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Review Article

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Antiquorum Sensing and Antibiofilm Activities of Natural Products Against *Bacillus cereus*.

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Abstract

Bacillus cereus (*B. cereus*) is a Gram-positive, spore-forming, and facultative anaerobic bacterium that is widely distributed in the environment. Commonly, *B. cereus* is a soil occupant and is generally isolated from food and food products. It is a human pathogen that causes two variant types of gastrointestinal diseases: diarrheal and emetic. Diseases caused by multidrug-resistant (MDR) bacteria are difficult to be treated. In addition, group of *B. cereus* has several virulence factors, which play their roles in pathogenesis, infectivity and its capability to form biofilms. *B. cereus* biofilm is grown on medical devices either abiotic or biotic surfaces. This biofilm avoids the bacteria from the effect of antibiotics and host immune system leading to chronic infections, persistence and mortalities. Thus, it is necessary to explore new antiquorum and antibiofilm agents better than the conventional therapy to eliminate the biofilm that reflect on controlling *B. cereus* infections. The present review will discuss *B. cereus* virulence attributes, antibiotic resistance profiles, and their ability to produce biofilm as well as its molecular regulation. The application of the antiquorum and antibiofilm approaches for infection control will be illustrated as well. Finally, we will spot the light on their consequence in food industry loses and human health risk.

KEYWORDS
B. cereus, Antibiotic resistance, Biofilm, Antibiofilm agents

INTRODUCTION

Bacillus cereus (*B. cereus*) is a Gram-positive, rod shape, motile, spore-forming bacterium that related to the genus *Bacillus* (Montville and Matthews, 2005). Under unfavorable environmental conditions, the microorganism form oval endospores that can persist for long periods even under extreme conditions (Delbrassinne *et al*., 2012).

Many *B. cereus* strains have been documented as the pathological agent of two forms of food poisoning: the emetic form (Ehling-Schulz *et al*., 2004) and the diarrheal form, and both are infrequently fatal (Dierick *et al*., 2005). Bacterial food-borne diseases are becoming a growing public health concern for the whole world, especially for the developing countries. Every year, 220 million children contract diarrheal diseases and 96000 die. An estimated 600 million, almost one person every ten people in the world affects after consumption unhygienic food and 420000 die every year (WHO, 2019). Foodborne infections or food poisoning can be occurred by bacterial biofilms formed in food matrix or equipment (Adame-Gómez *et al*., 2020).

Biofilm formation by *B. cereus* has also recently been investigated, since biofilms produced by this bacterium are considered a potential health hazard in the food industry (Lindsay *et al*., 2000). The talent of *B. cereus* to form biofilms on different substrata has an important concern in the food industry. Bacterial biofilm causes many economic losses and, raises the safety concerns through contamination of the food products (Vilain *et al*., 2006).

Bacillus cereus are able to develop multi-drug resistance (MDR) and to form biofilm that increases the difficulty of infections treatment and highlights the challenge for using new antiquorum and anti-biofilm approaches (Sadekuzzaman *et al*., 2015). Therefore, this review offers an overview of the virulence attributes of MDR *B. cereus* strains, quorum sensing regulators, its capacity for biofilm formation and alternatives approaches to diminish the biofilm development.

Features of *B. cereus* group

Belonging to the Family Bacillaceae, the genus *Bacillus* is a widely diverse group of strictly aerobic or facultative anaerobic rod-shape bacteria which sporulate under certain environmental conditions (Higgins and Dworkin, 2012). Bacillus sensu lato (*B. cereus* group) is currently composed of 13 species: *Bacillus cereus* (sensu stricto), *B. thuringiensis*, an entomopathogenic bacteria used as a bio pesticide; *B. anthracis*, the pathological agent of *anthrax*; *B. weihenstephanensis,* a phsycrotolerant species; *B. toyonensis*, a probiotic species used in animal ration including birds, mammals and fishes, that stabilizes intestinal microbiota and improves nutrient digestion; *B. mycoides*, with some strains providing a wide protection to plants against phytopathogens; *B. pseudomycoides*, isolated from soil; *B. cytotoxicus*, a thermo-

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tolerant bacteria rarely associated with food poisoning; *B. manliponensis* and *B. gaemokensis*, isolated from flat sediment of foreshore tidal of the Yellow Sea ; *B. bombisepticus*, pathogen of the main sink worm *Bombyx mori*, producing black chest septicemia; *B. bingmayonensis*, isolated from the pit soil of Emperor Qin's Terra-cotta warriors in China and *B. wiedmanii*, which is psychrotolerant and cytotoxic (Miller *et al*., 2016).

Virulence factors of *B. cereus* Group.

Two toxins are produced by *B. cereus*; diarrheal and emetic types causing two forms of illness (Ehling-Schulz *et al*., 2006). Enterotoxins formed by *B. cereus* lead to the diarrheal form and usually follow the ingestion of spore infected food, local bacterial growth and released toxin in the host intestines (Berthold-Pluta *et al*., 2015). It may be released in an average temperature of 10-43°C, with an optimal of 32°C. Toxin production occurs at pH 5.5 to 10, with an optimal 8 pH. The diarrheal form is stable at pH 4-11 and inactivated by heating to 56°C for 5 minutes (Jenson and Moir, 2003). Enterotoxins (Hbl, Nhe, and CytK) are tissue-destructive proteins that break the epithelial cells of mucous membrane of small intestine resulting in diarrhea (Senesi and Ghelardi, 2010). In addition to enterotoxins, *B. cereus* can secrete many other toxins (hemolysins HlyI and HlyII) and enzymes (phospholipases and proteases), which are controlled and directed to the bacterial surface, by the PlcR transcriptional activator (Gohar *et al*., 2008).

Cereulide toxin (emetic type) produced by *B. cereus* causes the vomiting form after ingestion of food containing toxin. The toxin remains stable for 80 minutes at 121°C and 60 minutes at 150°C (pH 9.5), thus it can resist cooking temperatures (Rajkovic, 2014).

Bacillus cereus strains commonly differ in their growing and surviving properties due to their genetic polymorphism. Both beneficial (Cutting, 2011) and pathogenic strains (Ehling-Schulz *et al*., 2015) are present. These strains were classified into mesophilic or psychrotrophic. Mesophilic types can grow at 37°C, psychrotrophic one grows at cold temperatures, below 10°C; but poorly grow at 37°C. *B. cereus* strain that produce emetic toxin is mesophilic in nature (Wijnands *et al*., 2006). The highest salt percentage for growth of *B. cereus* is 7.5% (Rajkowski and Bennett, 2003). *B. cereus* is well grown in presence of oxygen, but also can grow anaerobically. Aerobically grown *B. cereus* cells are poorly resistant to acid and heat than anaerobically or microaerobically grown *B. cereus* cells (Mols *et al*., 2009). Mesophilic *B. cereus* strains has a higher resistance to acid than psychrotrophic pathogens (Wijnands *et al*., 2006). Spores have a better tolerance to dry heat than moist heat. Spores are also more withstand to rays than vegetative cells (Jenson and Moir, 2003).

The most public health problem elicited by *B. cereus* is food poisoning; its virulence depends on the strain, pathogen doses and host variables, which explain why the physical incidence of *B. cereus* is not determinant to pathogenesis (Kamar *et al*., 2013). Pathogenesis is indefinable varying between 2 and 22% of total gastroenteritis cases reported, with a few data restricted only to a small group in developed countries (Dodd *et al*., 2017). However, its incidence is considered underestimated due to accumulative reasons: i) *B. cereus* generally produces low-mild symptoms, cases that do not need medical assistance and remain unnoticed in statistics; ii) the symptoms may be caused by thermostable toxins produced during food storage by bacteria which die in the digestive tract, making difficult to define the causative agent; iii) the existence of this bacteria in tests has been usually considered a contamination of samples; and iv) the 45-60% of gastrointestinal infections in hospitals are undefined (Glasset *et al*., 2016). Rice dishes have been usually related with *B. cereus* food poisoning, however, cases of intoxications has been also reported and originated from milk, meat, vegetables, potatoes, pasta, soups, spices and dehydrated meals. Attending to the symptoms, *B. cereus* food poisoning can be classified into emetic or diarrheic conditions, depending on the toxins involved (Saleh *et al*., 2012).

Emetic illness shows symptoms within 30 minutes to 6 hours and it is caused by the toxin cereulide, which shows multiple isoforms. The genetic cluster that is included in the formation of cereulide is hosted in a mega plasmid, which is only present in some strains of *B. cereus*. This toxin is non-ribosomaly synthetized and it is outstandingly resistant to acidic pH, proteolytic activity and heat (126°C/90 min). The physico-chemical properties of cereulide determine the physiopathology of this disorder, usually caused by the consumption of improperly conserved food with a subsequent bacteria proliferation and toxin production (Marxen *et al*., 2015).

On the other hand, vegetative cells of *B. cereus* are susceptible to the acidic pH of the stomach, what make this disease mainly produced by spores germinating in the intestine, which proliferate and produce the diarrheic toxins. Within these toxins, hemolysin BL, non-hemolytic enterotoxin and cytotoxic K are the main pore forming proteins implicated in this illness, although other toxins influence the virulence grade in addition to host variables. This condition takes 8-16 hours to show the first symptoms and usually solved within the next 24 hours (Kamar *et al*., 2013). The pathologies of *B. cereus* are mainly gastrointestinal (GI) syndromes as one of the most mutual pathogens of food poisoning outbreaks (Ehling-Schulz *et al*., 2015). *B. cereus* causes many different human infections; some of them are severely virulent or even lethal (Shimoyama *et al*., 2017). Nosocomial bacterial infections are the greatest commons among non-gastrointestinal disorders and are apparently related with the presence of vegetative cells or spores in hospital bed dressing, towels or uniforms. Further contamination of instruments or catheters permits *B. cereus* to reach immunosuppressed patients, although several cases have also been reported on non-immunocompromised patients (Gurler *et al*., 2012). *Bacillus thuringiensis* is an insect pathogen (Jensen *et al*., 2003). It secretes large crystal protein inclusions (δ-endotoxins) during sporulation. The latter insecticidal proteins are situated on large transmissible plasmids of the gene (Rasko *et al*., 2005). In general, *Bacillus anthracis* causes a fatal mammalian illness but in herbivores, it is known as anthrax (Mock and Fouet, 2001). Spores germinate into vegetative cells inside the host, which synthesize plasmid-encoded virulence factors that kill the infected host. Virulent bacteria carry two large plasmids, i.e. pX01 (181 kb) and pX02 (96 kb). Whereas pX01 harbors the genes encoding the tripartite fatal toxin and the pX02 encodes the genes able to synthesis the virulence factor, the poly-γ-D-glutamic acid capsule (Rasko *et al*., 2005).

Comparative genomic analyses of *B. cereus*, *B. thuringiensis* and *B. anthracis* were reported to carry different plasmids in a highly similar genetic background (Han *et al*., 2006). *B. anthracis* and *B. thuringiensis* are two species of the *B. cereus* group sensu lato which varies from *B. cereus* sensu stricto mostly by the existence of mega plasmids carrying genes encoding toxins that active against invertebrates or mammals, respectively (Majed *et al*., 2016).

Bacillus cereus grows as a saprophytic soil organism. Although *B. cereus* capable of vegetation and sporulation in a medium formed of a soil-extracted organic matter, but their spores that inoculated into the same medium capable of germination and following vegetative growth (Vilain *et al*., 2006). Notably, *B.*

cereus cells grown in this medium could shift to a multicellular phenotype that can form filaments and aggregate into macroscopic clusters. This was indifference with the single-celled growth observed in rich media. Furthermore, *B. cereus* has the ability of translocation through soil depending on flagellar motility. Translocation results from extension of the multicellular filaments through growth and cell division (Vilain *et al*., 2006). These data ensure that, *B. cereus* being primarily a soil saprophyte. Also, *B. cereus* displays a multicellular phenotype when growing in its natural environment, appearing as translocating bundled filaments in soil or as filaments adhered to the invertebrate intestine (Vilain *et al*., 2006).

Biofilm formation

Bacterial biofilm is consisted of groups of bacteria surrounded by an extracellular polysaccharide (EPS) matrix. The extracellular matrix (ECM) is consisted of water, polysaccharides, proteins, lipids, extracellular DNA (e DNA), membrane particles and ions (Karatan and Watnick, 2009).

Biofilm formation is not an attribute only to a few species, but a general ability of all microorganisms. Biofilm formation pathways vary from species to another affording to their environmental factors. However, there are common features of all biofilms: (i) cells are joined together in the biofilm by an extracellular matrix made of exo-polysaccharides, proteins, and occasionally nucleic acids; (ii) environmental and bacterial signals can initiate the biofilm formation; and (iii) the biofilm protects the bacteria from the surrounding environmental stresses, antibiotics and the immunological responses of the host (Lemon *et al*., 2008). Bacterial biofilms can form up on abiotic (such as metal, plastic, glass, etc.) or biotic (such as animals, plants and humans) surfaces (Moscoso *et al*., 2006 and Adame-Gómez *et al*., 2020).

Four plausible driving forces are suggested to act behind bacterial biofilm formation (Jefferson, 2004): (1) protection from health hazard in the host (defense), (2) restoration to a richly nutrient area (colonization), (3) utilization of cooperative benefits (community), (4) bacteria grow normally as biofilms in nature. The three dimensional complex of the biofilm is a coordinated community and allows the bacteria to adjust and survive in host environments. Bacteria in biofilms could detect the environmental changes and respond to them to survive in diverse and stressful conditions (Hall-Stoodley *et al*., 2004). Organisms surrounded by biofilms can resist antibiotics, nutrient depletion, pH changes, disinfectants and oxygen radicals more than planktonic organisms (Jefferson, 2004).

Bacterial biofilm is regulated by different environment signals including mechanical, nutritional, metabolic and host-derived signals; secondary messenger and protein transcriptional regulators are also involved (Karatan and Watnick, 2009).

Bacillus subtilis is one of the most famous strains in the Gram positive group, acting as a model organism for studies of cellular differentiation, sporulation, gene regulation or biofilm formation of motile bacteria (Sonenshein *et al*., 2001). Streptococcus pneumonia or *Staphylococcus aureus* (S. aureus) constitute a paradigm in the study of biofilm formation of non-motile Gram-positive bacteria (Moormeier and Bayles, 2017).

There are general steps of biofilm formation from studies with *Bacillus subtilis*. Initial reversible attachment is followed by a cell differentiation process into matrix producers, preventing detachment and favoring the formation of micro colonies. Further maturation steps lead to the three dimensional growth of bacterial community and control of its differentiation. In sporulating species, the process is triggered in a subpopulation within the biofilm. The cycle is completed with total or partial dispersion of biofilm of single individuals which can initiate a new planktonic phase (Vlamakis *et al*., 2013). Warmed by the relevance of biofilms, hundreds of studies have been directed at elucidating how bacteria sense signals, communicate and assemble the extracellular matrix; and how all this cellular machinery is regulated (McLoon *et al*., 2011). Up to date, most of the efforts to understand biofilm formation have been done with bacterial strains isolated in vitro and in controlled environmental conditions. Motile cells adhere to a surface, switching to a sessile life style characterized by bacterial growth in chains and differentiation into matrix producers. Maturation of the biofilm produces differentiation of a subpopulation into sporulating cells. Fully mature biofilms partially disassemble to colonize other niches and initiate a new life cycle (Vlamakis *et al*., 2013) and decrease motility in a strain surrounded by biofilms at the air liquid factors resulted in immersed biofilms (Hayrapetyan *et al*., 2015).

In the last years, some studies have been done in this direction with synthetic multispecies communities assembled in vitro, using a reduced number of strains as a model to mimic the natural environments (Niu *et al*., 2017). The main characteristic and visible feature of a bacterial biofilm is the incidence of an extracellular matrix surrounding the cells, which provides the community with outstanding stability and protection against external aggressions (López *et al*., 2010). However, for variant bacterial species, each of these structural elements (eps, protein and eDNA) acquires a particular relevance in the final architecture of the biofilm and thus the way they coordinate to form such architecture. For instance, matrix exopolysaccharides are very variable among species in terms of sugar composition of the main chain, chain length, ramification pattern, sugar composition of the ramifications or additional sugar modifications (Schurr, 2013).Such variability provides with different properties of adhering to the surface, cohesion of the community, rheology properties, or level of hydrophobicity, all of them affecting the matrix performance in different environmental conditions (Hussain *et al*., 2017). As introduced earlier, *B. subtilis*, closely associated to *B. cereus*, constitutes the most relevant model organism for studying of biofilm formation in Gram-positive bacteria and a reference in the study of biofilms in related species. Mutants in the exopolysaccharides (epsA-O) operon region-in charge of the biofilm synthesis exopolysaccharides- results in the lack of biofilm in liquid culture, revealing the significance of this component to the final biofilm architecture of floating pellicles or colony morphology in agar plates (Branda *et al*., 2004).

In motile bacterial species, flagella are very important structures for biofilm formation, playing a dual role either as: i) an element of the biofilm structure or ii) indirectly as a necessary functional element to reach the surface and generate the mechanical force for attachment, recruitment of new individuals or the formation of galleries inside the biofilm (Houry *et al*., 2010).

As a model of other elements, proteins with type collagen domains have been found essential for adhesion and biofilms in some *Bacillus* species (Zhao *et al*., 2015). Pellicles formed by *B. subtilis* show a wrinkled phenotype and robust resistance, which is also, conferred by the incidence of other structural components, the amyloid proteins TasA and TapA. These two proteins are included in the production of resistant and firm amyloid fibers (Diehl *et al*., 2018). Also the spermidine was also essential for activating expression of these matrix components (Hobley *et al*., 2017). Besides the amyloid protein and exopolysaccharides, biofilms of *B. subtilis* are also made by the hydrophobic protein BslA. Studies on this protein have shown the connection among hydrophobicity of BslA and its putative role giving protection

against external aggressions. This protein forms a plastic like cover of the biofilm community, which has been suggested as a raincoat protection against aqueous solutions in the soil when is polymerized. Besides, another function has been shown as a structural element of the matrix when is in a monomeric form (Arnaouteli *et al*., 2017).

Besides the protein and the exopolysaccharides, eDNA is being also recorded to be part of the extracellular matrix. Although, recent studies associated eDNA with colony morphology in *B. subtilis*, affecting the community extension where its production is not an effect of cell damage but needs both competence genes and the Opp oligopeptide permease, and is involved in horizontal gene transfer (Zafra *et al*., 2012). Furthermore, in other species, eDNA plays a determinant role in biofilm architecture as happens in *B. cereus* (Vilain *et al*., 2009).

Other components of the extracellular matrix of *B. subtilis* biofilm include the poly-γ-glutamic acid, one of the main produced polymeric compounds which are not determinant for biofilm synthesis, although, an alternative significant role in rhizosphere colonization has been offered for this polymer (Yu *et al*., 2016).

In the genus *Bacillus*, biofilms are also associated with sporulation, yielding resistant forms of life that can survive extreme conditions and constitute a perfect craft for bacterial dispersion (Branda *et al*., 2001).

Disruption of biofilm synthesis may also affect sporulation as both processes are connected. Cell differentiation into biofilm formation cell types is controlled by the levels of Spo0A-P, which induces sporulation when this level is high. Given that impairment in biofilm synthesis may maintain SpoA in a nonphosphorylated stage, biofilm disruption may affect sporulation (Fujita and Losick, 2005). *B. cereus* biofilms form preferentially at air liquid interfaces under static culturing conditions and that these biofilms function as a nidus for sporulation (Wijman *et al*., 2007). It was mentioned that the amount of biofilm formed on submerged surfaces were half of that formed at the air-liquid factors. Differences were also noted in the capability of different *B. cereus* strains to form biofilms (Laszlo *et al*., 1984). In support of this notion, Wijman *et al*. (2007) reported that a *B. cereus* mutant with reduced motility was indeed severely impaired in its aptitude to form biofilms at the air-liquid factors.

Several studies have reported that the biofilm cells of Gram-positive bacteria express adistinct transcriptome when compared to their planktonic species. A study by Beenken *et al*. (2004) showed that in S. aureus biofilm cells, the expressions of 48 genes were induced by a factor of more than two, while expression of 84 genes were repressed by a similar factor. Two independent studies have also reported differential gene expression in *B. subtilis* biofilm cells. Stanley *et al*. (2003) studied the gene expression of *B. subtilis* in the primary stage of surface biofilm synthesis (8, 12 and 24 hours after inoculation in batch culture), and recorded that 519 of the *B. subtilis* genes were expressed differentially in at least one time point as the planktonic cells transitioned to a biofilm mode of growth. Many of the genes differentially expressed during biofilm formation are included in motility and chemotaxis, phage-related functions, membrane bioenergetics and sugar catabolism. In contrast, Ren *et al*. (2004) studied gene expression in mature *B. subtilis* biofilms (5-days biofilms), and reported significant induction of 342 genes and repression of 248 genes in biofilm cells compared to planktonic cells. Genes that were highly expressed in the biofilm comprised sporulation genes, genes that have functions for transport, metabolism and antibiotic production.

Proteomic analysis, using two-dimensional SDS-polyacrylamide gel electrophoresis (2-DE), has consistently shown differences between the proteomes of biofilm and planktonic cells. Moreover, *B. cereus* DL-5 biofilm cells expressed at least 10 proteins as a result of surface attachment. Of these, four proteins were unique to the biofilm profile, while the other six proteins represented modified proteins forms found in both the biofilm and planktonic proteome profiles. Moreover, seven proteins were noticed to be expressed uniquely in planktonic cells (Oosthuizen *et al*., 2002). High percentage of proteins were expressed by biofilm cells that associated with cell attachment, peptidoglycan synthesis, fibrinogen-binding proteins, and enzymes involved in pyruvate and format metabolism, in comparison of the proteomic data with transcriptomic data, produced by the same group (Resch *et al*., 2006).

Due to the genetic similarities inside *B. cereus* group, particularities in biofilm synthesis are as subtle as those found among strains of the same species, therefore, knowledge in this bacterium is rationally extensive to the entire group, or at least may aid as a more reliable model than *B. subtilis*. Unfortunately, there are insufficient studies focused on biofilm synthesis in this bacterial species rather than the visual characterization of biofilm phenotypes. Among the three general components of the biofilms' extracellular matrix, eDNA has been studied in detail only in *B. cereus* as adhesion agent on polystyrene or glass surface (Vilain *et al*., 2009). Other studies reported the presence of exopolysaccharides and ascertained that, deletion of the *B. cereus* eps locus does not interfere with biofilm production however its origin is unknown but might be related to programmed cell death (Gao *et al*., 2015). In the same way, the occurrence of amyloid fibres in the biofilm of the *B. cereus* group had not been explored yet.

Unfortunately, cell differentiation within biofilm in *B. cereus* group species is still poorly understood. Comparatively with *B. subtilis*, the regulatory factors in *B. cereus* group are less understood. Although, several studies in *B. cereus* group species have recorded that the main routes that regulating biofilm formation are conserved. The phosphorelay that involve *Spo0A* is conserved, as well as the regulator *AbrB* and the antirepressor/repressor *SinI/SinR* pair act as a switch between biofilm synthesis and motility. Nevertheless, the regulons present some differences, as the exopolysaccharide biosynthesis operon *epsAO* is not under the control of *SinR* (Fagerlund *et al*., 2014). There is no paralog of *bslA* or *tapA* in the *B. cereus* strain, but *tasA* have two paralogs. One is *tasA*, involved in the *sipW-tasA* operon, and the other is *calY*, which is present next to *sipW-tasA* (Caro-Astorga *et al*.,2015). *TasA* and *CalY* are both included in the production of fibers, which can be observed by electron microscopy, and the deletion of their genes or of *sipW* leads to biofilm disorder resemble to that reported in *B. subtilis* (Caro-Astorga *et al*., 2015). Rather than surfactin, the lipopeptide shared in biofilm formation is the molecule kurstakin, which is included in the SinR regulon (Gélis-Jeanvoine *et al*., 2017). The phospholipase C regulator (PlcR) is absent in *B. subtilis*. It is in charge of sensing external signals like nutrients and population density through the peptide PapR. Its regulon comprises most of the virulent factors and it also sets biofilm synthesis through the initiation of the necrotrophic factor the neutral protease regulator (*NprR*), which induces kurstakin expression (Dubois *et al*., 2012 and Majed *et al*., 2016). The autoinducer (AI) mainly AI-2 plays a positive effect on biofilm synthesis in *B. subtilis*, however, *B. cereus* shows a contrary effect and induces bacteria liberation from the biofilm to the liquid medium (Duanis-Assaf *et al*., 2016). In *B. subtilis*, the subnetwork II and IV controlling biofilm formation involves the proteins *SlrA* and *DegU*, however both proteins has no homologue in *B. cereus* (Kobayashi, 2008). Even within the *B. cereus* group there are

many variances in the regulation of biofilm synthesis. In *B. cereus* ATCC14579, PlcR was reported to repress biofilm formation due to the interruption by a transposon of the *nprR* gene (Gélis-Jeanvoine *et al*., 2017). Although *B. anthracis* can form biofilms, only studies on phenotypes were found (Lee *et al*., 2007). In *B. thuringiensis*, it has been characterized the cell type differentiation into undifferentiated, virulent, necrotrophic and sporulating cells within the biofilm, reflective of the diversity and highly regulated genetic circuitry necessary to familiarize to all these divergent environmental destinies (Verplaetse *et al*., 2015). The exponential increase in the number of articles published on *B. cereus* biofilms illustrates the rising interest of the scientific community for this subject. Indeed, not only are biofilms a key issue in *B. cereus* life, they also display interesting specificities.

Biofilm and quorum sensing

Biofilms are multicellular surface-attached colonies of bacteria inserted in ECM. Quorum sensing (QS), a cell-to-cell communication, has been known to play serious roles in the synthesis of biofilm with its neighboring ECM (Li and Lee, 2017). The substratum surface presents host polymeric matrix, which is consisted mainly from proteins, nucleic acids, exopolysaccharides and other substances, enabling irreversible connection of the bacteria. It was stated that, the cell surface-associated proteins like Aap and SasG were involved in Staphylococcus epidermidis starting attachment and G5 domain of Aap protein that is important for the bacterial intercellular adhesion. Extracellular structures, including the extracellular glucan-binding protein, the surface-exposed protein and the glycosyltransferases (GtfE, GtfG and GtfH), are also important for cell adhesion property (Couvigny *et al*., 2018). Sortase A (SrtA) is a transpeptidase that can anchor cell surface proteins and prompts the biofilm synthesis during Gram-positive bacterial infection, such as S. aureus. Then, the attached bacteria multiplied into microcolonies. When the biofilm synthesis became mature, a complex construction of matrix was formed with water channels for inflow of nutrients and outflow of wastes (Roy *et al*., 2018) plus the bacteria could get out from the biofilm and can begin a new life cycle of biofilm formation by more attachment through purine biosynthesis and ClpYQ protease (Yan *et al*., 2017).Thus, many inhibitors that can prevent these adhesion-associated proteins and might give a good ability as anti-microbial and anti-biofilm activities were extensively established (Roy *et al*., 2018). Surrounding environments, such as oxygen or pH percentages, in biofilm funded to different gene expression profiles. Decreased oxygen amount within the biofilm could increase the programmed cell lysis (PCL) and stimulated biofilm synthesis in S. aureus. This progress was due to SrrAB and SaeRS-dependent upregulation of AtlA murein hydrolase, followed by relief of cytosolic DNA (Mashruwala *et al*., 2017). The mechanism underlying the role of QS in biofilm synthesis has been described indetails. Quorum sensing capable the bacteria to identify the population by sensing and evaluating the development of specific self-produced signal molecules secreted by the community, (Abisado *et al*., 2018). Meanwhile, it alters bacterial gene expression and activates cooperative responses by activating signaling pathways while the population density is high enough to prompt the level of accumulated signals in the surroundings. These genes express virulence factors, as proteases, elastases, exoenzymes, toxins, pyocyanine and bacteriocine, etc. Molecular mechanism convoluted in QS was broadly studied but was dissimilar between Gram-negative and Gram-positive bacteria. It is mediated by signal molecules, called autoinducers (AIPs), which secreted from Gram-positive bacteria and rise in amount as indicator of bacterial density (Papenfort and Bassler, 2016). Extracellular increase of the auto inducer to a minimal threshold stimulatory concentration result in determination of the signal by bacterial population members and subsequent alterations in gene expression. Bacteria can coordinate certain manners on a population-wide scale by using these signal-response systems and its function such as multicellular organisms (Waters and Bassler, 2005). These AIPs fix to the kinase receptors on the bacteria cell membrane to conduct signal to corresponding transcriptional elements, then finally initiate the associated genes expression such as accessory gene regulator (Agr) and RNAIII. Agr system was recognized as the most classical QS system in Gram-positive bacteria (Papenfort and Bassler, 2016). The second QS mechanism of Gram-positive bacteria is based on the direct binding of the oligopeptide to the cytoplasmic response regulator in the responder cell, particularly in bacteria from the Firmicutes phylum. These systems belong to the RNPP family – named from the key regulator members – Rap, NprR, PlcR and PrgX. Even if these proteins regulate various processes in altered bacterial species, they share two main features: the intracellular interaction with alinear processed oligopeptide (Phr, NprX, PapR, cCF10, respectively) that is reimported by oligopeptide permeases (Opp), and a similar construction of the regulators, which contain tetratricopeptide repeat (TPR) motifs (Pérez -Pascual *et al*., 2016).

Quorum sensing signaling affects the global gene expression of the entire bacterial population. Although, there are other signals considered a paracrine signaling, affecting a subpopulation within the bacterial colony, which is different from the population that sends the signal. This communication flux has been described in *B. subtilis* and relies on the surfactant molecule surfactin, which triggers the expression of extracellular matrix genes through Kinase C and the phosphorylation of Spo0A. Interestingly, the expression of surfactin synthesis genes is under the regulation of ComX pheromone contained in the surfacing synthesis operon (López *et al*., 2009).

Biofilm and antibiotic resistance

Clinically, biofilms are important because they reduce the susceptibility of bacteria to antimicrobials leading to persistent infections (Chen and Wen, 2011). Biofilm synthesis has been seen as one of the chief reasons contributing to antibiotic resistance (Olson *et al*., 2002). In natural, industrial and medical environment, bacteria are capable to adhere to surfaces and grow in biofilm communities. In fact, this is the predominant mode of bacterial growth in natural environment (Kaur *et al*., 2009). During biofilm life, bacteria become more accepting to conventional antibiotics and opsonophagocytosis (Stewart and Costerton, 2001), being 100–1000 times less predisposed to antibiotics than their planktonic counterparts (Donlan, 2000). This bacterial tolerance/ persistence cause the chronicity of a disease (Burki *et al*., 2015). Bacteria survive inside biofilms indicates a high adaptation and resistance to disinfectants and antibiotics. The rise of antibiotic resistance stands as an obstacle during treating biofilm-related acute and chronic infections (Li and Lee, 2017). Bacterial cells infections affected by biofilm synthesis are of a main public health concern, these bacterial cells might develop a biofilm-specific biocide-resistant phenotype. Because of biofilm heterogeneous nature, it is estimated that there are numerous resistance mechanisms work in a solitary population (Mah and O'Toole, 2001). Development of biocide resistance is not assumed, but recent studies have used to determine why and how biofilms are resistant to different antimicrobial agents as following: Failure of antimicrobial penetration into the biofilm exopolysaccharide matrix

or glycocalyx production and mediating bacterial gene expression (Hall Stoodley *et al*., 2004). It has been recommended that the matrix function is to prevent the access of antibiotics to the bacterial cells included in the population (Stewart, 1996). Stress response starvation of the bacterial community for a specific nutrient reduces its growth. Shift from exponential to slow or no growth is mostly accompanied by more resistant to antibiotics (Tuomanen *et al*., 1986). Slow bacterial growth has been detected in developed biofilms (Wentland *et al*., 1996). Heterogeneity of any bacterial cell inside the biofilm will experience a little different condition matched with other cells within the same biofilm and thus will grow at a different amount. Nutrients gradients, signaling factors and wastes permit for this heterogeneity inside the biofilm (Mah and O'Toole, 2001). General stress response has been submitted that the slow growth rate of some cells inside the biofilm is not due to the nutrient limitation, but to a general stress reaction started by growth inside a biofilm (Brown and Barker, 1999). This knowledge is possible since the stress reaction results in physiological changes that act to defend the cells from several environmental stresses. Thus, the bacterial cells are sheltered from the harmful effects of changes in pH, cold shock, heat shock and numerous chemicals (Hengge-Aronis, 1996). Induction of a biofilm phenotype has been based on slow down the influence of antimicrobial factors on cells growth in the biofilm. An emerging idea is that a biofilm-specific phenotype is prompted in a subpopulation of the colony that results in the expression of active mechanisms to fight the harmful effects of antimicrobial factors (Cochran *et al*., 2000). Additionally, biofilm synthesis can also be dangerous to host as they can stimulate the attraction of phagocytes and production of reactive oxygen, lysosomal enzymes and nitrogen bacterial species (Hermeyer *et al*., 2011). Morente *et al*. (2013) illustrated the role of biofilms in the progress and transfer of resistance supported by microbial communications occurring inside biofilm. From another point, the antibiotics misuse developed the drug resistance, which might exaggerate the bacterial infectious diseases. Thus, novel policies other than antibiotics should be settled to struggle the bacterial and biofilm synthesis. The previous novel trials, in the last two decades, in preventing the bacterial biofilm synthesis and QS have been broadly advanced and reported the natural products of plants. Many plant natural products have been confirmed chemo-preventive properties and antimicrobial (Tan and Vanitha, 2004). Extracts from plants were stated to obstruct QS and regulate biofilm formation. Regarding that thousands of herbs occurred in the world and the traditional medicinal herbs have a long history in treated infectious disease (Karbasizade *et al*., 2017).

Alternative approaches for mitigation of biofilm and antibiotic resistance

Bacterial infection can resist the first line of antimicrobials so the treatment would be switched to the second or third line drugs, which are always expensive. In many poor countries, such diseases are widespread and cannot be treated due to the high drug cost (WHO, 2019). Such a challenge needs to develop new different and unusual approaches for new antimicrobial drugs (Benzie and Wachtel-Galor, 2011).

Herbal medicinal products

World Health Organization (WHO, 2019) noted that common of the world's inhabitants depends on traditional healthcare medicine. Herbal medicines had been essential products for the developing countries to treat the mutual infectious diseases and defeat the difficulties of resistance and side effects of the presently available antimicrobial agents (Kianbakht and Jahaniani, 2003).

Plants introduced a new hope for unique drug ingredients to human well-being, as many plant herbal mixtures (Iwu *et al*., 1999). In consequence of their common use as medications for many infections, research on the antimicrobial action of plants are repeated (Betoni *et al*., 2006). Plants are rich in many secondary metabolites, such as terpenoids, tannins, flavonoids, alkaloids, which had been established in vitro to have antimicrobial characters (Lewis and Ausubel, 2006) and new studies found that many of these metabolites prevent the pathogenic bacterial growth (Benzie and Wachtel-Galor, 2011).

Moreover, plant-derived medications have the benefit of not inducing resistance after persistent exposure (Domadia *et al*., 2007). Nowadays, there are numerous serious threats about spreading the drug-resistant pathogens. Essential oils and other plant extracts have induced interest as natural antimicrobial products. They have been selected for their possible uses as alternative medications for the treatment of many virulent diseases (Tepe *et al*., 2004) showing antiviral, antibacterial, antifungal, insecticidal and antioxidant properties (Kordali *et al*., 2005).

The natural extracts have many anti-biofilm effects; mainly depending on the following features, the prevention of polymer matrix development, suppression of cell adhesion, disturbing ECM generation and decreasing virulence factors production, thereby blocking biofilm development and QS network. In the subsequent part, these antibiofilm agents extracted from natural plants, such as Cocculus trilobus, garlic and Coptis chinensis (Lan Lu *et al*., 2019).

In vitro studies have informed the combination of antibiotics and plant extracts, with significant decline in the minimal inhibitory concentrations (MICs) of the antibiotics against some resistant pathogen (Betoni *et al*., 2006). The therapeutic effect of natural plant extracts had been mentioned as resistance modifying/modulating activity (Gibbons, 2004).

This effect of plant extracts to counter antibiotics had not been well clarified. It is suggested that prevention of drug outflow and substitute mechanisms of action could cause the synergistic interactions between antibiotics and plant extracts (Lewis and Ausubel, 2006).

Nano approaches to overcome MDR bacteria

Over the last few years, nanoparticles (Nps) drew attention of some investigation groups since these structures can be used as transmission vehicles for antimicrobial agents (Dehkordi *et al*., 2011). Nanotechnology has proved to be a useful tool for solving biomedical problems. Silver nanoparticles (AgNPs) have been widely studied as anti- microbial agents, including their use against MDR bacteria (Theophel *et al*., 2014).

Nanoscale improves the antibacterial action of silver even at low concentration; nanometer metallic particles show different chemical, physical and biological properties associated to conventional silver, due to their high surface to volume ratio (Herman and Herman, 2014). Furthermore, AgNPs have been described to be of low toxicity of silver ions to host (De Lima *et al*., 2012).

Numerous data have recently reported that the toxicity of NPs against bacteria depend on particle shape, size, composition and concentration where AgNPs concentration ≥75µg/mL usually obstructs the bacterial growth (Tajkarimi *et al*., 2014).

The exact mechanisms of silver nanoparticle toxicity to bacteria are not completely identified, thus a growing consent regarding the candidate effects. First, the silver nanoparticles action

happens by the silver ion release (Ag+) and from potential disturbance or destruction to the cell membrane by these particles (Mijnendonckx *et al*., 2013).

AgNPs have been illustrated to be defensive agents contrary to numerous species of bacteria, including B.cereus, *Escherichia coli*, Enterococcus faecalis, S. aureus, and others (Duncan, 2011). However, AgNPs give potent antimicrobial effect, silver-resistant bacteria have been reported; these microorganisms can rapidly progress resistance to AgNPs by genetic modifications (Graves *et al*., 2015).

Many antimicrobials' combinations appear to be the best policy for overcoming the antibiotic resistant microorganisms (Bass *et al*., 2015). Therefore, the synergistic antibacterial effects of AgNPs collective with alternatives (phenazine-1-carboxamide, eugenol and cinnamaldehyde) or conventional (kanamycin, ampicillin, erythromycin, chloramphenicol, ciprofloxacin, amoxicillin and moxifloxacin) antimicrobial combinations have been stated to be good for treatment of persistent infections (Biasi-Garbin *et al*., 2015). The synergistic effect of AgNPs and antibiotics was showed a successful combination against S. aureus using antibiotics that inhibit protein translation, such as erythromycin (Kazemi *et al*., 2014).

CONCLUSION

B. cereus spp. are recently dangerous food borne pathogens that may cause severe outbreaks. *B. cereus* can form biofilms, which protect the pathogen from the host immune responses and from antibiotics, enhancing its persistence on epithelial tissues and medical device surfaces. Recent approaches like using the natural extracts, their essential oils, nanotechnology and quorum sensing inhibitors obstruct the biofilm synthesis that enhancing food industry and consequently relief *B. cereus* human risk hazards.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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