095110. We report for the first time Gram-positive *L. monocytogenes* strain BN3 producing iron-chelating siderophores by chrome azurol S (CAS) agar test. Also, this is a first report which reveals that *L. monocytogenes* strain BN3 responds to *N*-hexanoyl-homoserine lactone molecule (C_6-HSL) by gradual increase in their biofilm-forming potential with a gradual increase in AHL (C_6-HSL) concentration (250, 500 nM–1 µM) as compared to control revealed by crystal violet assay (CV) in microtiter plate. These results were further confirmed by scanning electron microscopy (SEM). A significant decrease in biofilm formation was observed when *L. monocytogenes* strain BN3 was treated with 10 µg/ml (*R*)-2-(2-hydroxynaphthalen-1-yl) thiazolidine-4-carboxylic acid, but when 250 and 500 nM AHL molecules were added, biofilm formation in strain BN3 was found to be enhanced as compared to control even in the presence of antibacterial compound, (*R*)-2-(2-hydroxynaphthalen-1-yl)thiazolidine-4-carboxylic acid. These results revealed that AHL molecules nullify the effect of antimicrobial compound and promote biofilm formation in *L. monocytogenes* strain BN3.

Keywords *Listeria monocytogenes* · Biofilm · AHL · Siderophores

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Introduction

Listeria monocytogenes are Gram-positive, short rod bacteria, responsible for food-borne disease called listeriosis in humans (Farber and Peterkin 1991; Doijad et al. 2011, 2015; Cherifi et al. 2017). The disease exhibits high hospitalization rate (91%), high neonatal death rate (50%) and case fatality rate (20–30%). Sources of *L. monocytogenes* infection are contaminated foods such as raw meat, raw milk, milking utensils, vegetables, dairy products, sausages, fruit juices, and ready-to-eat and packed foods (Low and Donachie 1997; Farber and Peterkin 1991; Swaminathan and Gerner-Smidt 2007; Doijad et al. 2010). Unhygienic milking of cow, improperly washed milking utensils and milk-processing machines contribute to loads of organisms including pathogenic *Listeria* sp. in milk-processing plants (Doijad et al. 2011). *Listeria monocytogenes* are capable of surviving and multiplying in phagocytic cells as well as non-phagocytic cells of humans (Vázquez-Boland et al. 2001). In large numbers, they infect monocytes in blood which are also phagocytic cells and increase the number of monocytes in the blood of the infected host; thus the name *monocytogenes* was suggested by Murray (Vázquez-Boland et al. 2001; Camejo et al. 2011).

In *L. monocytogenes*, the first virulent gene that was identified is the hemolysin gene (hly) (Vázquez-Boland et al. 2001) which encodes listeriolysin O (LLO) (Doijad et al. 2010; Camejo et al. 2011). When phagocytized by host monocytes, the bacterium lyses the vacuole and escapes into cytoplasm by encoding listeriolysin O (LLO), phosphatidylinositol-specific phospholipase c (plcA) and phosphatidylcholine-specific phospholipase c (plcB) (Vázquez-Boland et al. 2001; Camejo et al. 2011 Doijad 2014). Most humans at risk are infants with weak immunity, pregnant women, aged people with weakened immunity, people having AIDs and HIV, individuals having diabetes or chronic liver disease and group of people routinely exposed to agriculture-associated animals (Malik et al. 2002; Raorane et al. 2015). This bacterium is a well-known psychrophile and thus has the ability to grow on raw milk and meat kept in the refrigerator to increase shelf life. Thus, these foods are at risk of getting contaminated with *L. monocytogenes* (Doijad 2014). The resistance of *L. monocytogenes* biofilms to clean-in-place (CIP) procedure has been reported by Doijad (2014). The CIP procedure consists of six steps: washing food industry equipment with hot water; alkali wash, acid wash, alkali wash, hot water wash and sanitization, followed by cold water wash to remove biofilm-forming bacteria (Eide et al. 2003). Equipment with large surfaces like cutting and slicing boards, coolers, butchers knives, milk cans and milk containers are hard to clean completely. Studies have shown that food-processing equipment and utensil surfaces are contaminated with *L. monocytogene* in food-processing plants (Oliveira et al. 2010). Stronger biofilm formers form multiple layers that prevent the acid/alkali/heat applied during the CIP procedure; therefore, the cells that are situated deep in the biofilm on utensils get protected. To eliminate biofilm-forming *L. monocytogenes* from the food industry, pasteurization and treatment with bactericidal compounds are carried out (Doijad 2014; Doijad et al. 2015), but biofilm of *L. monocytogenes* are very difficult to eliminate. The manifestation of food-borne diseases originated due to bacterial ability to form biofilm on food surfaces and utensils, leading to health issues (Farber and Peterkin 1991; Oliveira et al. 2010; Doijad et al. 2015). Therefore, if food/dairy industry can control biofilms, then it is easy to control food-borne *L. monocytogenes* infections to a great extent.

Acyl homoserine lactone (AHL) quorum sensing system was first reported in *Vibrio fischeri*; it could produce 3-oxo-hexanoyl homoserine lactone and is extensively seen in Gram-negative organisms (Irie and Parsek 2008).

Bioluminescence genes in marine *V. fischeri* are well known to be activated by the transcription factor LuxR in combination with a diffusible signal compound, *N*-(3-oxo-hexanoyl) homoserine lactone, identified as an autoinducer. Quorum-sensing AHLs molecules are well known to involve in virulence gene expression and biofilm formation in Gram-negative bacteria and peptides-based QS system (*agr* QS system) in Gram-positive bacteria (Novick and Muir 1999; Irie and Parsek 2008; Garmyn et al. 2009; Zetzmann et al. 2016). AHL-based quorum-sensing system has been reported to be present only in Gram-negative bacteria, but there is only one report on marine Gram-positive *Exiguobacterium* sp. with the presence of the AHL quorum sensing system (Biswa and Doble 2013). *Exiguobacterium* sp. AT1b (GenBank CP001615.1) was reported to produce C3-oxo-octanoyl homoserine lactone (OOHL) using bioreporters, namely *Chromobacterium violaceum* CV026, *Agrobacterium tumefaciens* A136 and *E. coli* JM 109(psb1075). There is no report of AHL-based quorum sensing system in *L. monocytogenes* and involvement of AHL in biofilm formation. Also till date, no report on *L. monocytogenes* response to AHL. Therefore, the study of biofilm-forming *L. monocytogenes* from the dairy industry and the response of *L. monocytogenes* to the AHL molecule is very crucial in understanding the persistence of *L. monocytogenes* in the dairy industry, where both Gram-positive and Gram-negative bacteria are present in the same econiche.

In the present investigation, we have aimed to isolate biofilm-forming *L. monocytogenes* from milk-collecting utensils from the local dairy at Usgao, which collects milk for the Goa dairy. We also explore their potential for siderophore production, AHL quorum sensing molecule production and response of *L. monocytogenes* to exogenous AHL molecules.

Materials and methods

Isolation of biofilm-forming *Listeria monocytogenes* from the local dairy industry

Swabs were taken from utensils used by the local milk collection dairy society at Usgao, Goa, collecting milk for the Goa dairy using sterile swabs dipped in 0.85% sterile saline. Swabs taken were put in sterile 50 ml centrifuge tubes containing 10 ml sterile saline (0.85%) and carried to the laboratory in an ice box.

Sample enrichment

As per modified United States Department of Agriculture (USDA) method, the swab along with the saline was inoculated into 150 ml of University of Vermont media-I broth (UVM I) for primary enrichment and incubated at 37 °C for 24–48 h. After primary enrichment, 1 ml from UVM I was inoculated into 50 ml UVM II broth for secondary enrichment and was incubated at 37 °C for 24–48 h (Doijad et al. 2010, 2011; Doijad 2014).

Isolation of *Listeria* on PALCAM agar

From enriched UVM II broth, a loopful was streaked on polymyxin–acriflavine–lithium chloride–ceftazidime–aesculin–mannitol agar (PALCAM agar) plate and was incubated at 37 °C for 24–48 h. The colonies assumed to be *Listeria* by grayish shrunken centered and showing aesculin hydrolysis, indicated by black zone surrounding colonies. The isolated colonies were picked and purified on PALCAM agar followed by subculturing every month on brain heart infusion agar slants and stored at 4 °C (Doijad et al. 2010, 2011; Doijad 2014). The bacterial isolate was designated as BN3.

Identification of bacterial isolate BN3 using biochemical tests

Listeria isolates on PALCAM agar showing grayish shrunken center and black zone surrounding colonies were further tested for phosphatidyl-inositol phospholipase C (ALOA test) and hemolysis and Christie Atkins Munch Petersons test (CAMP) (Doijad et al. 2010, 2011; Doijad 2014; Dudhe et al. 2014). Also, biochemical tests such as sugar fermentation, Gram staining and motility at 25 and 37 °C were conducted and, by referring to Bergey's manual of systematic bacteriology, bacteria were identified (Sneath et al. 1986).

Identification of bacterial isolate BN3 by 16S rRNA sequencing

The 16S rDNA gene was PCR amplified using the following eubacterial primers: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) using genomic DNA of *L. monocytogenes* strain BN3 as template. PCR amplification was performed using the PCR amplification kit (Bangalore Genei, India) and sequencing was done at Yaaz Xenomics, Madurai, Tamilnadu, India. 16S rDNA sequence was compared against GenBank database using NCBI BLAST search.

Antibiotic susceptibility test

Antibiotic susceptibility test of *L. monocytogenes* isolate was performed following Kirby–Bauer disc diffusion method (Bauer et al. 1966), using Muller–Hinton agar plates and antibiotic discs. HiMedia Hexa G-plus disc containing ciprofloxacin (10 µg/disc), gentamicin (10 µg/disc), linezolid (30 µg/disc), penicillin-G (10units/disc), streptomycin (10 µg/disc) and vancomycin (30 µg/disc) was used.

Siderophore production by *Listeria monocytogenes* strain BN3

Listeria monocytogenes was never reported to produce its own siderophores (Simon et al. 1995; Barchini and Cowart 1996). Siderophore production potential of *L. monocytogenes* strain BN3 was tested by spot inoculating *L. monocytogenes* strain BN3 on Chrome Azurol S agar plates (blue agar plates) and incubating at 37 °C for 24–48 h (Schwyn and Neilands 1987).

AHL molecule detection assay using reporter bacteria *Chromobacterium violaceum* MCC2216

Reporter *C. violaceum* strain MCC2216 was ordered from Microbial Culture Collection (MCC) at National Centre for Cell Science (NCCS), Pune. *C. violaceum* MCC2216 was streaked on Luria–Bertani agar plate. AHL quorum sensing molecule production by *L. monocytogenes* was tested by cross-streaking *L. monocytogenes* strain BN3 against *C. violaceum* MCC2216 and incubating at 24–48 h at 37 °C. Wild *C. violaceum* produce violet pigment on LB agar, but *C. violaceum* MCC2216 is a mutated strain of *C. violaceum* which does not produce the violet pigment in the absence of external AHL molecules. Therefore, if *L. monocytogenes* produces AHL QS molecules, then the violet pigment will be produced by *C. violaceum* MCC2216 at the streak of *L. monocytogenes* strain BN3, indicating AHL production (Lim et al. 2014). *Vibrio harveyi* is a bioluminescent marine bacterium isolated from marine quid and was used as positive control for AHL production.

Response of *L. monocytogenes* strain BN3 to AHL molecule (C_6-HSL)

N-Hexanoyl-homoserine lactone molecule (C_6-HSL, i.e., AHL) was procured from Sigma-Aldrich, USA. Stock 1 M was prepared, filter sterilized and stored at 4 °C. The biofilm-forming potential of *L. monocytogenes* strain BN3 was tested using Crystal Violet Assay (CV assay) in 48-well sterile polystyrene microtiter plate. 500 µl of sterile BHI broth was added to a sterile polystyrene microtiter plate and to this 15-h-old *L. monocytogenes* strain BN3 grown in brain heart infusion (BHI) broth was added (500 µl). Plain 1 ml BHI broth (without inoculum) in polystyrene microtiter plate was kept as negative control. Plates were then incubated at 37 °C for 48 h. The culture broth from microtiter wells was drained and unbound cells were removed by gently washing the microtiter plate with phosphate-buffered saline (PBS) and then gently washed twice with 1 ml sterile distilled water, followed by drying of the microtiter plate by inverting on tissue paper for 15–30 min. Then, 1 ml of 0.1% of crystal violet solution

was added to each well and kept for 45 min at room temperature. Excess dye was washed off by rinsing gently with 1 ml sterile distilled water two to three times. The plate was dried and then 1 ml of 30% acetic acid was added into each well to extract crystal violet and OD was taken at 600 nm using UV–Vis spectrophotometer (Shimadzu, Model–UV 2450, Japan) by keeping 30% acetic acid as blank (Doijad 2014; Doijad et al. 2015).

Similarly, the response of *L. monocytogenes* strain BN3 to varying concentrations of C_6-HSL was tested using the CV assay in 48-well sterile polystyrene microtiter plate in BHI broth. *L. monocytogenes* strain BN3 was exposed to different concentrations of AHL molecule (250, 500 nM and 1 µM) and its effect on biofilm-forming ability checked using the CV assay. Three replicates were maintained for each concentration. BHI broth with *L. monocytogenes* strain BN3 without AHL is considered as control and plain BHI broth as negative control. Also, the effect of antibacterial compound A(4)2015, i.e., (*R*)2-(2-hydroxynaphthalen-1-yl)thiazolidine-4-carboxylic acid) (10 µg/ml) on the biofilm-forming ability of *L. monocytogenes* BN3 was studied in a microtiter plate using the CV assay. Thiazolidine derivatives are well known for their antibacterial and other biological activities (Jain et al. 2012). Furthermore, the effect of A(4)2015 on biofilm formation in *L. monocytogenes* BN3 in the presence of AHL molecule was also studied. Here A(4)2015 (10 µg/ml) was added to the microtiter wells containing different concentrations of AHL molecule (250 and 500 nM), and *L. monocytogenes* strain BN3 was inoculated and the biofilm-forming potential was examined using crystal violet assay. Thiazolidine derivative, A(4)2015, was synthesized by Dr. Chinmay Bhat, Department of Chemistry, Goa University (Online Resource 1).

The biofilm-forming ability of *L. monocytogenes* strain BN3 in the presence of AHL molecule (C_6-HSL) and antibacterial compound A(4)2015 was also tested by scanning electron microscopy (SEM). Here, clean and sterile 0.5 cm^2 size coverslips were placed in microtiter wells containing different concentrations of AHL molecule (C_6-HSL) and A(4)2015 10 µg/ml and also in positive and negative control microtiter wells. After 48 h of incubation at 37 °C, coverslips were removed using sterile forceps and gently washed with sterile saline to remove BHI broth. Then all the coverslips were fixed in 3% glutaraldehyde overnight with 50 mM PBS at 4 °C. Coverslips were gently washed thrice with PBS and then dehydrated in gradually increasing concentrations of ethanol, i.e., 10, 20, 50, 70, 80, 90, 95, and 100% for 15 min each (Naik and Dubey 2011), air dried and stored in a vacuum chamber prior to SEM analysis. The air-dried coverslips were then sputter coated with gold to a thickness of 10 nm approximately and examined under SEM (Zeiss EVO18). SEM analysis was carried out within 2 days.

Results and discussion

Isolation of biofilm-forming *Listeria monocytogenes* from the local dairy industry

After enriching *Listeria* spp. from swabs taken from milk utensils from the local dairy society in UVM I and UVM II broth, a loopful of UVM II broth which turned into black color due to esculin hydrolysis was streaked on PALCAM agar. After incubation at 37 °C for 48 h, one isolated colony showing grayish color with shrunken center and blackening of medium in the vicinity of the colony due to esculin hydrolysis was picked and purified on PALCAM agar (Online Resource 2) and tentatively named as BN3. Bacterial isolate BN3 was subcultured every month on BHI agar slants and stored at 4 °C.

Identification of bacterial isolate BN3 using biochemical tests and 16S rRNA sequencing

Bacteria isolate BN3 showed phosphatidyl-inositol phospholipase C activity (Online Resource 3), weak β-hemolysis and enhanced hemolytic activity of *S. aureus* through CAMP test. Isolate BN3 was found to be Gram-positive short rods, non-motile at 37 °C, but motile at 25 °C. The bacterium showed the ability to ferment rhamnose, while they cannot ferment xylose and mannitol. When all the biochemical tests were compared with Bergey's manual of systematic bacteriology, isolate BN3 was identified as *L. monocytogenes*. Moreover through 16S rDNA sequence analysis and comparing against GenBank database using NCBI BLAST search, isolate BN3 was confirmed to be *L. monocytogenes* strain BN3 with GenBank accession number MF095110.

Antibiotic susceptibility test

Listeria monocytogenes strain BN3 showed resistance to ciprofloxacin (10 µg/disc) and penicillin-G (10 units/disc) antibiotics, which divulge that infection caused by *L. monocytogenes* strain BN3 will be difficult to treat.

Production of siderophore by *Listeria monocytogenes* strain BN3

The appearance of an orange halo around the bacterial colony on Chrome Azurol S agar plate demonstrated siderophore production by *L. monocytogenes* strain BN3 (Online Resource 4). Utilization of exogenous siderophores and natural catechols by *L. monocytogenes* was reported (Simon et al. 1995), but *L. monocytogenes* was never reported to produce its own siderophores. This is a maiden report on *L. monocytogenes* producing siderophores to survive in iron-starved conditions and may chelate iron from human host to

weaken their immune system and survive in monocytes and multiply, since iron is reported to be an important element for a stronger immune system (Soyano and Gomez 1999).

AHL molecule detection assay using reporter *Chromobacterium violaceum* MCC2216

Chromobacterium violaceum MCC2216 did not produce violet pigment when *L. monocytogenes* strain BN3 was cross-streaked against reporter strain *C. violaceum* MCC2216. But when *V. harveyi* bioluminescent bacteria isolated from marine quid cross-streaked against reporter strain *C. violaceum* MCC2216, it was observed that reporter *C. violaceum* MCC2216 produced the violet pigment at the streak region (Online Resource 5). This confirmed that *L. monocytogenes* strain BN3 is negative for AHL production. Till date, there is no report on *L. monocytogenes* producing AHL molecules and our results also go hand in hand with earlier reports saying *L. monocytogenes* have only *agr* QS system (Novick and Muir 1999; Garmyn et al. 2009; Gray et al. 2013; Zetzmann et al. 2016).

Response of *L. monocytogenes* strain BN3 to AHL molecule (C_6-HSL)

Listeria monocytogenes are well known to be negative for AHL production and till date there are no reports on whether *L. monocytogenes* can respond to exogenous (external) AHL molecules. In the present study through crystal violet assay in microtiter plate, it was observed that on addition of 250 nM AHL there was 24% enhancement in biofilm formation in *L. monocytogenes* strain BN3 (Fig. 1) (Online Resource 6). This proved that *L. monocytogenes* strain BN3 responds to AHL (C_6-HSL) and there is a positive effect of AHL on biofilm formation in *L. monocytogenes* strain BN3. Likewise on addition of 500 nM AHL molecule, there was a rise in biofilm formation by 34% as compared to the control. Furthermore, increase in biofilm formation by 47% as compared to control was observed in the presence of 1 µM AHL molecules; so there is a gradual increase in biofilm formation with gradual increase in AHL concentration (Fig. 1). Therefore, it is confirmed that AHL plays an important role in biofilm formation in *L. monocytogenes*, although it is negative for AHL production.

On addition of 10 µg/ml A(4)2015 (thiazolidine derivative), there was a decrease in biofilm formation in *L. monocytogenes* strain BN3 by 37%. This reveals that the A(4)2015 compound has a negative effect (antimicrobial property) on biofilm formation in *L. monocytogenes* strain BN3. However on addition of 250 nM AHL molecule in the presence of 10 µg/ml A(4)2015, there was 3% increase in biofilm formation as compared to the control. Also, there was rise in biofilm formation by 24% as compared to the control, when 500 nM AHL molecule was added in the presence of 10 µg/ml A(4)2015. This reveals that the AHL molecules nullify the effect of A(4)2015 and there is significant increase in biofilm formation even in the presence of antilisterial compound A(4)2015.

Scanning electron microscopy results also support our results obtained using crystal violet assay. Here, we observed that as the concentration of AHL molecules was increased (250 and 500 nM), the biofilm-forming potential of *L. monocytogenes* BN3 was also enhanced (Fig. 2a–c) as compared to the control. The control showed isolated *L. monocytogenes* cells, but when the AHL concentration was increased to 250 nM, cells started forming aggregates (biofilm), and when AHL concentration was further increased to 500 nM there was a thick biofilm formation, which proved the positive effect of AHL molecule on biofilm formation (Fig. 2a–c). When *L. monocytogenes* strain BN3 was exposed to thiazolidine derivative A(4)2015 (10 µg/ml), marked reduction in biofilm formation was observed

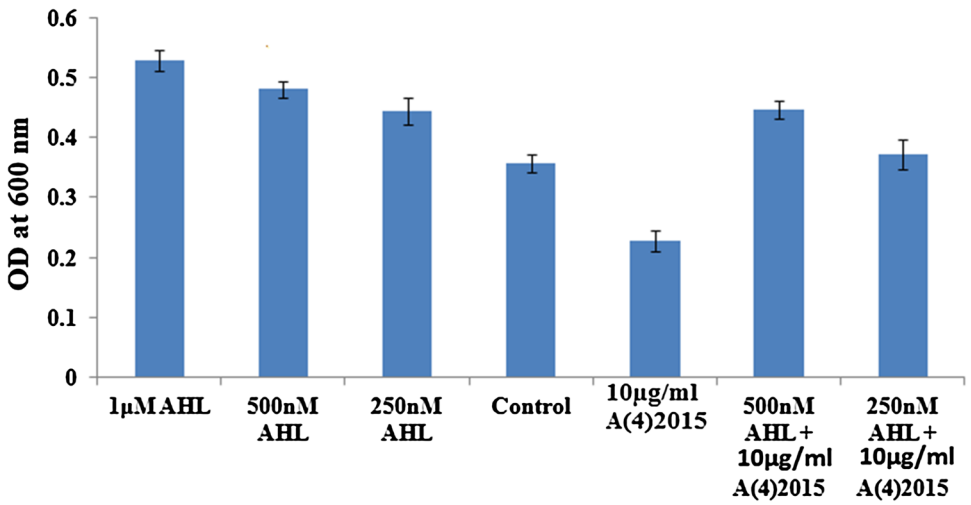

Fig. 1 Response of *L. monocytogenes* strain BN3 to AHL molecule (C_6-HSL) by biofilm formation

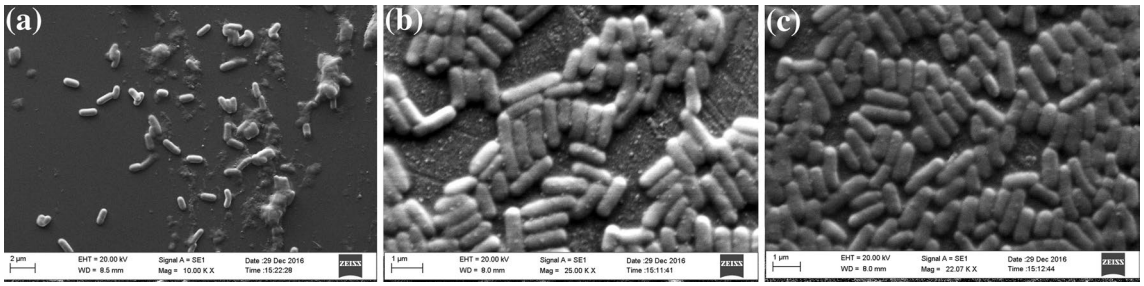

Fig. 2 Biofilm formation potential of *L. monocytogenes* strain BN3 in the presence of **a** 0 nM AHL (control), **b** 250 nM AHL and **c** 500 nM AHL in BHI medium in a microtiter plate. A sterile and clean glass coverslip (0.5 cm^2 size) was inserted into each microtiter well and incubated for 48 h and then monitored for biofilm formation on glass coverslip using SEM analysis

(Fig. 3a). But when *L. monocytogenes* strain BN3 was exposed to 10 µg/ml of antimicrobial compound A(4)2015 in the presence of 500 nM AHL molecule, there was no significant enhancement in biofilm formation, which suggests the role of AHL molecule in biofilm formation and the biofilm formed results in resistance of *Listeria* to antimicrobial compound A(4)2015 (Fig. 3b). Therefore, crystal violet assay and SEM analysis revealed the role of AHL in biofilm formation and resistance of *L. monocytogenes* stain BN3 to antimicrobial compounds and thus persistence of *L. monocytogenes* in dairy industry.

Gram-positive bacteria mostly produce short linear or cyclic peptides. These peptides act as autoinducers that transmit signals for the purpose of quorum sensing and virulence gene expression (Novick and Muir 1999; Garmyn et al. 2009). Gram-positive bacteria (including *L. monocytogenes*) have been reported to possess *agr* quorum sensing system (*agr BDCA* operon) (Garmyn et al. 2009), but there is only one report on marine Gram-positive *Exiguobacterium* sp. having the presence of the AHL quorum sensing system, which was reported to produce C3-oxo-octanoyl homoserine lactone (OOHL) (Biswa and Doble 2013). *Staphylococcus aureus* has never been reported to produce the AHL quorum sensing system, but reported to respond negatively to external AHL molecules (Qazi et al. 2006). In this report, external *N*-acyl homoserine lactone was reported to antagonise virulence gene expression and quorum sensing in *S. aureus*. Also 3-oxo-C_{12}-HSL is reported to inhibit *agr* QS expression in *S. aureus* as reported by Qazi et al. (2006). Till date, there is no report on *L. monocytogenes* producing AHLs and our results also confirmed the same. In the present study, we report for the first time that although *L. monocytogenes* strain BN3 does not produce AHLs, it responds to exogenous AHL molecules (C_6-HSL) positively by gradual increase in their biofilm-forming potential with gradual increase in AHL molecule concentration in a concentration-dependent manner (250, 500 nM–1 µM). Also, the biofilm-forming potential of *L. monocytogenes* is unaffected by antibacterial thiazolidine derivative in the presence of exogenous AHLs (C_6-HSL). To control biofilm formation by *L. monocytogenes* and other Gram-positive pathogens, researchers target only *agr* quorum sensing system (Gray et al. 2013),

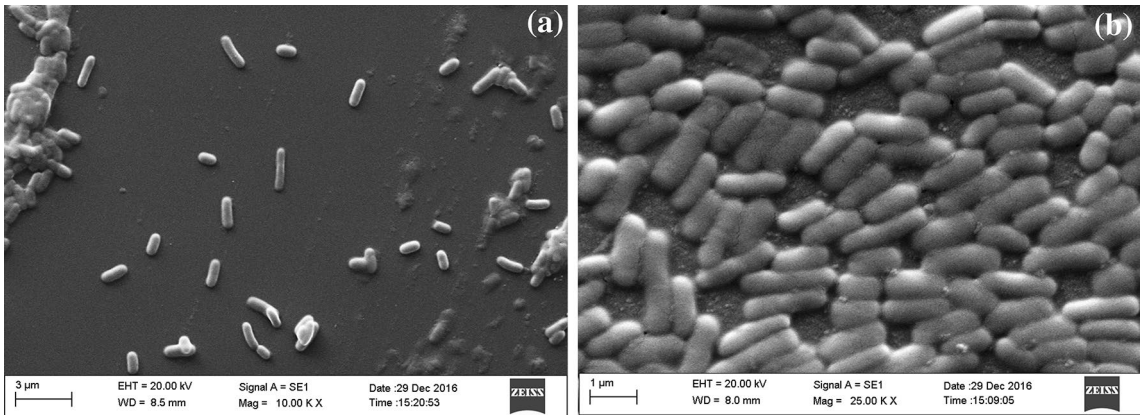

Fig. 3 Biofilm formation potential of *L. monocytogenes* strain BN3 in the presence of **a** 10 µg/ml A(4)2015 and **b** 10 µg/ml A(4)2015 + 500 nM AHL in BHI medium on microtiter plate. A sterile and clean glass coverslip (0.5 cm^2 size) was inserted into each microtiter well and incubated for 48 h and then monitored for biofilm formation on a glass coverslip using SEM analysis

but it is very difficult to control *L. monocytogenes* biofilm. Therefore, our study suggests targeting AHL quorum sensing system along with *agr* quorum sensing system for better results. This investigation will definitely help in future to understand how Gram-negative and Gram-positive bacteria interact with each other when present in the same ecological niche. It also helps to understand the mechanism by which AHL molecules produced by Gram-negative bacteria influence biofilm formation in *L. monocytogenes* on milk-collecting utensils in dairy industries and food-processing plants and this will help to develop a strategy to tackle *L. monocytogenes* biofilm in the dairy industry.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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