Engineering 6 (2020) 393–405

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Food Safety and Health—Review

Insights into *Cronobacter sakazakii* Biofilm Formation and Control Strategies in the Food Industry

Na Ling [a,b], Stephen Forsythe [c], Qingping Wu [a,*], Yu Ding [d], Jumei Zhang [a], Haiyan Zeng [a]

[a] *Guangdong Institute of Microbiology & Guangdong Academy of Sciences & State Key Laboratory of Applied Microbiology Southern China & Guangdong Provincial Key Laboratory of Microbiology Culture Collection and Application & Guangdong Open Laboratory of Applied Microbiology, Guangzhou 510070, China*
[b] *School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, China*
[c] *foodmicrobe.com, Keyworth NG12 5GY, UK*
[d] *Department of Food Science and Technology, Jinan University, Guangzhou 510632, China*

ARTICLE INFO

Article history:
Received 2 January 2019
Revised 23 April 2019
Accepted 7 May 2019
Available online 28 February 2020

Keywords:
Biofilm
Control
Cronobacter sakazakii
Formation
Removal

ABSTRACT

Cronobacter sakazakii (*C. sakazakii*) is a foodborne opportunistic pathogen that can cause life-threatening invasive diseases, such as necrotizing enterocolitis, meningitis, and sepsis in infants. The potential risk of *C. sakazakii* contamination of powdered infant formula (PIF) is an issue that has attracted considerable attention from manufacturers, regulators, and consumers. *C. sakazakii* biofilms on the surfaces of equipment and in diverse food-production environments constitute a mode of cell growth that protects the pathogen from hostile environments, and are an important source of persistent contamination of food products. Bacterial biofilms are difficult to remove due to their resistant properties. Conventional cleaning and sterilizing procedures may be insufficient for biofilm control, and may lead to further biofilm development and dispersal. Consequently, novel control strategies are being developed, such as nanotechnology-based delivery systems, interspecies interactions, antimicrobial molecules of microbial origin, natural extracts, and phages. This review focuses on describing the mechanisms underlying the biofilm formation and behavior of *C. sakazakii* and discussing potential control strategies.

1. Introduction

The *Cronobacter* genus is composed of seven species, which collectively were previously known as the foodborne pathogen *Enterobacter sakazakii*. *Cronobacter sakazakii* (*C. sakazakii*) is the most infectious species among the *Cronobacter* spp. [1]. These pathogens are Gram-negative rods that are motile with peritrichous flagella, and can grow under aerobic and anaerobic conditions. Infections occur in all age groups, and particularly occur in neonates, infants below six months in age, and the elderly. *C. sakazakii* has drawn the attention of regulatory authorities due to its association with outbreaks of life-threatening infections in neonates. The International Commission on Microbiological Specifications for Foods (ICMSF) had ranked *Cronobacter* as a "severe hazard for restricted populations, life threatening or substantial chronic sequelae of long duration" [2]. Life-threatening symptoms in primarily low-birth-weight neonates and infants below six months in age (pre-weaning) include meningitis, necrotizing enterocolitis, and respiratory infections, with neurological sequelae or rapid death having a mortality ranging between 40% and 80% of cases [3–7]. *C. sakazakii* infects other age groups and predominantly causes infections in adults who are typically immunocompromised, although an acute gastroenteritis outbreak has been reported among students [8]. In adults, *C. sakazakii* can lead to a wide range of symptoms such as bacteremia, appendicitis, septicemia, osteomyelitis, pneumonia, splenic abscesses, wound infections, and urinary tract infections [5,9].

C. sakazakii have been isolated from various environments, including powdered infant formula (PIF), vegetables, ready-to-eat foods, water, dried foods, medicinal plants, spices, and flies [10–19]. A major ecological niche for *Cronobacter* may be plants due to the organism's desiccation resistance, production of polysaccharide capsule, and secretion of yellow carotenoid pigment that protects it from oxygen radicals [20]. Moreover, the organism is found in household environments [21,22] and food-production facilities [23,24].

In response to their varying growth environment, bacteria can form spatially organized dynamic ecosystems called biofilms. This is a major adaptation survival strategy by which foodborne

* Corresponding author.
E-mail address: wuqp203@163.com (Q. Wu).

https://doi.org/10.1016/j.eng.2020.02.007

pathogens can protect themselves against environmental stresses [25–27]. Furthermore, bacteria in biofilms differ from their planktonic counterparts in that they exist as a community with cell–cell cooperation and competition, transmit signal molecules, and conduct horizontal gene transfer with each other—thus maintaining a mutual relationship as "both friends and enemies" [28]. The distinctive morphology, structure, and physiology of the biofilm vary according to the resident microorganisms and their environmental conditions. Changes within the biofilm are achieved by the organisms' ability to sense and respond to various external environmental signals. By means of an intersystem cross talk in response to these environmental signals, bacteria are able to orchestrate their gene expression and adapt to the changing environment.

C. sakazakii can adhere to abiotic materials such as silicon, latex, polycarbonate, stainless steel, glass, and polyvinyl chloride (PVC), and thereafter form biofilms on these surfaces [29,30]. It is noteworthy that the survival and persistence of *C. sakazakii* in PIF, which has been linked to outbreaks of *C. sakazakii*, require the organism's ability to adapt to harsh osmotic and dry conditions. Beuchat et al. [31] found that cells in biofilms of *C. sakazakii* were more persistent than planktonic cells when exposed to low moisture conditions. In milk formula processing factories, the organism has been found in the environment (i.e., on air filters, floor, shoes, trucks, and roofs) and processing equipment (i.e., in roller dryers and air filters), and may persist for several months. Biofilms of *C. sakazakii* also form on neonatal enteral feeding tubes, which may lead to an increased risk of exposure to the neonate [32]. Recently, the biofilm state has been considered to be the default lifestyle of bacteria, and it is thought that free-living planktonic cells may only be a transitional stage in bacterial life [33]. Microbial contamination of food products is largely linked to biofilm formation on food-contact surfaces. Such surfaces can provide a solid substrate for the development and persistence of biofilms throughout the food processing chain; these biofilms are difficult to remove, leading to bacterial contamination of food and potential health risks to consumers.

Research in this field has been focused on gaining a deeper understanding of biofilms and identifying strategies to avoid the contamination of foodstuffs. Recently, advances in multi-omic and imaging technologies have revealed complex spatial organization and processes within bacterial biofilms. Our improved understanding of biofilms will lead to improved strategies for their removal. Nevertheless, the control of *C. sakazakii* biofilms remains poor, since our understanding is currently at an initial stage. The aim of this review is to present a panoramic view of our current knowledge, including the mechanisms involved in biofilm formation, conventional and innovative biofilm-controlling strategies that have been proposed to combat the challenges caused by biofilms, and how these may be applied for the control of *C. sakazakii* biofilms in the food industry.

2. Mechanism of biofilm formation

"Prevention is better than a cure"; therefore, an in-depth understanding of biofilm formation can contribute significantly to designing the food-production environment in such a way as to minimize biofilm formation. Biofilms are composed of complex microflora coated with an extracellular protective matrix made of various types of biopolymers, and can form on a variety of surfaces [25]. *C. sakazakii* biofilm formation is a dynamic process with a sequence of events that involves attachment, maturation, dispersal, and the next round of attachment, as revealed through scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Planktonic bacteria initially stick to the surface of the carrier and secrete large amounts of extracellular polymers. Bacteria clump together to form a thickened biofilm with the growth of cells, and the growth of the biofilm reaches its maximum after 72 h. After 96 h, the biofilm detaches from the surface and releases bacteria (Fig. 1). The processes governing biofilm formation by *C. sakazakii* are similar to those of other bacteria [34]. In order to generate the biofilm, bacteria have a number of mechanisms enabling intercellular communications and attachment to abiotic surfaces, and consequently grow as resilient structures.

2.1. Environmental factors influencing C. sakazakii biofilm development

2.1.1. Culture conditions

Biofilm formation is a complex process that is influenced by the changeable environmental surroundings. Biofilm-forming ability and exopolysaccharide production by *C. sakazakii* differ according to environmental conditions, which include the contact surface properties, nutrient availability, and relative humidity [35]. Ye et al. [36] evaluated the biofilm formation of 23 *C. sakazakii* isolates under diverse pH values, temperatures, and incubation periods. The biomass and properties of polysaccharides in the biofilm formation of *C. sakazakii* were strain-specific dependent and were affected by the culture's microenvironments. The growth of biofilms during the production of dairy products can also be linked to bacterial extracellular polymeric substances (EPSs) and milk residues—mostly proteins and calcium phosphate. Milk components also affect *Cronobacter* biofilm formation. Whey protein and casein have been shown to have a more important influence on *Cronobacter* biofilm formation in skim milk than lactose, and the nitrogen source was found to be more important than carbohydrate [37]. Ye et al. [38] assessed the effects of Ca^{2+} and Mg^{2+} on the biofilm formation of *C. sakazakii* isolated from PIF. The divalent cation concentrations influenced the total biomass formation, with 1.50% $MgCl_2$ and 0.25% $CaCl_2$ giving optimal growth conditions. In addition, the biomass properties of the extracellular matrix were found to be altered by the divalent cations.

2.1.2. Contact surface

Contact surface is an indispensable part of the preconditioning of biofilm formation. The properties of the surface will affect the cell adhesion and subsequent biofilm formation. Electrostatic interaction can be established between the two substances when the cell contacts and adheres with the surface. Since most bacteria have a negative charge on their surface, a positive charge on the contact surface is conducive to biofilm formation [39]. In practical situations, the contact surface may also be exposed to a highly ionic medium composed of ions and small molecules that adsorb to the surface through diffusion and mass transfer processes, thus changing the properties of the contact surface. Interaction between the contact surface and bacterial cells is subsequently altered, and electrostatic interactions are built up. The distance between the cell and the contact surface interface is shortened when microbial cells initially adsorb to the surface through electrostatic interaction.

After non-polar and low-surface-energy hydrophobic groups at the contact surface interface interact with the bacterial cells, the adhesion capacity of the cells on the surface becomes fixed due to the tendency to change the hydrophobicity of the contact interface [40,41]. Davidson and Lowe [42] suggested that hydrophobic groups on the contact surface interface were favorable for the formation and stability of biofilms. Nevertheless, Heistad et al. [43] reported that there was no obvious relationship between interfacial hydrophobicity and biofilm formation. *C. sakazakii* cells attached more readily to silicone and polycarbonate than to steel ($P < 0.05$), and hydrophilic materials appeared to be more favorable

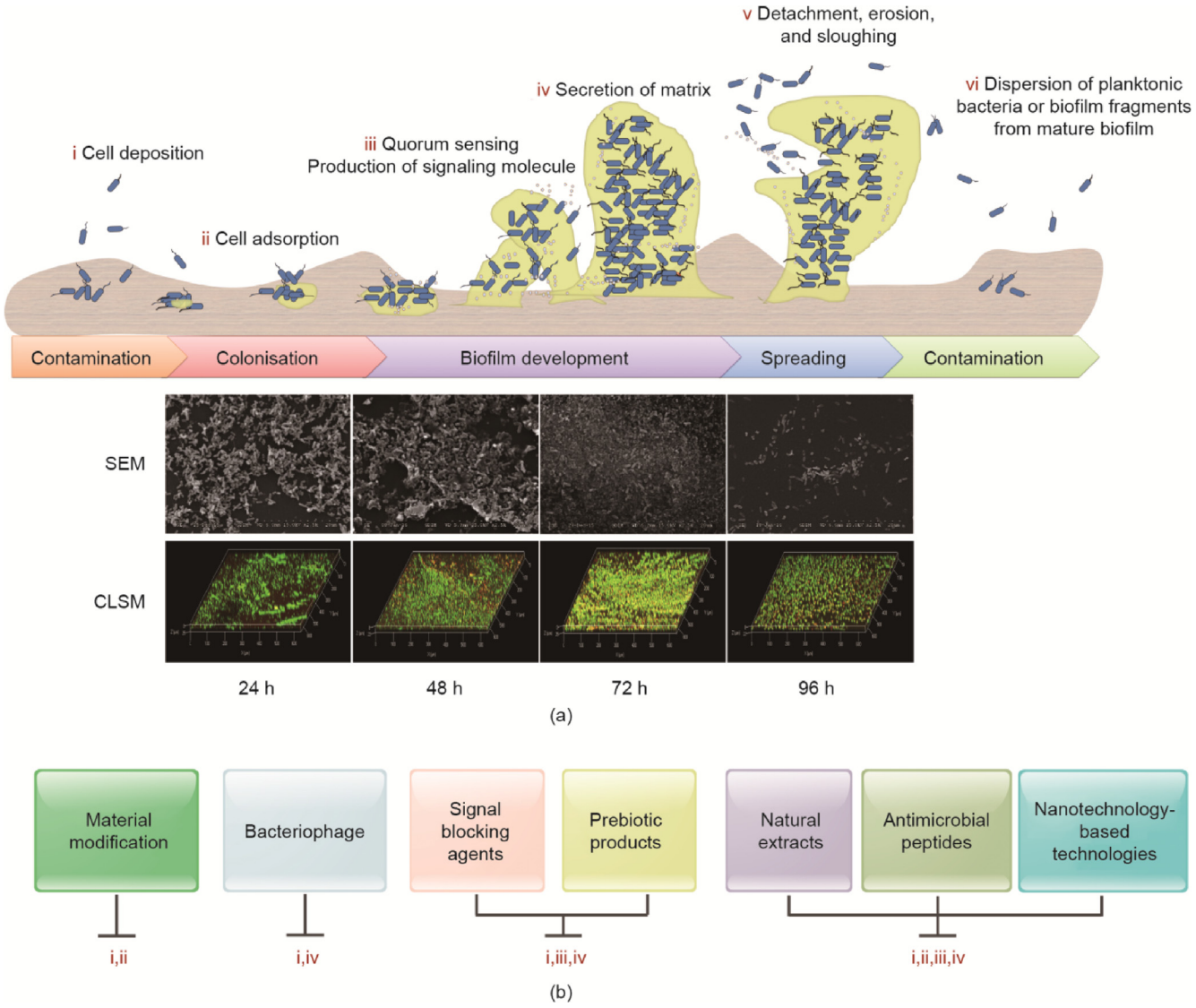

Fig. 1. The inhibition of biofilm formation by various substances. (a) Dynamic process of biofilm formation in a periodic manner that involves attachment, maturation, dispersal, and the next round of attachment; the periodic process usually contains six steps, as shown here. (b) The removal roles of substances in diverse steps of biofilm formation, as shown by SEM images (×2500). Biofilms were stained with propidium iodide and SYTO9 and were observed by CLSM.

to biofilm formation by *C. sakazakii* [29]. Therefore, the question of whether or not there is a link between biofilm formation and interfacial hydrophobicity is a controversial issue, and more experiments focused on the adhesive properties of *C. sakazakii* are required.

Surface roughness can reduce the hydraulic shear force on bacterial cells in the liquid mobile phase. Consequently, roughness will protect the bacterial cells from being removed by water flow, and will enhance the initial adhesion of the cells. However, there is a considerable difference across cell particle sizes for different bacteria, leading to variation in the optimal contact surface roughness for different bacteria.

2.2. Cell self-regulation

2.2.1. Quorum sensing

In the majority of environments, biofilms live as a mixed population rather than as a monoculture. A mixed population generates physiological functions that are not present in monocultures. Interspecies regulatory mechanisms within the biofilm include quorum sensing (QS) [44,45]. Such QS systems involve low-molecular-weight signaling molecules called autoinducers, which will be expressed according to the number of bacteria in the surrounding environment [46]. When the amount of bacteria in the biofilm reaches a sensory threshold concentration, the bacteria release signaling molecules to change and coordinate their behavior. Changes in specific gene expression managed by QS systems include the transport of nutrients and the removal of metabolic end-products. This behavior can reduce the limitations that result from the lack of space and nutrients caused by excessive bacteria growth in order to facilitate biofilm formation.

Gram-negative bacteria mostly use *N*-acyl-*L*-homoserine lactones (AHLs) to communicate with each other, and these bacterial pheromones are often produced as mixtures of several AHLs (Fig. 2). The basic QS circuit usually harbors two indivisible proteins (LuxI and LuxR) that function within the synthesis and recognition of the autoinducer, respectively. LuxI is a synthase that induces the synthesis of the QS self-inducible introns AHLs and their derivatives, which can diffuse freely in and out of the cell. When the concentration of AHLs reaches the critical value along

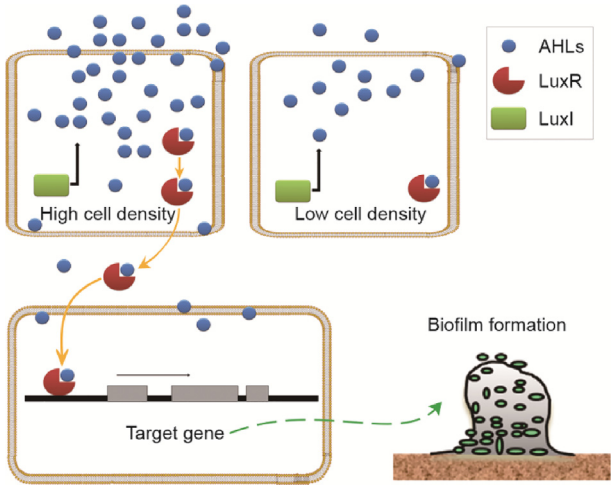

Fig. 2. Simplified diagram of AHL-mediated QS in Gram-negative bacteria. Gram-negative bacteria possess two regulatory components: LuxI and LuxR. LuxI is required for AHLs biosynthesis and LuxR encodes for AHLs binding protein. Once a threshold concentration has been reached, AHLs will freely enter into neighboring cells. The complex consisting of LuxR and AHLs will activate the expression of downstream genes. Biofilm formation is subsequently affected through target genes regulated by AHL-mediated QS.

with the increasing bacterial density, the AHLs bind to LuxR in order to activate the expression of downstream genes, thereby causing a physiological reaction of the bacteria to adapt to their surroundings [47]. AHLs are generally composed of an end made of an acetyl chain of 4–18 carbons and a conserved head made of high serine lactone. The main differences between various AHLs are based on the length of the N-terminus side chain and on the existence of substituents or unsaturated bonds at C-3 [48]. At present, three signaling molecules (*N*-heptanoyl-AHL, *N*-dodecanoyl-AHL, and *N*-tetradecanoyl-AHL) are known to be produced by *C. sakazakii* [49]. Lehner et al. [30] suggested that *Cronobacter* can regulate biofilm formation by expressing QS AHLs. Two different types of AHLs (3-oxo-C6-AHL and 3-oxo-C8-AHL) have also been found by means of thin-layer chromatography. Using high-performance liquid chromatography (HPLC) and liquid chromatography–high-resolution mass spectrometry (LC–HRMS), significant expression levels of long-chain AHLs with chain lengths of C6–C18 have been identified. LC–HRMS analysis of AHLs confirmed the presence of *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 in biofilm-forming strains of *C. sakazakii* [50].

Further studies should be centered on the mechanism underlying the formation and development of *C. sakazakii* biofilms, as regulated by the QS system. This will provide a basis for the exploration of attractive novel tactics against pathogenic bacteria based on QS.

2.2.2. Genetic elements and physicochemical properties of the cell

The microorganisms in biofilms are encapsuled in the self-produced matrix of EPS. EPS mainly consist of extracellular polysaccharides, proteins, and extracellular DNA (eDNA) and its substrates; this matrix mediates the adhesion on surfaces and coagulates the cells together (Fig. 3). The matrix fills the space between bacteria, resulting in a mechanical cohesive stability. Due to this stability, microbes within biofilms possess a high level of survival and high persistence potential. Biofilm heterogeneity is dependent on the concentration, cohesion, charge, and absorptive capacity of diverse extracellular polymer components, as well as on the density, pore size, and channel of the matrix. Biofilms accordingly exhibit miscellaneous morphological features, and may be smooth, flat, coarse, fluffy, or filamentous; they can even form agglomerations like mushrooms surrounded by water-filled voids [28].

Researchers recently investigated the difference between the biofilm and planktonic phases of *Cronobacter* using a comparative proteome approach, and confirmed that the differentially expressed proteins were enriched in the pathways of flagella, cellulose synthesis, amino acid metabolism, and carbohydrate metabolism [51–53]. The *Cronobacter* capsule is composed of *O*-antigen, *K*-antigen, colanic acid, cellulose, and the enterobacterial common antigen. The ratio of components probably changes according to growth condition, although it has not been studied to date. This is an area that warrants more research, especially

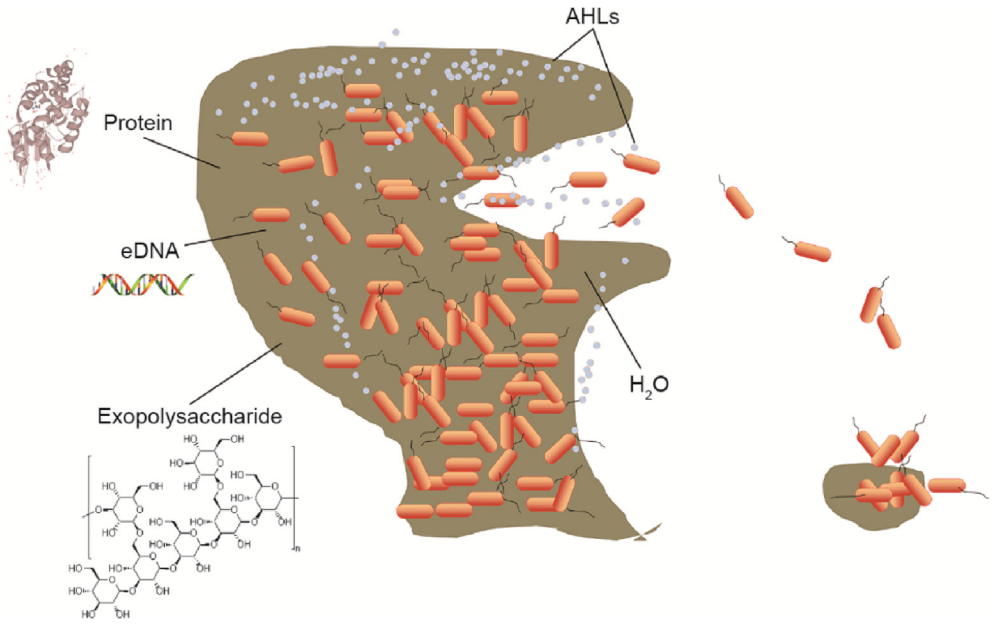

Fig. 3. Basic components of a biofilm with Gram-negative bacteria.

since particular capsule profiles are associated with *Cronobacter* isolates from neonatal meningitis cases [54]. Cellulose produced by bacteria is generally an extracellular component that imparts both mechanical and chemical protection to the cell against adverse environments. Cellulose is also a structural component of biofilms, as it forms a hydrogel with strong water-adsorption capabilities. Cellulose is synthesized and excreted by a cellulose synthase complex consisting of BcsA and BcsB. While *bcsA* encodes for cellulose synthase catalytic subunit, *bcsB* encodes for a cyclic dimeric guanosine monophosphate (c-di-GMP) binding protein. Two genetic elements—the *bcsABZC* and *bcsEFG* operons—are required for biosynthesis and regulation of cellulose (Fig. 4). The cellulose gene cluster comprises nine genes (bcsGFERQABZC) and is present in almost all *C. sakazakii* strains [54]. Hu et al. [55] compared the prevalence of cellulose synthase-related genes in *Cronobacter* isolated from clinics, foods, and the natural environment. The results indicated that cellulose synthase operon genes (*bcsABZC*) were present in all clinical isolates and in most of the food and environmental isolates of *Cronobacter*. Moreover, biofilm formation and bacterial cell–cell aggregation were significantly reduced in *bcsA* and *bcsB* mutants compared with the parental strain, and *bcsA* and *bcsB* mutants did not produce cellulose. *bcsR* negatively regulated cellulose synthesis, but exhibited positive regulation of biofilm formation, adhesion, and invasion in *C. sakazakii*. Upregulation of crucial cellulose synthesis genes (*bcsA*, *B*, *C*, *E*, *Q*), flagellar assembly genes (*fliA*, *C*, *D*), and virulence-related genes (*ompA*, *ompX*, *hfq*) in the Δ*bcsR* mutant were also verified via reverse transcription polymerase chain reaction (RT-PCR). Among these genes, the expressions of *fliC* and *ompA* in the Δ*bcsR* mutant were remarkably reduced in comparison to those in the wild-type. Furthermore, variations in biofilm components including carotenoids, fatty acids, and amides were shown by Raman spectrometry to be significantly reduced after *bcsR* knockout [56].

In addition, bacterial extracellular organelles (flagella, cilia and fimbria, etc.) can contribute to overcoming the electrostatic repulsion and strengthening bacterial accumulation at the carrier interface [57]. Ten putative fimbriae gene clusters have been identified by means of a comparative analysis of *Cronobacter* genomes [58]. Gene clusters encoding for β-fimbriae are unique to *C. sakazakii*, whereas the genomes of the other six species encode for curli fimbriae. Curli fimbriae are thin, coiled, highly aggregative amyloid fibers that provide an amorphous matrix. They are involved in rugosity, cell aggregation, adhesion, biofilm formation, and environmental persistence [59]. Besides biofilm formation, fimbriae are significant in *C. sakazakii* pathogenicity, as the route of infection is probably through attachment and invasion of the intestinal cells. Hartmann et al. [60] confirmed that the curli-fimbria-associated genes were absent in *C. sakazakii*. Since *C. sakazakii* strains dominate clinical isolates, it can be deduced that curli fimbriae are not essential for *Cronobacter* pathogenicity. Together with cellulose, curli fimbriae form a honeycomb-like structure that facilitates the establishment of biofilms [61]. The biosynthesis and assembly of curli fimbriae are directed by proteins encoded within two divergently transcribed operons, *csgDEFG* and *csgBA*. The *csgBA* operon encodes for the major curli structural subunit CsgA and the nucleator protein CsgB [62]. Hu [63] detected genetic elements related to curli synthesis in 180 *C. sakazakii*, suggesting that no *csgA* and *csgG* genes were present in all *C. sakazakii* strains. In addition, the curli *csgBAC* and *csgDEFG* operons were not present in *C. sakazakii* strains. Nevertheless, *csgA* and *csgB* positively regulate curli fimbriae, biofilm formation, and cell–cell aggregation in other species of *Cronobacter*, with the exceptions of *C. sakazakii* and *C. muytjensii*. Therefore, *C. sakazakii* might express different proteins in other pathways conducive to biofilm formation and cell–cell aggregation that have efficacities similar to those of curli fimbriae.

Motility enables movement across the attachment surface to areas that are more suitable for growth, and considered to be closely related to biofilm formation [64]. Planktonic cells are motile due to the presence of rotating flagella (Fig. 4). FlhA and FliG proteins structure the basal body in flagella, and the deletion of FlhA results in a lack of flagellar components. Along with FliN and FliM, FliG forms the C ring of the basal body [65]. The motors (MotA and MotB stator proteins) located at the base of the flagellum propels the cell through diverse environments using the proton motive force to strengthen the rotation of the helical filament [66]. The Δ*flhA*, Δ*fliG*, Δ*fliC*, and Δ*flaA*Δ*fliC* strains rendered the cells aflagellate and nonautoaggregate. Conversely, the Δ*motAB* and Δ*flaA* strains retain the structural components (flagella) and aggregation ability. Δ*flaA* is motile, while Δ*motAB* is not motile. In addition, *C. sakazakii* biofilm formation on PVC is not mediated by FliC. In contrast, cells within a biofilm generally aggregate by being wrapped in an extracellular matrix. Researchers believed that motility and

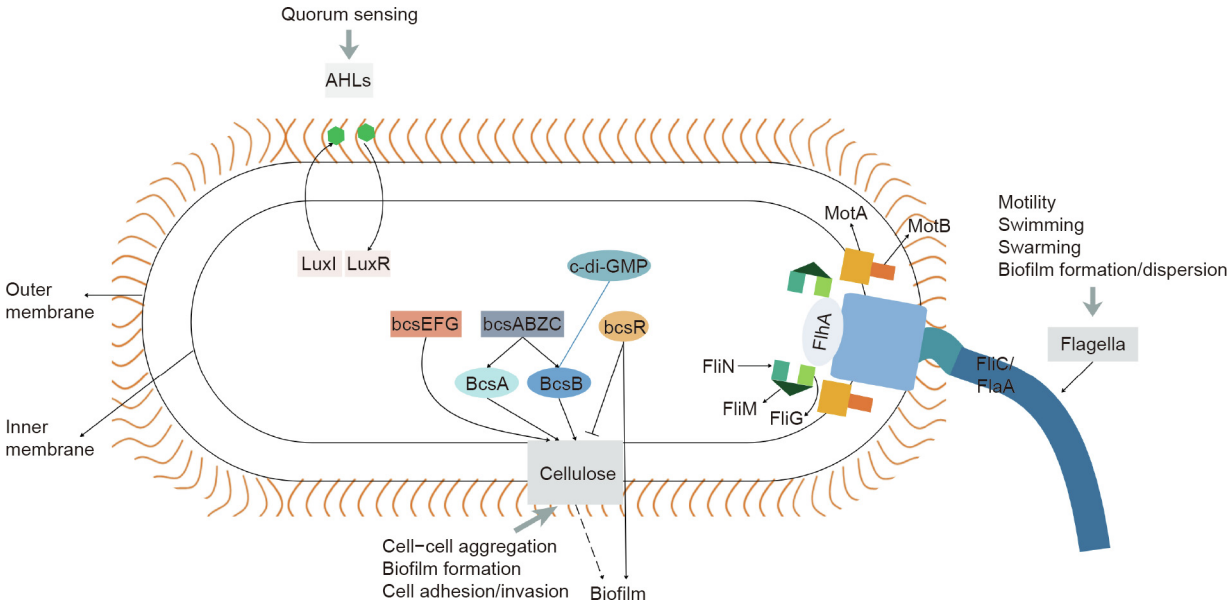

Fig. 4. Regulation networks of biofilm formation for *C. sakazakii*, as previously reported [58–66].

matrix synthesis are often oppositely and coordinately regulated. However, the results of defective mutants of flagella and some regulated genes in *C. sakazakii* have indicated that the relationship between motility and biofilms is strain-specific dependent. In addition, it is still controversial whether flagella have positive or negative effects on biofilm formation, and factors other than motility could also influence biofilm development [67].

Research on microbial populations has expanded considerably due to high-throughput functional genomics. This is primarily due to advances in DNA sequencing, which is now invaluable for probing microbial community composition and function in diverse environments at high resolution [68]. Metabolomics is an evolving research area that can provide a better understanding of the mechanisms that underlie phenotypes beyond biomarker discovery [69]. Multiple "omic" data types, such as transcriptomics, proteomics, and metabolomics, can capture the systems-level profiles of a microbial community. Thanks to innovative developments in informatics and analytical technologies, in addition to the omic approaches in an integrated framework, the "multi-omics" approach has generated novel insights into microbial physiology [70]. Bioinformatic analysis of large omic datasets can show regulatory pathways and pivotal targets related to phenotypic traits. Subsequently, specific DNA regions can be directly altered by applying reverse genetics techniques to verify the visualized effects of the alterations on physiological phenomena. In this way, the goal of a better understanding of bacterial biofilms, including their formation and dispersal, can be achieved (Fig. 5).

3. Biofilm removal and control

Cronobacter has been isolated from various inert and bioactive surfaces, and is widely found in the food industry [24,71–73]. The organism can be isolated from the production environment, and specific strains are reported to persist in factories for several months or even years. As infants are frequently fed PIF and follow-up formula (FUF), these foods have been the focus of attention to reduce infant infection by *Cronobacter* [20]. PIF is generally fed to infants, who are defined as persons below 12 months of age, while FUF is a weaning diet for infants from 6 months month on and for young children, where young children are defined as persons aged 12 months to 3 years [74,75]. Products may be subjected to high temperature and drying during the manufacturing process, and these treatments contribute to the microbiological safety of the products [76,77]. Nevertheless, contamination by *C. sakazakii* can occur in post-processing during storing and packaging (e.g., on roller dryers, packing machines, drying towers, and air filters) (Fig. 6). Obviously, during the manufacturing process, the bacteria are subjected to various challenges to their survival. Biofilm formation represents an important strategy to withstand external stresses, and the protective effect of biofilm should not be neglected. There is good evidence to indicate that *Cronobacter* cells in biofilms have higher resistance against cleaning agents and disinfectants than planktonic cells do [78]. At present, controlling or even eliminating biofilms from dairy and other food processing equipment is a challenging task.

3.1. The clean-in-place system

Cleaning is an extremely important procedure for limiting the adhesion of microbes to contact surfaces during food production. Manual cleaning has been gradually replaced by the clean-in-place (CIP) system, in which production facilities are automatically or semi-automatically cleaned without being dismantled. In the CIP system, detergent solution is sprayed on to the equipment and other items by means of pressure or mass flow. This method has been extensively adapted throughout the various stages of food-production plants [79].

Bremer et al. [80] investigated the effectiveness of eliminating biofilms attached to stainless steel surfaces in the dairy industry by CIP. They reported that a standard CIP system did not reproducibly ensure the removal of bacterial biofilms, and proposed that biofilm removal in a dairy manufacturing plant could be enhanced by changing the CIP conditions. The factors responsible for CIP efficacy are cleaning temperature, time, chemical composition, concentration, surface characteristics, and biofilm layer [81–83]. Kumari and Sarkar [84] mimicked biofilm formation by *Bacillus cereus* in dairy chilling tanks on a laboratory scale in order to optimize CIP regimes. The optimized CIP achieved 4.77 log reduction per square centimeter by altering the concentration of the agent and the treatment time of the CIP process, while the reference CIP caused 3.29 log reduction per square centimeter. Optimization of CIP regimes not only improves food safety, but also improves pro-

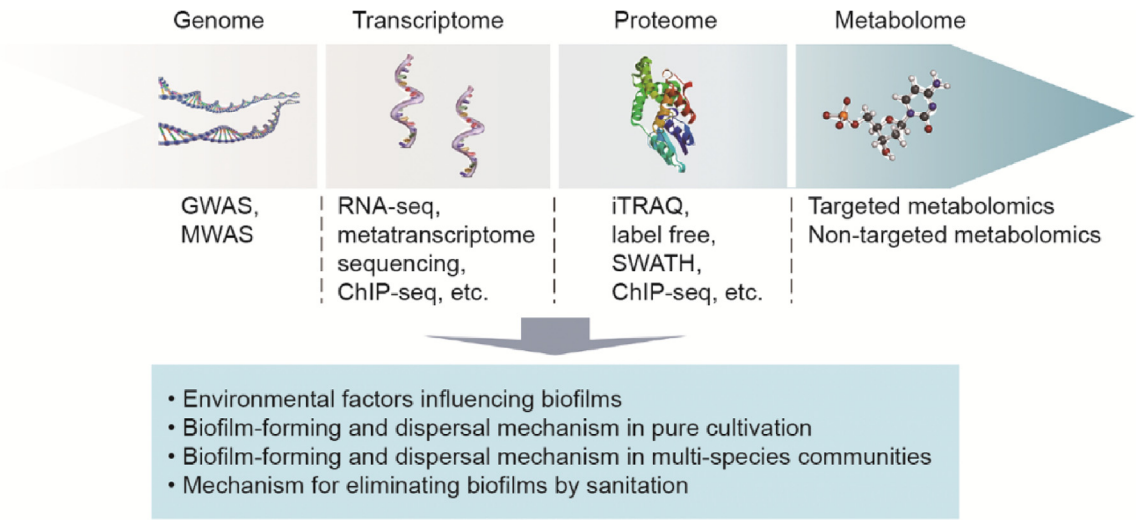

Fig. 5. Multiple "omic" approaches used in biofilm research. GWAS: genome-wide association study; MWAS: metagenome-wide association study; RNA-seq: RNA sequencing (including messenger RNA, small RNA, long non-coding RNA, and circular RNA); ChIP-seq: chromatin immunoprecipitation sequencing; iTRAQ: isobaric tags for relative and absolute quantitation; SWATH: sequential window acquisition of all theoretical mass spectra.

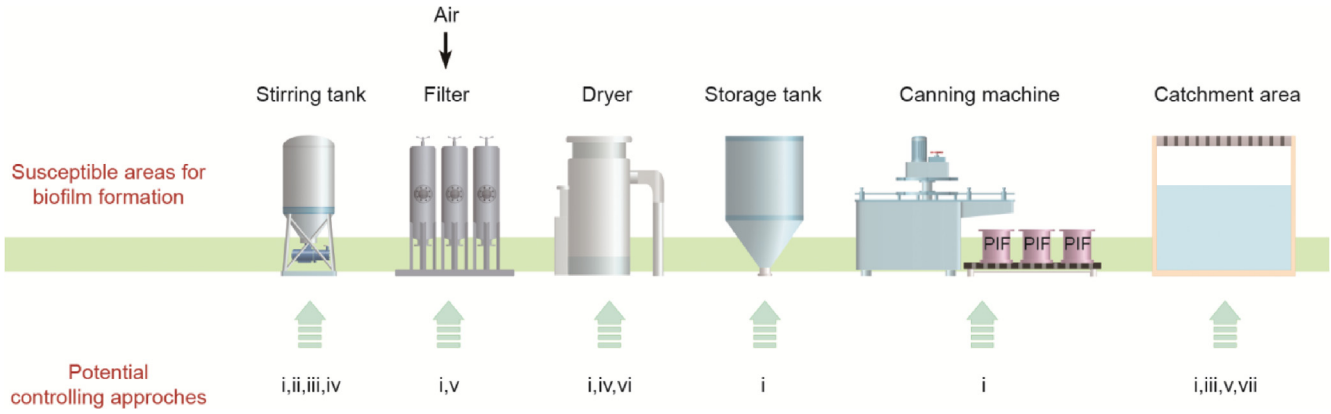

Fig. 6. Popular locations for biofilms and potential approaches against biofilms in PIF processing. i: Coating; ii: prebiotic products; iii: bacteriophages; iv: natural extracts (including signal blocking based on natural extracts); v: ultrasonic cleaning; vi: nanotechnology-based delivery systems; vii: sanitizers.

duct quality and plant performance, and has good economic returns. However, little is known on the optimization of CIP regimes in the dairy industry for the control of *C. sakazakii*.

3.2. Physical treatment

Physical methods used to remove bacterial biofilms generally include mechanical, ultrasonic, and electric methods. Equipment design and choice of surface materials play a prominent role in preventing biofilm formation. Copper alloy is widely utilized in industrial food processing and has antimicrobial properties against antibiotic- and copper ion-resistant bacteria [85]. Copper alloys can release copper ions from the surface to change bacterial cell membrane permeability, leading to copper influx into the cell and subsequent damage to iron-sulfur proteins [86]. Elguindi et al. [87] found that *C. sakazakii* cells suspended in Tryptic Soy Broth were killed within 10 min on 99.9% moist copper alloys and within 1 min of drying on 99.9% copper alloys. These results provided evidence that copper can be effectively applied in the drying process in order to kill *C. sakazakii* before/during packaging and subsequent storage.

Cronobacter is highly resistant to desiccation, and is able to survive in PIF for more than two years [88]. However, it is not a spore-former and is therefore not particularly resistant to heat [89]. Nazarowec-White and Farber [90] determined the *D*-values of a pool of ten strains (five clinical isolates and five food isolates) of *Cronobacter*. *D*-values of 54.8, 23.7, 10.3, 4.2, and 2.5 min were obtained for 52, 54, 56, 58, and 60 °C. The overall calculated *z*-value was 5.82 °C. In addition, Iversen et al. [91] compared the thermotolerance of the *Cronobacter*-type strain and the capsulated strain. However, no statistical difference was shown in the *D*- and *z*-values between these two types of strains as the large standard deviation. The *D*-value of the cells suspended in infant formula milk was determined to be between 54 and 62 °C. The *D*-values ranged from 10.2 to 16.4 min at 54 °C, and from 0.2 to 0.4 min at 62 °C. The resultant *z*-value was 5.7 °C. Awadallah et al. [92] found that *Cronobacter* isolates were thermotolerant when subjected to temperature up to 64 °C, with a *D*-value of 13.79 and *z*-value of 14.42, while Ueda [93] indicated that isolates would be completely inactivated within 2–5 min at temperatures greater than 60 °C. The Food and Agriculture Organization/World Health Organization (FAO/WHO) has proposed that PIF should be reconstituted with water at temperatures greater than 70 °C and should be used immediately before any survivors can multiply to significant levels [78]. Nevertheless, heat shock during food processing may induce protection mechanisms, and the organism is able to survive spray-drying [94].

Another disinfection method adopted in CIP is electromagnetic wave radiation. The electromagnetic wave method inactivates bacteria by destroying or altering the structure of biological macromolecules. Mahmoud [95] investigated the viability of *Cronobacter* treated by X-rays, and demonstrated that *Cronobacter* was significantly ($P < 0.05$) reduced to below detectable limits (< 1 log CFU·mL^{-1} (CFU: colony forming units)) in skim milk at 5.0 kGy and in milk with 1% fat content after treatment with X-rays. Ultraviolet (UV)-C (200–280 nm wavelength), acidic electrolyzed, and neutral electrolyzed water are frequently applied in microbiological controls, where UV-C illumination is more effective at eliminating the *C. sakazakii* population. The viable count reductions ranged from 2 to 2.4 log CFU·g^{-1} after the application of 7.5 and 10 kJ·m^{-2} doses of UV-C [96]. A combination of near-infrared (NIR) heating and UV radiation treatment for 7 min achieved a 2.79-log-unit CFU reduction of *C. sakazakii* and showed a strong synergistic bacteriocidal effect. This synergistic effect was mainly caused by the disruption of the bacterial cell membrane [97]. Although irradiation is an effective and safe alternative technology to decontaminate *C. sakazakii* in the dairy industry, it may cause losses of vitamins and lipids, which are sensitive to irradiation, leading to a reduction in nutrient content. Differences in the type of food, dose of irradiation, temperature, amount of oxygen, and type of vitamin can generate varying degrees of vitamin loss after irradiation. From the perspective of nutrient maximization and the balance between food safety and health, superior green technology should be exploited and improved.

Ultrasonication is another biofilm-removal method that is efficient in comparison with conventional swabbing methods [98]. Nevertheless, bacteria cannot be completely eliminated using this technology, so a combination of methods is used. Adekunte et al. [99] performed inactivation studies of *C. sakazakii* inoculated in reconstituted infant formula under the synergetic conditions of temperature and ultrasound, and reported that a combination of these conditions could drastically reduce *C. sakazakii* levels. Manosonication (MS) is the use of ultrasonic waves under pressure; the lethal effect of this technique to bacteria depends on the cavitation intensity. The *D*-value of *C. sakazakii* under standard treatments (35 °C, 117 μm, 200 kPa, citrate-phosphate buffer pH 7.0) was 0.41 min, which was higher than that of *Yersinia enterocolitica* (D = 0.19 min) and lower than those of *Salmonella enterica* serovar Enteritidis (D = 0.61 min), *Listeria monocytogenes* (D = 0.86 min), and *Enterococcus faecium* (D = 1.2 min) [100].

Physical processing is undoubtedly one of the primary biofilm-controlling strategies, but it can suffer from incomplete removal and from a lack of access to all stages of food processing. Therefore,

chemical or biological controlling approaches are required to complement or collaborate with the physical treatments.

3.3. Chemical treatment

3.3.1. Sanitizers

An effective and safe antibacterial substance achieves economic effectiveness if the agent is highly cost effective, environmentally friendly, and does not leave residue. Kim et al. [78] compared the survival of *Cronobacter* in suspension and in biofilms dried on the surface of stainless steel after being exposed to 13 disinfectants. These surfaces had been chosen based on their use in hospitals, daycare centers, and food service kitchens. Quaternary ammonium compounds and peroxyacetic acid/hydrogen peroxide-associated disinfectants were found to be more effective against *Cronobacter* biofilms. Their use resulted in viable count reductions of 69%, 73%, and 51% for benzalkonium chloride, peroxyacetic acid, and chlorine dioxide, respectively, at 400 $mg \cdot L^{-1}$ for 15 min. However, these treatments only caused bacteriostatic action for up to 18% of the biofilm biomass, as determined using a crystal violet assay [101]. Although these sanitizers play an important role in food safety, it should be noted that excessive use of sanitizers may give rise to resistant isolates that can exert cross-resistance to clinically important drugs [102]. The threat of antibiotic resistance in bacteria is a global problem that can restrict the effectiveness of clinical treatment.

3.3.2. Natural extracts

Conventional sanitization procedures are not fully effective against biofilms, and may induce the selection of resistant phenotypes. Therefore, there is a need for further development and application of "green" (or eco-friendly) biocides, which are generally considered to be chemical residue-free. Natural compounds extracted from plants are now being evaluated for their potential to eliminate biofilms. These compounds have the ability to penetrate bacterial biofilms, are easily degraded in the environment, and are almost nontoxic.

Trans-cinnamaldehyde (TC), which is extracted from cinnamon bark and is an ingredient in cinnamon oil, is a food-grade substance approved by the US Food and Drug Administration (FDA). The inhibitory effects of TC on biofilms adhered to the surfaces of polystyrene plates, stainless steel coupons, feeding bottle coupons, and enteral feeding tube coupons were investigated by Amalaradjou and Venkitanarayanan [103]. TC inactivated the *C. sakazakii* biofilms on all the tested matrices. *C. sakazakii* was attenuated by > 4.0 and 3.0 log $CFU \cdot mL^{-1}$ after 96 h of exposure to 38 $mmol \cdot L^{-1}$ and 750 $\mu mol \cdot L^{-1}$ TC, respectively. In addition, TC significantly suppressed the expression of the genes responsible for biofilms, as well as the virulence that is critical for motility, host tissue adhesion and invasion, macrophage survival, and lipopolysaccharide synthesis in *C. sakazakii*. Consequently, the adhesion and invasion of intestinal epithelial cells and brain microvascular endothelial cells were significantly ($P \leq 0.05$) inhibited, and *C. sakazakii* survival in human macrophages was reduced after TC treatments [104].

Polyphenols have human health benefits, such as strengthening blood vessel walls, promoting gastrointestinal digestion, reducing blood fat, inhibiting the proliferation of bacteria and cancer cells, and performing antioxidant activity to remove free radicals that are harmful to human health. Recently, muscadine seed extracts have been shown to exert a high level of lethal activity against *Cronobacter*, reducing an initial population (approximately 6 log $CFU \cdot mL^{-1}$) to a undetectable level (minimum detection limit, 10 $CFU \cdot mL^{-1}$) within 1 h. Polar and polyphenol fractions may be the main antimicrobial constituents of muscadine seed extracts [105]. Blueberry juice and blueberry polyphenols also have inhibiting effects against *C. sakazakii*, with *C. sakazakii* strains being reduced to undetectable levels from approximately 8.30 log $CFU \cdot mL^{-1}$, respectively with exposure to blueberry juice (pH 2.8) or blueberry proanthocyanidins for 1 h [106].

3.3.3. Antimicrobial peptides

Antimicrobial peptides (AMPs) are innate immune system molecules that serve as a first line of defense in fighting invading pathogens. These natural proteins can be found extensively in a diverse group of organisms including animals, plants, insects, and bacteria [107]. Natural AMPs are typically relatively short, consisting of 12–100 amino acids. AMPs can be classified into four types based on secondary structure—namely, β-sheet, α-helical, loop, and extended peptide—with the first two classes being the most common in nature [108]. Since bacteria have developed resistance as a result of the overuse of chemical preservatives, traditional food preservation methods are inadequate for food safety. AMPs were recently applied as a natural bio-preservative to enhance the shelf-life of food [109]. Many researchers have pointed out that AMPs demonstrate activity against several food-borne pathogens without altering the food properties and or being nocuous to humans [110]. In the search to address the persistent contamination caused by bacterial biofilms, AMPs have been considered as potential alternative agents to conventional antibiotics. These peptides can combat biofilms at their different stages of formation [111]. Eliminations of bacteria occur through penetration, or by the formation of pores on the surface of cell membranes by AMPs [112]. Research has shown that AMPs can also inhibit the cell wall, nucleic acid, and protein biosynthesis. Human cathelicidin LL-37 disrupted the development of biofilms formed by *Staphylococcus epidermidis*. LL-37 can significantly reduce both the attachment of bacteria to the surface and the biofilm mass at concentrations that do not kill or inhibit the growth of planktonic bacteria, which indicates a direct effect of the peptide on biofilm production [113]. AMPs have also been reported as stimulating twitching motility and influencing the downregulation of genes related to biofilm development [114].

A number of natural, semi-synthetic, and synthetic AMPs have been shown to be active against microbial biofilms. biofilm-active AMPs (BaAMPs) is a database designed to collect data on AMPs specifically tested against microbial biofilms. The AMPs in this database can be used as references for intervening in the biofilm-forming process. Nevertheless, optimization of AMP antibiofilm activity for *Cronobacter* biofilms and the discovery and synthesis of novel peptides are highly desirable. BaAMPs can be freely accessed via the Internet at http://www.baamps.it. There are many methods to design AMPs; however, the safety of synthetic AMPs should be taken into consideration when these peptides are applied in food.

3.4. Biological processes

The multiple interspecies interactions and metabolites produced by microorganisms can interfere with biofilm formation and development. Two of the most frequently used approaches to eradicate bacteria and their derivatives through microbe–microbe interactions are discussed below.

3.4.1. Probiotic products

Kefir (Body Ecology, Inc., USA) is a dairy probiotic product with antimicrobial activity against *C. sakazakii* [115]. Culture supernatants derived from the lactic acid bacteria isolated in kefir—including *Lactobacillus kefiri* (*L. kefiri*) DH5 and *L. kefiranofaciens* DH101—significantly inhibited the proliferation of *C. sakazakii* ATCC 29544. *L. kefiri* DH5 exerted a higher degree of deactivation activity, as it could disrupt the cellular membrane integrity of *C. sakazakii*.

Awaisheh et al. [116] investigated the antimicrobial activity of lactic acid bacteria against different strains of *C. sakazakii*, and discovered that probiotic lactic acid bacteria isolated from healthy infants produced bacteriocins that could suppress *C. sakazakii*. However, these bacteriocins cannot be used in the industry due to their heat lability. The inclusion of prebiotic products in infant formula milk powder will not only contribute to the healthy growth of infants, but also inhibit potential contamination by *C. sakazakii*.

3.4.2. Bacteriophages

The application of phages to control biofilms can be a practicable, natural, harmless, and specific approach to control the diverse organisms involved in biofilm formation [117]. For example, the addition of the QS molecule AI-2 led to prophage induction and promoted the dispersal of bacteria from established *E. faecalis* biofilms [118]. The first phage product for food safety, ListShield (Intralytix, Inc., USA), was approved by the FDA in 2006; thus, phages have been recognized as a safety additive [119]. Since then, an increasing number of phage products have been approved as food biocontrol agents. These advances highlight the potential of phages for controlling foodborne pathogenic bacteria and spoilage organisms. Phage-related control methods have the following advantages over traditional antibiotic therapy:

(1) **High safety:** Phages cannot infect mammalian cells.

(2) **Accessibility:** Phages are ubiquitous in the environment and are therefore relatively easily obtained.

(3) **Strong specificity:** Specific phages that target pathogenic bacteria would not interfere with other normal microflora in the human body or with inherent microflora in the food matrix.

In order to ensure infant formula safety, Nestlé proposed the use of a nontoxic lytic phage to reduce contamination by *Cronobacter* [120]. Endersen et al. [121] showed that a phage cocktail had a relatively broad host range covering 73% of the *C. sakazakii* strains tested. The viability of *C. sakazakii* ($\leq 10^4$ CFU·mL^{-1}) was markedly reduced to below the limit of detection (< 10 CFU·mL^{-1}) by the phage mixture (3×10^8 CFU·mL^{-1}). The phage cocktail also inhibited biofilm formation for more than 48 h after treatment. Phage multiplication occurred at 4–37 °C and a pH range of 6–8. In addition, all the phages were free of lysogenic properties, highlighting the potential therapeutic application of these phages. These results demonstrate the potential application of phages for the biocontrol of *C. sakazakii* contamination in reconstituted infant formula and for use as preventative agents against biofilm formation.

3.5. Potential approaches

3.5.1. Nanotechnology

The development of nanomaterials and corresponding technologies provides a novel opportunity for the development of antimicrobial agents to control microbial biofilms [122]. Nanomaterials such as nanometer silver, titanium dioxide, and copper oxide show good antibacterial activities [123,124]. These nanoparticles have numerous useful properties including stability, tolerance of sterilization, lower toxicity, resistance elimination, and high specific surface area giving more active sites for nanoparticles to interact with bacteria. When used as a coating on equipment, these materials can effectively control bacteria growth during food processing.

Nanotechnology-based delivery systems are also important strategies for enhancing anti-biofilm activity. Bactericide efficacy against biofilms is usually limited due to poor penetration into the biofilm matrix. However, nanotechnology-based drug delivery systems can enable drugs to directly interact with the complex architecture of biofilms, due to their direct interaction between the nanoparticles and membranes of the microorganisms. At present, the main types of nanosystems used to deliver bioactive substances include liposomes (LIPs), microemulsions, nanoemulsions, cyclodextrins, solid lipid nanoparticles, polymeric nanoparticles, and metallic nanoparticles [125]. Robinson et al. [126] compared cationic and anionic LIPs with respect to sterilization by delivering hydrophobic bactericide triclosan (TCS), which is an effective enoyl-acyl-carrier protein (ACP) reductase inhibitor. The anionic liposome exhibited both greater efficacy of delivery to the pure *S. sanguis* C104 biofilms and higher effectiveness of the liposomal TCS. The delivery of TCS by cationic LIPs was only effective in inhibiting the growth of *S. sanguis* C104 biofilms, and was not effective against mixed-flora biofilms. These findings demonstrate the significance of electrostatic interaction for the delivery of TCS, as the use of LIP formulations had a substantial effect on both single bacteria and the mixed species of biofilms.

Nanozymes are defined as nanomaterials with enzyme-like activity; they can catalyze the substrates of natural enzymes following similar reaction kinetics behaviors under physiological conditions [127]. Based on the activities of the oxidase and peroxidase in nanozymes, these materials are able to catalyze the production of a free radical cloud that will damage the integrity of the cell membrane, degrade the nucleic acid, inactivate various proteins, and eventually cause bacterial death. Moreover, artificial enzymes can circumvent the shortcomings of natural enzymes, as the natural catalytic activity of natural enzymes can easily be affected or suppressed by their surroundings, and they can be digested by proteases. Tao et al. [128] constructed a mesoporous silica-supported gold nanoparticles (MSN-AuNPs)-based antibacterial system that possesses dual enzyme activities similar to those of peroxidase and oxidase. The peroxidase-like activity originating from MSN-AuNPs can catalyze hydrogen peroxide (H_2O_2) decomposition into ·OH, and its oxidase-like activity can generate reactive oxygen species (ROS). Due to their prominent enzyme activities, MSN-AuNPs exhibit excellent antibacterial capacity and biofilm elimination against both Gram-negative and Gram-positive bacteria. Ferromagnetic nanoparticles (Fe_3O_4, MNPs) with peroxidase-like activity can efficiently degrade biological molecules such as DNA, proteins, and polysaccharides by catalyzing the degradation of hydrogen peroxide. In addition, Fe_3O_4, MNPs have the capacity to potentiate the efficacy of H_2O_2 in biofilm elimination. Treatment with MNP–H_2O_2 resulted in (18 ± 6.17)% of the biofilm remaining after treatment, while (49 ± 7.37)% of the biofilm remained after treatment with H_2O_2 alone [129]. This difference indicates that MNP–H_2O_2 is more efficient at reducing bacterial viability than H_2O_2.

3.5.2. Signal blocking

QS is one of the mechanisms that has been proposed to govern the development and sustenance of biofilm communities. Therefore, quorum sensing inhibition (QSI) has been developed to interfere with QS-regulated behaviors such as cell adherence and the secretion of extracellular polymers. Hence, QS therapeutics usually disable cells by modulating bacterial communication pathways. The process of QS can be disrupted by different mechanisms: ① inhibiting the synthesis of QS signal molecules, ② reducing the activity of QS signal molecules, ③ degrading QS signal molecules, and ④ designing analogues of signal molecules that will competitively bind to the receptor proteins [130]. Among the methods of QS inhibition, degrading signal molecules and inhibiting the synthesis of AHLs are currently the more promising directions for biofilm control. Singh et al. [131] investigated nine plant extracts (*Piper nigrum, Cinnamomum verum, Coriandrum sativum, Cuminum cyminum, Allium sativum, Myristica fragrans, Zingiber officinale, Syzygium aromaticum*, and *Trigonella foenum graecum*) for their inhibition of QS-mediated biofilm formation by *C. sakazakii* strains. Of the nine plant extracts, *Piper nigrum* and *Cinnamomum verum* at 100 parts per million (ppm) led to a decrease of 78% and 68% in

QS-controlled production, as measured using the bioindicators *Chromobacterium violaceum* 026 and *Agrobacterium tumefaciens* NTL4 (pZLR4), respectively. Accordingly, these two extracts displayed higher inhibitory effects (> 50%) on biofilm formation in *C. sakazakii*, whereas moderate (25%–50%) and minimal (< 25%) suppressed activities were found using the other extracts. This study highlights the use of *Piper nigrum* and *Cinnamomum verum* for their anti-QS potential to inhibit *C. sakazakii* biofilm formation; this study would be the important foundation and basis for novel bioactive molecules. Certainly, further studies could focus on QS disruption by other plant extracts.

4. Suggestions regarding *Cronobacter* biofilms in food processing factories

Cronobacter contamination of infant formula can be due to the production environment, which includes ingredients as well as personnel. Storage tanks, equipment, and operation rooms are generally the most popular locations for microorganisms (Fig. 6). "Dead zones" within the plant, such as cracks, corners, joints, and gaskets, are places where biofilms can remain after cleaning. Apart from equipment that is prone to biofilm residue, incoming aerosol particles via filtration and catchment areas give rise to the retention of bacteria and, thereafter, the formation of biofilms. In view of the diverse practical application and operation of appliances (machine size, texture, function, etc.), it might not be possible to eliminate bacteria in food-production facilities using one method; therefore, combinations of techniques are recommended. The emergence of surface modifications of materials that incorporate technologies that target adherence is a promising approach to prevent microbial adhesion and subsequent biofilm formation. These strategies may show further potential when the ecological complexity of biofilms in food environments is addressed. This—together with an improved knowledge of the mechanisms involved in biofilm formation—plays a crucial part towards the goal of achieving a novel, highly practical, economic, and environmentally friendly tactic to ensure food safety.

5. Conclusion and perspective

High-throughput DNA sequencing technologies greatly facilitate a comprehensive understanding of the molecular mechanism of biofilm formation. Biofilm formation can be considerably reduced by the application of various approaches. To be specific, modifications of contact materials to interfere with the stages of biofilm formation result in both prevention and control of biofilm. The most pursued and successful strategy, which combines antibacterial substances and materials, has been surface coating—a strategy that directly manages the contamination sites for biofilms. In addition, QS signal-blocking compounds are promising future approaches, as an impaired QS system would further influence cell adsorption, matrix secretion, and prevention of its infection and pathogenicity.

Our understanding of biofilms has progressed considerably since this term was first formally defined in the mid-1980s. However, we are only now beginning to understand the onset of *Cronobacter* biofilm formation, and many questions concerning the molecular mechanisms and functions of *Cronobacter* biofilms remain to be revealed in future studies using a combination of biochemical, cellular, molecular, and genetic approaches. The following questions related to biofilms in *C. sakazakii* might be reserved for further consideration and clarification.

(1) What is the unique mechanism of fimbriae synthesis in *C. sakazakii*? And how do fimbriae affect biofilms?

(2) What is the mechanism of extraordinary resistance to the desiccation of *C. sakazakii* biofilm?

(3) Does the drying process change the composition of *C. sakazakii* biofilm so that it has a strong dry-resistance ability that other bacteria do not achieve?

(4) Research indicates that *C. sakazakii* pathovar with K2:CA2 capsule is strongly associated with life-threatening neonatal meningitis. Is this capsule composition enabling the organism to persist in powdered infant formula, and in the stomach?

(5) How does *Cronobacter* retain communication with other species within the biofilm? Can it be postulated that there are shared quorum sensing mechanisms across different bacterial species?

Acknowledgements

We gratefully acknowledge the financial support of National Key Research and Development Program of China (2017YFC1601200) the Science and Technology Planning Project of Guangdong Province (2017A070702018) the Science and Technology Planning Project of Guangzhou (201604020003) and Guangdong Academy of Sciences Special Project of Science and Technology Development (2017GDASCX-0201).

Compliance with ethics guidelines

Na Ling, Stephen Forsythe, Qingping Wu, Yu Ding, Jumei Zhang, and Haiyan Zeng declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Iversen C, Lehner A, Mullane N, Bidlas E, Cleenwerck I, Marugg J, et al. The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1. BMC Evol Biol 2007;7(1):64.

[2] International Commission on Microbiological Specifications for Food. Microbiological testing in food safety management. Report. New York: Kluwer Academic/Plenum; 2002.

[3] Arseni A, Malamou-Ladas E, Koutsia C, Xanthou M, Trikka E. Outbreak of colonization of neonates with *Enterobacter sakazakii*. J Hosp Infect 1987;9(2):143–50.

[4] Centers for Disease Control Prevention. *Cronobacter* species isolation in two infants—New Mexico, 2008. MMWR Morb Mortal Wkly Rep 2009;58:1179–83.

[5] Lai KK. *Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature. Medicine 2001;80(2):113–22.

[6] Ries M, Harms D, Scharf J. Multiple cerebral infarcts with resulting multicystic encephalomalacia in a premature infant with *Enterobacter sakazakii* meningitis. Klin Padiatr 1994;206(3):184–6.

[7] Van Acker J, De Smet F, Muyldermans G, Bougatef A, Naessens A, Lauwers S. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J Clin Microbiol 2001;39(1):293–7.

[8] Yong W, Guo B, Shi X, Cheng T, Chen M, Jiang X, et al. An investigation of an acute gastroenteritis outbreak: *Cronobacter sakazakii*, a potential cause of food-borne illness. Front Microbiol 2018;9:2549.

[9] Patrick ME, Mahon BE, Greene SA, Rounds J, Cronquist A, Wymore K, et al. Incidence of *Cronobacter* spp. infections, United States, 2003–2009. Emerg Infect Dis 2014;20(9):1520–3.

[10] Belal M, Al-Mariri A, Hallab L, Hamad I. Detection of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) from medicinal plants and spices in Syria. J Infect Dev Ctries 2013;7(2):82–9.

[11] Fei P, Jiang Y, Gong S, Li R, Jiang Y, Yuan X, et al. Genotyping occurrence and antibiotic susceptibility of *Cronobacter* spp. in drinking water and food samples from Northeast, China. J Food Prot 2018;81(3):456–60.

[12] Forsythe SJ. *Enterobacter sakazakii* and other bacteria in powdered infant milk formula. Matern Child Nutr 2005;1(1):44–50.

[13] Ling N, Li C, Zhang J, Wu Q, Zeng H, He W, et al. Prevalence and molecular and antimicrobial characteristics of *Cronobacter* spp. isolated from raw vegetables in China. Front Microbiol 2018;9:1149.

[14] Ueda S. Occurrence of *Cronobacter* spp. in dried foods, fresh vegetables and soil. Biocontrol Sci 2017;22(1):55–9.

[15] Vasconcellos L, Carvalho CT, Tavares RO, De Mello Medeiros V, De Oliveira Rosas C, Silva JN, et al. Isolation, molecular and phenotypic characterization of *Cronobacter* spp. in ready-to-eat salads and foods from Japanese cuisine commercialized in Brazil. Food Res Int 2018;107:353–9.

[16] Xu X, Wu Q, Zhang J, Ye Y, Yang X, Dong X. Occurrence and characterization of *Cronobacter* spp. in powdered formula from Chinese retail markets. Foodborne Pathog Dis 2014;11(4):307–12.

[17] Ye Y, Li H, Wu Q, Chen M, Lu Y, Yan C. Isolation and phenotypic characterization of *Cronobacter* from dried edible macrofungi samples. J Food Sci 2014;79(7):M1382–6.

[18] Hamilton JV, Lehane MJ, Braig HR. Isolation of *Enterobacter sakazakii* from midgut of *Stomoxys calcitrans*. Emerg Infect Dis 2003;9(10):1355–6.

[19] Pava-Ripoll M, Pearson RE, Miller AK, Ziobro GC. Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and *Listeria monocytogenes* associated with the body surfaces and guts of individual filth flies. Appl Environ Microbiol 2012;78(22):7891–902.

[20] Iversen C, Forsythe S. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends Food Sci Technol 2003;14(11):443–54.

[21] Kandhai MC, Reij MW, Gorris LG, Guillaume-Gentil O, Van Schothorst M. Occurrence of *Enterobacter sakazakii* in food production environments and households. Lancet 2004;363(9402):39–40.

[22] Killer J, Skřivanová E, Hochel I, Marounek M. Multilocus sequence typing of *Cronobacter* strains isolated from retail foods and environmental samples. Foodborne Pathog Dis 2015;12(6):514–21.

[23] Arts M. *Enterobacter sakazakii* in factories and households. Lancet 2004;364 (9432):414–5.

[24] Fang R, Wang Q, Yang B, Zhang J, Cao B, Geng W, et al. Prevalence and subtyping of *Cronobacter* species in goat milk powder factories in Shaanxi Province, China. J Dairy Sci 2015;98(11):7552–9.

[25] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2 (2):95–108.

[26] Prosser JI, Bohannan BJM, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, et al. The role of ecological theory in microbial ecology. Nat Rev Microbiol 2007;5 (5):384–92.

[27] Boles BR, Thoendel M, Singh PK. Self-generated diversity produces "insurance effects" in biofilm communities. Proc Natl Acad Sci USA 2004;101(47):16630–5.

[28] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010;8 (9):623–33.

[29] Iversen C, Lane M, Forsythe SJ. The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. Lett Appl Microbiol 2004;38(5):378–82.

[30] 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 Prot 2005;68(11):2287–94.

[31] Beuchat LR, Kim H, Gurtler JB, Lin LC, Ryu JH, Richards GM. *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. Int J Food Microbiol 2009;136(2):204–13.

[32] Hurrell E, Kucerova E, Loughlin M, Caubilla-Barron J, Forsythe SJ. Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii, Salmonella* serovars and other *Enterobacteriaceae*. Int J Food Microbiol 2009;136 (2):227–31.

[33] Kragh KN, Hutchison JB, Melaugh G, Rodesney C, Roberts AE, Irie Y, et al. Role of multicellular aggregates in biofilm formation. MBio 2016;7(2): e00237–316.

[34] Ling N, Zhang J, Li C, Zeng H, He W, Ye Y, et al. The glutaredoxin gene, *grxB*, affects acid tolerance, surface hydrophobicity, auto-aggregation, and biofilm formation in *Cronobacter sakazakii*. Front Microbiol 2018;9:133.

[35] Jung JH, Choi NY, Lee SY. Biofilm formation and exopolysaccharide (EPS) production by *Cronobacter sakazakii* depending on environmental conditions. Food Microbiol 2013;34(1):70–80.

[36] Ye Y, Ling N, Jiao R, Wu Q, Han Y, Gao J. Effects of culture conditions on the biofilm formation of *Cronobacter sakazakii* strains and distribution of genes involved in biofilm formation. Lebensm Wiss Technol 2015;62(1):1–6.

[37] Dancer GI, Mah JH, Kang DH. Influences of milk components on biofilm formation of *Cronobacter* spp. (*Enterobacter sakazakii*). Lett Appl Microbiol 2009;48(6):718–25.

[38] Ye Y, Ling N, Jiao R, Wu Q, Han Y, Gao J. Effects of Ca^{2+} and Mg^{2+} on the biofilm formation of *Cronobacter sakazakii* strains from powdered infant formula. J Food Saf 2015;35(3):416–21.

[39] Soni KA, Balasubramanian AK, Beskok A, Pillai SD. Zeta potential of selected bacteria in drinking water when dead, starved, or exposed to minimal and rich culture media. Curr Microbiol 2008;56(1):93–7.

[40] Pringle JH, Fletcher M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. Appl Environ Microbiol 1983;45 (3):811–7.

[41] Thomas WE, Nilsson LM, Forero M, Sokurenko EV, Vogel V. Shear-dependent, "stick-and-roll" adhesion of type 1 fimbriated *Escherichia coli*. Mol Microbiol 2004;53(5):1545–57.

[42] Davidson CAB, Lowe CR. Optimisation of polymeric surface pre-treatment to prevent bacterial biofilm formation for use in microfluidics. J Mol Recognit 2004;17(3):180–5.

[43] Heistad A, Scott T, Skaarer AM, Seidu R, Hanssen JF, Stenström TA, et al. Virus removal by unsaturated wastewater filtration: effects of biofilm accumulation and hydrophobicity. Water Sci Technol 2009;60(2):399–407.

[44] Nadell CD, Xavier JB, Levin SA, Foster KR. The evolution of quorum sensing in bacterial biofilms. PLoS Biol 2008;6(1):e14.

[45] Teschler JK, Zamorano-Sánchez D, Utada AS, Warner CJ, Wong GC, Linington RG, et al. Living in the matrix: assembly and control of *Vibrio cholerae* biofilms. Nat Rev Microbiol 2015;13(5):255–68.

[46] Jayaraman A, Wood TK. Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. Annu Rev Biomed Eng 2008;10 (1):145–67.

[47] Fuqua C, Winans SC, Greenberg EP. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu Rev Microbiol 1996;50(1):727–51.

[48] Dickschat JS. Quorum sensing and bacterial biofilms. Nat Prod Rep 2010;27 (3):343–69.

[49] Da Silva Araújo FD, Esper LMR, Kuaye AY, Sircili MP. Marsaioli AJ. *N*-acyl-homoserine lactones from *Enterobacter sakazakii (Cronobacter* spp.) and their degradation by *Bacillus cereus* enzymes. J Agric Food Chem 2012;60 (2):585–92.

[50] Singh N, Patil A, Prabhune A, Raghav M, Goel G. Diverse profiles of *N*-acyl-homoserine lactones in biofilm forming strains of *Cronobacter sakazakii*. Virulence 2017;8(3):275–81.

[51] Du XJ, Wang F, Lu X, Rasco BA, Wang S. Biochemical and genetic characteristics of *Cronobacter sakazakii* biofilm formation. Res Microbiol 2012;163(6–7):448–56.

[52] Yang JL, He YP, Jiang J, Chen WY, Gao Q, Pan LW, et al. Comparative proteomic analysis by iTRAQ-2DLC-MS/MS provides insight into the key proteins involved in *Cronobacter* spp. biofilm formation. Food Control 2016;63:93–100.

[53] Ye YW, Jiao R, Gao JN, Li H, Ling N, Wu QP, et al. Proteins involved in responses to biofilm and planktonic modes in *Cronobacter sakazakii*. Lebensm Wiss Technol 2016;65:1093–9.

[54] Ogrodzki P, Forsythe S. Capsular profiling of the *Cronobacter* genus and the association of specific *Cronobacter sakazakii* and *C. malonaticus* capsule types with neonatal meningitis and necrotizing enterocolitis. BMC Genomics 2015;16(1):758.

[55] Hu L, Grim CJ, Franco AA, Jarvis KG, Sathyamoorthy V, Kothary MH, et al. Analysis of the cellulose synthase operon genes, *bcsA, bcsB*, and *bcsC* in *Cronobacter* species: prevalence among species and their roles in biofilm formation and cell–cell aggregation. Food Microbiol 2015;52:97–105.

[56] Gao JX, Li P, Du XJ, Han ZH, Xue R, Liang B, et al. A negative regulator of cellulose biosynthesis, *bcsR*, affects biofilm formation, and adhesion/invasion ability of *Cronobacter sakazakii*. Front Microbiol 2017;8:1839.

[57] Bullitt E, Makowski L. Structural polymorphism of bacterial adhesion pili. Nature 1995;373(6510):164–7.

[58] Joseph S, Desai P, Ji Y, Cummings CA, Shih R, Degoricija L, et al. Comparative analysis of genome sequences covering the seven cronobacter species. PLoS ONE 2012;7(11):e49455.

[59] DeBenedictis EP, Liu J, Keten S. Adhesion mechanisms of curli subunit CsgA to abiotic surfaces. Sci Adv 2016;2(11):e1600998.

[60] Hartmann I, Carranza P, Lehner A, Stephan R, Eberl L, Riedel K. Genes involved in *Cronobacter sakazakii* biofilm formation. Appl Environ Microbiol 2010;76 (7):2251–61.

[61] Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, et al. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science 2002;295(5556):851–5.

[62] Hammar M, Arnqvist A, Bian Z, Olsén A, Normark S. Expression of two *csg* operons is required for production of fibronectin- and Congo red-binding curli polymers in *Escherichia coli* K-12. Mol Microbiol 1995;18 (4):661–70.

[63] Hu L. Prevalence of curli genes among *Cronobacter* species and their roles in biofilm formation and cell–cell aggregation. Int J Food Microbiol 2018; 265:65–73.

[64] Lemon KP, Higgins DE, Kolter R. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. J Bacteriol 2007;189(12):4418–24.

[65] Zhao X, Norris SJ, Liu J. Molecular architecture of the bacterial flagellar motor in cells. Biochemistry 2014;53(27):4323–33.

[66] Macnab RM. How bacteria assemble flagella. Annu Rev Microbiol 2003;57 (1):77–100.

[67] Hoeflinger JL, Miller MJ. *Cronobacter sakazakii* ATCC 29544 autoaggregation requires FliC flagellation, not motility. Front Microbiol 2017;8:301.

[68] Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A, Abu-Ali G, Morgan XC, et al. Sequencing and beyond: integrating molecular "omics" for microbial community profiling. Nat Rev Microbiol 2015;13(6):360–72.

[69] Papenfort K, Förstner KU, Cong JP, Sharma CM, Bassler BL. Differential RNA-seq of *Vibrio cholerae* identifies the VqmR small RNA as a regulator of biofilm formation. Proc Natl Acad Sci USA 2015;112(7):E766–75.

[70] Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 2016;17(7):451–9.

[71] Fei P, Man C, Lou B, Forsythe SJ, Chai Y, Li R, et al. Genotyping and source tracking of the *Cronobacter sakazakii* and *C. malonaticus* isolated from powdered infant formula and an infant formula production factory in China. Appl Environ Micob 2015;81(16):5430–9.

[72] Jacobs C, Braun P, Hammer P. Reservoir and routes of transmission of *Enterobacter sakazakii (Cronobacter* spp.) in a milk powder-producing plant. J Dairy Sci 2011;94(8):3801–10.

[73] Reich F, König R. Von Wiese W, Klein G. Prevalence of *Cronobacter* spp. in a powdered infant formula processing environment. Int J Food Microbiol 2010;140(2–3):214–7.

[74] FAO. Code of hygienic practice for powdered formulae for infants and young children. Report. Geneva: Food and Agriculture Organization of the United Nation; 2008. Report No.: CAC/RCP 66-2008.

[75] Cordier JL. Production of powdered infant formulae and microbiological control measures. In: Farber JM, Forsythe SJ, editors. Emerging issues in food safety: *Enterobacter sakazakii*. Washington, DC: ASM Press; 2007.

[76] World Health Organization, Food and Agriculture Organization of the United Nations. *Enterobacter sakazakii* and other microorganisms in powdered infant formula. Geneva: World Health Organization; 2004.

[77] Arku B, Fanning S, Jordan K. Heat adaptation and survival of *Cronobacter* spp. (formerly *Enterobacter sakazakii*). Foodborne Pathog Dis 2011;8(9):975–81.

[78] Kim H, Ryu JH, Beuchat LR. Effectiveness of disinfectants in killing *Enterobacter sakazakii* in suspension, dried on the surface of stainless steel, and in a biofilm. Appl Environ Microbiol 2007;73(4):1256–65.

[79] Thomas A, Sathian CT. Cleaning-in-place (CIP) system in dairy plant—review. J Environ Sci Toxicol Food Technol 2014;8(6):41–4.

[80] Bremer PJ, Fillery S, McQuillan AJ. Laboratory scale clean-in-place (CIP) studies on the effectiveness of different caustic and acid wash steps on the removal of dairy biofilms. Int J Food Microbiol 2006;106(3):254–62.

[81] Boulange-Petermann L, Jullien C, Dubois PE, Benezech T, Faille C. Influence of surface chemistry on the hygienic status of industrial stainless steel. Biofouling 2004;20(1):25–33.

[82] Lelievre C, Faille C, Benezech T. Removal kinetics of *Bacillus cereus*, spores from stainless steel pipes under CIP procedure: influence of soiling and cleaning conditions. J Food Process Eng 2001;24(6):359–79.

[83] Lelièvre C, Legentilhomme P, Gaucher C, Legrand J, Faille C, Bénézech T. Cleaning in place: effect of local wall shear stress variation on bacterial removal from stainless steel equipment. Chem Eng Sci 2002;57(8):1287–97.

[84] Kumari S, Sarkar PK. *In vitro* model study for biofilm formation by *Bacillus cereus* in dairy chilling tanks and optimization of clean-in-place (CIP) regimes using response surface methodology. Food Control 2014;36(1):153–8.

[85] Elguindi J, Moffitt S, Hasman H, Andrade C, Raghavan S, Rensing C. Metallic copper corrosion rates, moisture content, and growth medium influence survival of copper ion-resistant bacteria. Appl Microbiol Biotechnol 2011;89(6):1963–70.

[86] Grass G, Rensing C, Solioz M. Metallic copper as an antimicrobial surface. Appl Environ Microbiol 2011;77(5):1541–7.

[87] Elguindi J, Alwathnani HA, Rensing C. Rapid inactivation of *Cronobacter sakazakii* on copper alloys following periods of desiccation stress. World J Microbiol Biotechnol 2012;28(4):1837–41.

[88] Edelson-Mammel SG, Porteous MK, Buchanan RL. Survival of *Enterobacter sakazakii* in a dehydrated powdered infant formula. J Food Prot 2005;68(9):1900–2.

[89] Breeuwer P, Lardeau A, Peterz M, Joosten HM. Desiccation and heat tolerance of *Enterobacter sakazakii*. J Appl Microbiol 2003;95(5):967–73.

[90] Nazarowec-White M, Farber JM. Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. Lett Appl Microbiol 1997;24(1):9–13.

[91] Iversen C, Druggan P, Forsythe S. A selective differential medium for *Enterobacter sakazakii*, a preliminary study. Int J Food Microbiol 2004;96(2):133–9.

[92] Awadallah MAI, Ahmed HA, Merwad AMA, Abou Elez RMM, Saleh KMA. Molecular characterization of *Cronobacter sakazakii* in Egypt, survival and thermoresistance at different temperatures: a potential public health risk. Vector Borne Zoonotic Dis 2018;18(2):101–7.

[93] Ueda S. The effects of temperature on the growth and heat resistance of *Cronobacter* spp. Biocontrol Sci 2017;22(2):125–9.

[94] Arku B, Mullane N, Fox EM, Fanning S, Jordan KN. *Enterobacter sakazakii* survives spray drying. Int J Dairy Sci 2008;61(1):102–8.

[95] Mahmoud BS. Inactivation effect of X-ray treatments on *Cronobacter* species (*Enterobacter sakazakii*) in tryptic soy broth, skim milk, low-fat milk and whole-fat milk. Lett Appl Microbiol 2009;49(5):562–7.

[96] Santo D, Graça A, Nunes C, Quintas C. Survival and growth of *Cronobacter sakazakii* on fresh-cut fruit and the effect of UV-C illumination and electrolyzed water in the reduction of its population. Int J Food Microbiol 2016;231:10–5.

[97] Ha JW, Kang DH. Synergistic bactericidal effect of simultaneous near-infrared radiant heating and UV radiation against *Cronobacter sakazakii* in powdered infant formula. Appl Environ Microbiol 2014;80(6):1858–63.

[98] Oulahal-Lagsir N, Martial-Gros A, Bonneau M, Blum LJ. Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment—validation and application to a dairy factory. J Appl Microbiol 2000;89(3):433–41.

[99] Adekunte A, Valdramidis VP, Tiwari BK, Slone N, Cullen PJ, Donnell CP, et al. Resistance of *Cronobacter sakazakii* in reconstituted powdered infant formula during ultrasound at controlled temperatures: a quantitative approach on microbial responses. Int J Food Microbiol 2010;142(1–2):53–9.

[100] Arroyo C, Cebrián G, Pagán R, Condón S. Inactivation of *Cronobacter sakazakii* by ultrasonic waves under pressure in buffer and foods. Int J Food Microbiol 2011;144(3):446–54.

[101] Torlak E, Gokmen M, Aydemir S. Efficacy of disinfectants against *Cronobacter* biofilm on plastic surfaces. Qual Assur Saf Crop 2015;7(5):819–23.

[102] Condell O, Iversen C, Cooney S, Power KA, Walsh C, Burgess C, et al. Efficacy of biocides used in the modern food industry to control *salmonella enterica*, and links between biocide tolerance and resistance to clinically relevant antimicrobial compounds. Appl Environ Microbiol 2012;78(9):3087–3097.

[103] Amalaradjou MAR, Venkitanarayanan K. Effect of trans-cinnamaldehyde on inhibition and inactivation of *Cronobacter sakazakii* biofilm on abiotic surfaces. J Food Prot 2011;74(2):200–8.

[104] Amalaradjou MA, Kim KS, Venkitanarayanan K. Sub-inhibitory concentrations of trans-cinnamaldehyde attenuate virulence in *Cronobacter sakazakii in vitro*. Int J Mol Sci 2014;15(5):8639–55.

[105] Kim TJ, Silva JL, Weng WL, Chen WW, Corbitt M, Jung YS, et al. Inactivation of *Enterobacter sakazakii* by water-soluble muscadine seed extracts. Int J Food Microbiol 2009;129(3):295–9.

[106] Joshi SS, Howell AB, D'Souza DH. *Cronobacter sakazakii* reduction by blueberry proanthocyanidins. Food Microbiol 2014;39:127–31.

[107] Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006;24(12):1551–7.

[108] Melo MN, Ferre R, Castanho MARB. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. Nat Rev Microbiol 2009;7(3):245–50.

[109] Rai M, Pandit R, Gaikwad S, Kövics G. Antimicrobial peptides as natural bio-preservative to enhance the shelf-life of food. J Food Sci Technol 2016;53(9):3381–94.

[110] Upendra RS, Khandelwal P, Jana K, Ajay Kumar N, Gayathri Devi M, Stephaney ML. Bacteriocin production from indigenous strains of lactic acid bacteria isolated from selected fermented food sources. Int J Pharma Res Health Sci 2016;4(1):982–90.

[111] Jorge P, Lourenço A, Pereira MO. New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 2012;28(10):1033–61.

[112] Di Luca M, Maccari G, Nifosì R. Treatment of microbial biofilms in the post-antibiotic era: prophylactic and therapeutic use of antimicrobial peptides and their design by bioinformatics tools. Pathog Dis 2014;70(3):257–70.

[113] Hell Ã, Giske CG, Nelson A, Römling U, Marchini G. Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of *Staphylococcus epidermidis*. Lett Appl Microbiol 2010;50(2):211–5.

[114] Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 2008;76(9):4176–82.

[115] Kim DH, Jeong D, Song KY, Kang IB, Kim H, Seo KH. Culture supernatant produced by *Lactobacillus kefiri* from kefir inhibits the growth of *Cronobacter sakazakii*. J Dairy Res 2018;85(1):98–103.

[116] Awaisheh SS, Al-Nabulsi AA, Osaili TM, Ibrahim S, Holley R. Inhibition of *Cronobacter sakazakii* by heat labile bacteriocins produced by probiotic LAB isolated from healthy infants. J Food Sci 2013;78(9):M1416–20.

[117] Kudva IT, Jelacic S, Tarr PI, Youderian P, Hovde CJ. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl Environ Microbiol 1999;65(9):3767–73.

[118] Rossmann FS, Racek T, Wobser D, Puchalka J, Rabener EM, Reiger M, et al. Phage-mediated dispersal of biofilm and distribution of bacterial virulence genes is induced by quorum sensing. PLoS Pathog 2015;11(2):e1004653.

[119] Bren L. Bacteria-eating virus approved as food additive. FDA Consum 2007;41(1):20–2.

[120] Breeuwer P, Boissindelaporte C, Joosten H, Lardeau A. Isolated phages and their use as disinfectant in food or for sanitation of factory environment. Switzerland patent WO2005049813A1P. 2003 Nov 19.

[121] Endersen L, Buttimer C, Nevin E, Coffey A, Neve H, Oliveira H, et al. Investigating the biocontrol and anti-biofilm potential of a three phage cocktail against *Cronobacter sakazakii* in different brands of infant formula. Int J Food Microbiol 2017;253:1–11.

[122] Rizzello L, Cingolani R, Pompa PP. Nanotechnology tools for antibacterial materials. Nanomedicine 2013;8(5):807–21.

[123] Besinis A, Hadi SD, Le HR, Tredwin C, Handy RD. Antibacterial activity and biofilm inhibition by surface modified titanium alloy medical implants following application of silver, titanium dioxide and hydroxyapatite nanocoatings. Nanotoxicology 2017;11(3):327–38.

[124] Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Rev 2013;65(13–14):1803–15.

[125] Dos Santos Ramos MA, Da Silva PB, Spósito L, De Toledo LG, Bonifácio BV, Rodero CF, et al. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. Int J Nanomed 2018;13:1179–213.

[126] Robinson AM, Bannister M, Creeth JE, Jones MN. The interaction of phospholipid liposomes with mixed bacterial biofilms and their use in the delivery of bactericide. Colloid Surface A 2001;186(1–2):43–53.

[127] Wei H, Wang E. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chem Soc Rev 2013;42(14):6060–93.
[128] Tao Y, Ju E, Ren J, Qu X. Bifunctionalized mesoporous silica-supported gold nanoparticles: intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. Adv Mater 2015;27(6):1097–104.
[129] Gao L, Giglio KM, Nelson JL, Sondermann H, Travis AJ. Ferromagnetic nanoparticles with peroxidase-like activity enhance the cleavage of biological macromolecules for biofilm elimination. Nanoscale 2014;6(5):2588–93.
[130] Amara N, Krom BP, Kaufmann GF, Meijler MM. Macromolecular inhibition of quorum sensing: enzymes, antibodies, and beyond. Chem Rev 2011;111(1):195–208.
[131] Singh N, Patil A, Prabhune A, Goel G. Inhibition of quorum-sensing-mediated biofilm formation in *Cronobacter sakazakii* strains. Microbiology 2016;162(9):1708–14.