EDITORIAL

Diverse profiles of N-acyl-homoserine lactones in biofilm forming isolates of Cronobacter sakazakii

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Quorum sensing (QS) is a cell-to-cell physiological communication system in bacteria that depends on the production, secretion, and detection of a signaling molecule or autoinducer (AI) within a population of cells in response to changes in that population’s cell density in the presence of a given concentration of the AI. Quorum sensing systems were first described by Nealon and Hastings in 1979 in 2 bioluminescent Gram-negative bacterial species, Vibrio fischeri and Vibrio harveyi. In Gram-negative bacteria, AI molecules are comprised of several chemical groupings including acyl homoserine lactones (AHLs), alkylquinolones, α-hydroxyketones and small diffusible signal factors which mimic fatty acid-like compounds. These signaling molecules are synthesized from common metabolites such as fatty acids, anthranilate, and S-adenosylmethionine (SAM), either with a single synthase or through a series of enzymatic reactions. AI molecules are secreted extracellularly, and their concentration increases proportionally to cell population density. It is thought that as the concentration of AI molecules reaches a stimulatory threshold level in the surrounding environment, these small molecules bind to specific cell receptors which act as transcriptional regulators and sequentially alter the gene expression profile of bacteria in a synchronized, cell density-dependent fashion. This activity allows for the coordinated expression by the bacterial population of specific sets of genes involved in group behaviors such as nutrient uptake, genetic competence development and exchange, biofilm formation, sporulation, toxin secretion, bioluminescence, and expression of virulence factors. Bacterial species living within natural environments depend on QS to regulate important cellular processes that are essential for survival, persistence, and adaptation to their ever changing environments. In this issue, Singh et al. investigated the interaction between biofilm formation, extracellular polysaccharide substance (EPS) production and AHL production during biofilm formation in Cronobacter sakazakii, a re-emerged foodborne pathogen that causes a variety of infections in all age groups; however, neonates and elderly individuals are at a higher risk and remain the most susceptible age groups for life-threatening, invasive disease. While infantile Cronobacter infections are often linked to the consumption of reconstituted, temperature-abused, intrinsically or extrinsically contaminated powdered formulas, these organisms are also found associated with other foods and food production environments, such as that of manufacturing facilities of dried foods, thereby posing a risk to susceptible consumers. Previous studies by Lehner et al. and da Silva Araujo et al. reported the presence of 2 and 3 QS molecules in different Cronobacter spp., respectively. Singh et al. identified a group of long chain AHLs, of C6-C18 in length, using High Performance Liquid Chromatography and Liquid Chromatography-High Resolution Mass Spectrometry in combination with 2 different QS screening bioassays based on Chromobacterium violaceum CV026 and Agrobacterium tumefaciens NTL4(pZLR4). The C. sakazakii strains produced colorless colonies with the CV026 bioassay and produced blue green pigmented colonies with the A. tumefaciens NTL4(pZLR4) bioassay indicating that the strains were expressing long chain AHL QS signaling molecules which were identified include N-undecanoyl-L-AHL, N-dodecanoyl-L-AHL, N-tetradecanoyl-L-AHL, N-pentadecanoyl-L-AHL, N-(β-ketocaproyl)-L-AHL, N-octanoyl-L-AHL, N-3-oxo-octanoyl-L-AHL and N-octadecanoyl-L-AHL. The in vitro results demonstrate that the stimulatory concentrations of these AHLs appeared in amounts sufficient to be detected after 6 h of incubation. These authors also showed that strains which had
significant levels of long chain AHLs synthesis also produced significantly more EPS and could form significantly more biofilms. To quantitate biofilm formation these authors used a novel mathematical formula called the specific biofilm formation (SBF) index. The SBF index was calculated as follows: SBF = (AB-CW)/G in which AB is the OD540 nm reading of stained and attached bacteria and CW is the OD540 nm reading of stained control wells containing bacteria-free medium only and G is OD600 nm reading of cell growth in suspended culture. A strong SBF index was observed for all 4 strains.

This study has some limitations in that only 4 strains of C. sakazakii were evaluated. Although C. sakazakii is the primary pathogen involved in infections, a question arises as to whether the QS system described is unique only to C. sakazakii. Another obvious inquiry that arises from this study addresses the molecular mechanism(s) that causes these phenotypic changes to occur and whether other pleotropic effects are triggered through this QS system that may be important in survival, persistence, and in disease causation.

This study opens up numerous collaborative avenues for further investigation. For example the authors could use the mutants described by Suppiger et al. to see if such mutants respond in similar fashion in the screening bioassays and whether this QS system is found in these strains. Furthermore, besides the phenotypic and genotypic evidence of EPS and biofilm formation of C. sakazakii that Singh et al. provide, a comprehensive transcriptomic investigation is warranted. This line of investigation will shed light on the extent of the global regulatory circuitry that may be involved and determine whether differences exist among this QS system and those of previously noted QS systems. Finally, to ascertain the role of this QS system in disease causation, infectivity studies using appropriate mutants and wildtype strains should also be conducted.

To the best of our knowledge, this is the first report describing the expression of long chain AHLS in C. sakazakii and confirms the presence of 3-oxo-C8-AHL. As QS regulates the expression of many important phenotypes including virulence production in bacteria, the investigations of QS systems in Cronobacter spp. may lead to a better understanding of its survival in different environments and hosts.

This study makes an interesting contribution to the concept of how bacteria regulate expression of factors involved in survival, which are driven by environmental signals such as AHLS and their role in possibly enhancing the production of virulence factors that contribute to the virulence of Cronobacter. Further work into the mechanisms driving these changes, and how they may influence infection, will be enlightening. To date, QS mechanisms have been investigated in too few bacterial species, an inadequate sample size, for understanding the detailed interaction and physiologic circuitry involved among different bacterial communities, especially those which are involved in foods. In the foreseeable future, this knowledge can be applied in the development of assays designed to detect pathogenic bacterial strains in different food production sectors such as the dairy industry, food manufacturing and in measuring contaminants in water.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Nealson KH, Hastings JW. Bacterial bioluminescence: its control and ecological significance. Microbiol Rev 1979; 43:496-518; PMID:396467
[2] Hawver LH, Jung SA, Ng WL. Specificity and complexity in bacterial quorum-sensing systems. FEMS Microbiol Rev 2016; 40(5):738-52; PMID:27354348
[3] Singh N, Patil A, Prabhune A, Raghav M, Goel G. Diverse profiles of N-acyl-homoserine 1 lactones in biofilm forming strains of Cronobacter sakazakii. Virulence 2017; 8(3): 275-281; http://dx.doi.org/10.1080/21505594.2016.1226713
[4] Yan QQ, Condell O, Power K, Butler F, Tall BD, Fanning S. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium. J Appl Microbiol 2012; 113:1-15; PMID:22420458; http://dx.doi.org/10.1111/j.1365-2672.2012.05281.x
[5] Tall BD, Yan QQ, Gopinath GR, Grim CJ, Jarvis KG, Chen Y, Fanning S, Lampel KA. Cronobacter: an emergent pathogen causing meningitis to neonates through their feeds. Science Progress 2014; 97:154-72; PMID:25108996
[6] Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B, Stephan R. Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various Enterobacter sakazakii strains: aspects promoting environmental persistence. J Food Protect 2005; 68:2287-94
[7] da Silva Araujo FD, Esper LMR, Kuaye Y, Sircili MP, Marsaioi AJ. N-acyl homoserine lactones from Enterobacter sakazakii (Cronobacter spp) and their degradation by Bacillus cereus enzymes. J Agr Food Chem 2012; 60:585-92; http://dx.doi.org/10.1021/jf203846f
[8] Suppiger A, Eshwar AK, Stephan R, Kaever V, Eberl L, Lehner A. The DSF type quorum sensing signaling system RpfF/R regulates diverse phenotypes in the opportunistic pathogen Cronobacter. Scientific Reports 2016; 6:18753; PMID:26725701; http://dx.doi.org/10.1038/srep18753