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Bacillus cereus biofilm: Implications for food and diseases

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ABSTRACT

Microbes play an important function in human undertakings that goes beyond spoilage and disease. *Bacillus cereus* is a Gram-positive bacterium that may generate biofilms with a variety of contributions. Bacteremia, endocarditis, endophthalmitis, gastrointestinal diseases, and a variety of other illnesses are caused by the external insertion or interference of *B. cereus*. In addition, it has aided the bioremediation of pollutants, petroleum spills, metal contamination, other oil spills, biocontrol agents in plants, biosurfactant manufacturing, antibiotic storage, and many more. The exopolysaccharide of biofilm can be used to extract sugars, polysaccharides, lipids, and other important components for polymeric fabrications. As *B. cereus* biofilm contributes to food toxicity, illnesses, and is also beneficial, it necessitates further investigation. The objective of this study is to provide a comprehensive look at *B. cereus* and its biofilm formation, mechanism, regulatory details, resistance capabilities, impact on food and diseases, and positive potential, as well as potential research prospects, all in one place.

1. Introduction

A biofilm is a collection of bacterial cells encased in a self-produced macromolecular matrix primarily made up of exopolysaccharides (EPS), proteins, and nucleic acids. Biofilm is produced by nearly 80 % of all bacterial cells on the planet (Muhammad et al., 2020). Biofilm's primary role is to combine bacterial cells and shield them from the hostile environment and also serve other functions such as metabolite waste disposal and antimicrobial protection (Yin et al., 2019). Contamination of biofilm in medical devices and tools, particularly surgical devices such as pacemakers, catheters, artificial heart valves, orthopedic implantations, and contact lenses are one of the most significant contaminants, as it leads to post-surgical infections, persistent inflammations, wound infections. Plaque formation in teeth is a type of bacterial colonization that results in biofilm formation (GM, 2021). Additionally, biofilm formation over food processing machinery and the adhesion of various biofilm-producing bacteria, play a crucial role in food spoilage and dairy industries.

B. cereus is a gram positive, facultatively anaerobic, toxin producing bacteria (S.E. McDowell and Friedman, 2023). Widely distributed *B. cereus* is capable of colonizing many different niches in the environment, such as soil and ocean water, where it can live as a saprophyte or while migrating from other ecological habitats. Additionally, this bacterium has been reported to coexist with plant tissues, either commensally or in symbiosis as the root zone dweller (Hu et al., 2017). In addition, *B. cereus* has a niche in the guts of mammals and arthropods, where it can exist as a pathogen or as a commensal, either opportunistic or not (Fiedoruk et al., 2017). These bacteria's adaptability indicates their capacity for resistance in various environmental circumstances (von Stetten et al., 1999). The bacterial species *B. thuringiensis* and *B. anthracis*, which are diversified in their host impact and effect both human and insect, belong to the *B. cereus sensu lato* group, which bears the same name. These species are phylogenetically similar (Helgason et al., 2000). Certain strains of *B. cereus* are beneficial to plants, acting as biocontrol agents or promoters of growth (PGP) against microbial illnesses. Conversely, other strains have been suggested as probiotics for humans as well as livestock (Anwar et al., 2014; Zhu et al., 2016). Nonetheless, other strains are accountable for human diseases, which are primarily brought on by food poisoning, tainted food products, or even food deterioration (Wan et al., 2018; Schultz et al., 2011; Rajasekar et al., 2007). Human concerns about *B. cereus* are furthered by its corrosive effects, clogging, and biofouling in industrial equipment (Rajasekar et al., 2007). The majority of the above-mentioned scenarios are thought to be connected to how bacterial cells organize in biofilms,

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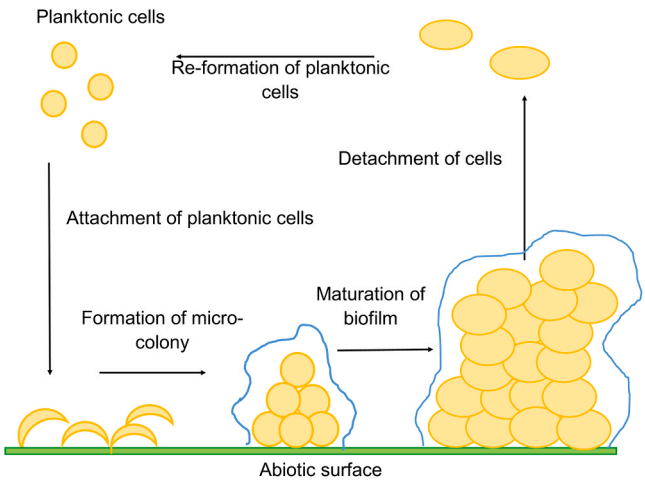

Fig. 1. The schematic representation of biofilm formation.

regardless of the outcomes. Most bacterial species are known to undergo biofilm formation as a critical stage in their life cycles, and this process is linked to disease outbreaks, antibiotic resistance, and pollution of industrial and medical equipment (Gurler et al., 2012; Caro-Astorga et al., 2020).

B. cereus which is majorly responsible for dairy industry spoilage and other food contamination are the major concern in food industry due to the formation of spores, biofilms, and diarrhea and/or emetic toxins. Hence, study of *B. cereus* and its biofilm formation and its related aspects can unfold many significant information. Therefore, this review will focus on the biofilm formation of *B. cereus* and detailed mechanism behind it.

2. Stages of biofilm formation

There are four stages of bacterial biofilm formation such as (i) surface attachment, (ii) bacterial proliferation, (iii) formation of mature biofilm structure, (iv) detachment, or dispersal. They are discussed below. The schematic representation of biofilm formation is shown in Fig. 1.

(i) Microorganism adhesion to abiotic surfaces: The adhesion of biofilm-forming bacteria to abiotic surfaces has been extensively explored. Bacterial properties such as hydrophobic interactions and van der Waals forces of attraction are physicochemical in nature and determine the amount of adherence to these surfaces (Monds and O'Toole, 2009). Bacteria detect changes in environmental parameters such as temperature, oxygen saturation, ionic strength, osmotic pressure, iron levels, and other elements that promote biofilm formation. Following that, the cells use physicochemical forces to adhere to the substrate surface. Furthermore, if biofilms persist in the clinical setting, their attachment to abiotic surfaces may play a key role in disease transmission (Joo and Otto, 2012).

(ii) Bacterial proliferation: After attaching to the surface, bacterial biofilms grow and produce an extracellular substance in the second stage. The purpose of extracellular substances is to allow bacterial cells to cling to one another, enabling for the formation of multilayered biofilms.

(iii) Formation of mature biofilm structure: At this point, the biofilms have fully grown and have a mushroom-like appearance, with channels that are thought to be important for transporting nutrients to cells in the deeper layers of the biofilm (Jahid and Ha, 2012). This shows that for biofilm growth, cell-cell interactions, such as increased cell density and genetic factors are essential.

(iv) Detachment: During the dispersal phase, single cells and or multicellular complexes can be released from biofilms by sensing environmental stimuli and transmitting them through highly regulated networks (Guilhen et al., 2017). Polysaccharide-degrading enzymes such as β-mannanase, chitinases, and a variety of proteases and nucleases aid biofilm dispersion (Cui et al., 2016; Lister and Horswill, 2014; Martí et al., 2010; Ranjith et al., 2021).

3. Biofilm formation by *B. cereus*

B. cereus have highly diverse niches and lifestyle and enjoys significant role as pathogenic and beneficial strain. Though it found widespread in the environment, whether it is living organism or inert surface but is predominant in food industry. It can also produce varieties of biofilm which are different in architecture and process of formation reflecting adaptation to the environment. *B. cereus* can form biofilms in both static and dynamic environments; however, dense biofilms of *B. cereus* occur most frequently at the air-liquid interface (Shemesh and Chai, 2013). Depending over the strains of the *B. cereus*, it can also form floating or immersed biofilms and can produced various enzymes, toxins, metabolites, and many more which effects both the biofilm and the environment. The spores of the *B. cereus* are reported to be increasingly adhesive and resistant towards detergents and antibiotics. As previously reported by Shemesh and Chai in 2013, biofilms produced by numerous *B. cereus* strains were less persistent, varied, and exhibited few physically distinctive traits than those produced by *Bacillus subtilis* (Shemesh and Chai, 2013). *B. cereus* is frequently found in mixed biofilms with other bacteria in industries such as food and beverage, clinics, paperboard, petroleum, and chemical factories. Exopolysaccharides (EPS) play a crucial role in biofilm growth, and are produced in distinct amounts by different *B. cereus* strains (Vilain et al., 2009). As reported by Faille et al. in 2014, *Bacillus* biofilms are not constituted of homogenous monolayers of microbial cells, rather, there were substantial differences between strains (Faille et al., 2014). *B. cereus* 5832 was primarily made up of individual cells, tiny flat clusters, and its biofilms produced a large amount of extracellular matrix. Observation under confocal microscopy by the authors, revealed the presence of large number of spores and individual cells within the biofilm which confirms the non-homogenous monolayers of microbial cells (Faille et al., 2014).

Biofilm generation is acknowledged as a severe food safety hazard in the food processing sectors (Srey et al., 2013). Occasionally, high rates of *Bacillus* isolates within biofilms have been observed along food processing lines. *Bacillus* isolates, including *B. cereus*, have been found in dairy businesses at high concentrations of up to 25 %. Hence, understanding the factors affecting the biofilm formation is immensely required (Faille et al., 2014).

4. Factors influencing biofilm formation

Biofilm formation of bacteria helps them to adjust and flourish in the stress environment, diversify its habitat and lifestyle. Bacterial biofilm development may be influenced by a range of environmental parameters, including nutritional composition, pH, temperature, oxygen level, co-factors found on attachment surfaces, osmolality, strain, and maturation time. Each factors influencing the biofilm formation are discussed below.

4.1. *Effect of flagellar motility over biofilm formation*

Flagella is a bacterial appendage which helps in motility over liquid or solid surface. This allows the bacteria to find suitable place for attachment. The same bacteria which once is motile can form non-motile multicellular colonies entrapped by the biofilm synthesized by themselves. This transition from motile to non-motile bacteria occurs by inhibition of motility. Transition of motile to biofilm forming stage involves majorly two steps: short-term control and long-term control (Guttenplan and Kearns, 2013). In case of short-term control, the regulation is imposed over either the flagellar rotation is inhibited, or the basal flagellar reversal rate is modulated. Whereas in case of

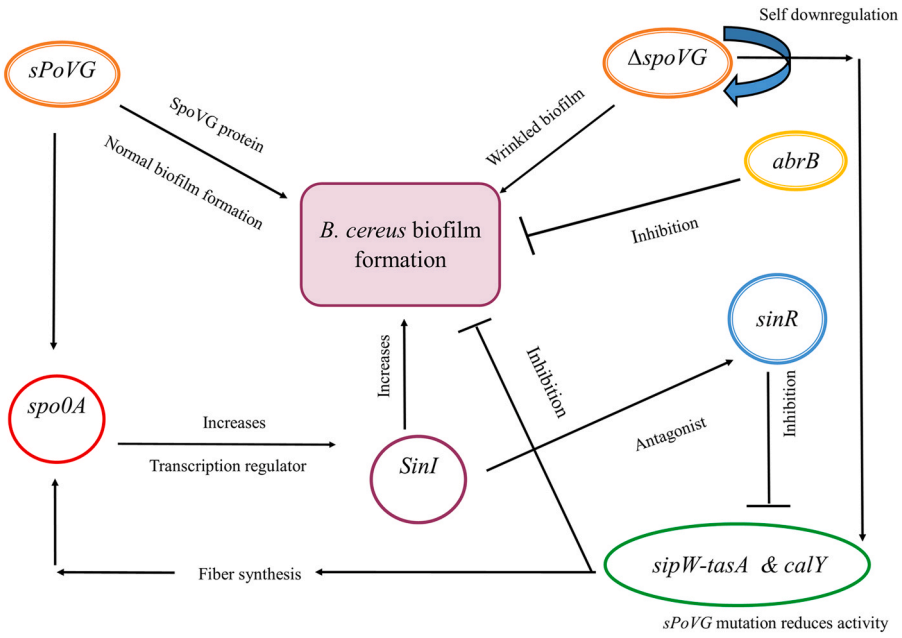

Fig. 2. The schematic representation of regulatory mechanism of biofilm formation.

long-term control, the gene responsible for the transcription of the flagella is inhibited and in absence of any new regulatory system, flagella are disappeared slowly in the process of evolution. Thus, both the short-term and long-term control for the inhibition of flagella is important for the stabilization of the cell aggregates over the surface for proper investment of the nutrients for the biofilm formation (Guttenplan and Kearns, 2013). Other than that, involvement of flagella over formation of biofilm by *B. cereus* is also determined by the surface attachment. According to Houry et al., 2010, flagella hinder between the interaction of bacterial cell wall and surface which results into decrease adherence of the bacteria to the glass surface. It was also noticed that in different environmental condition bacterial flagella plays important role in biofilm formation. Flagella helps to travel to the right place for biofilm formation such as in case of glass tube it helps to reach air-liquid interphase in static condition whereas in case of flow cells it can sediment over the glass slide and form biofilm. There are two to five flagellin genes (*fla* genes) all of which are located in the same chromosomal region in opposite directions and are limited by genes that run in the same manner (Houry et al., 2010). Mot A and Mot B are the two proteins that make up the flagellar motor. The expression of *flaA* in strain 407 was tracked by Houry et al. in 2010 using transcriptional merging between the promoter regions of *flaA* and *lacZ* (Houry et al., 2010). Deletion of the *fla* locus resulted in non-flagellated and non-motile bacteria, whereas elimination of the *motA* gene resulted in flagella-free bacteria. Furthermore, the *fla* and *motA* mutants were unable to form biofilms on microtiter plates and glass tubes. The *motA* mutant, on the other hand, produced more biofilm than the *fla* mutant in both trials. Hence, flagella are required to form biofilm in glass tubes and microtiter plates but not in flow cells. Another advantage of the motility of bacteria with respect to biofilm is, it allows involvement of planktonic bacteria which increases the mass of the biofilm. The mobile bacteria also create channels helpful for exchange and discard of nutrients and waste materials simultaneously. The motile bacteria present at the boundary of the biofilm also leads to extension of the biofilm to further surface (Majed et al., 2016a). Therefore, depending on the environmental condition the presence of flagella over biofilm formation can be determined.

4.2. Regulatory networks

The formation of the biofilm matrix is regulated by various genes. *B. cereus* shares many common genetic features with other *Bacillus* species such as *B. subtilis* but just a few genes are involved in *B. cereus* biofilm formation. The *epsA-O* operon product is vital for the proper biofilm formation in case of *B. subtilis* and absence of it produces brittle biofilm pellicle (Lemon et al., 2008). But deletion of this same *eps* locus in *B. cereus* does not affect its biofilm production (Gao et al., 2015). Hence, role of *eps* locus in *B. cereus* is still unknown. Other than this, the structural protein responsible for biofilm production in *B. subtilis* is also similar with *B. cereus*. The structural proteins play important role in biofilm matrix formation. One of such protein which is responsible for hydrophobic biofilm surface layer over biofilm is BslA (Hobley et al., 2013). Some fiber networks are also present in the biofilm to strengthen it, which is created by the association of TasA into amyloid-like fibers fixed to the cell wall of the bacterium by TapA (Vlamakis et al., 2013). In *tapA-sipW-tasA* operon, *sipW* synthesizes signal peptidase which is involved in the release of two proteins TapA and TasA. These proteins are responsible for fiber synthesis not only in *B. subtilis* but also in *B. cereus* (Caro-Astorga et al., 2014). Though there is no paralog of the gene *bslA* or *tapA* in case of *B. cereus* but there are paralog of the gene *tasA* which is *sipW-tasA* operon or *calY* present next to the operon responsible for fiber synthesis (Caro-Astorga et al., 2014). The SinR regulator suppresses the transcription of the *sipW-tasA* and *calY*. The SinI is antagonist to SinR and whose absence creates hypermotility of the bacteria, but biofilm is not formed whereas if SinR is absent then no motility of bacteria is seen, and biofilm is overproduced (Fagerlund et al., 2014; Kearns et al., 2005). Hence, both SinI and SinR together regulates biofilm production and motility of the bacteria. Other than this, Spo0A is important for production of biofilm whereas AbrB suppresses the production of biofilm in *B. subtilis* and *Bacillus thuringiensis* and same is seen in *B. cereus* (Fagerlund et al., 2014; Majed et al., 2016b). SpoVG is a DNA-binding protein that is a sporulation-related component in *B. subtilis* and is generally conserved, particularly among Gram-positive bacteria such as *Bacillus anthracis* , *B. subtilis*, and *B. cereus* (Chen et al., 2020). Sporulation and biofilm formation of *Bacillus* species specially *B. cereus* is related to each other. The role of SpoVG in the production of biofilms in wild type strain *B. cereus* was reported by Huang et al. in 2021. It is present upstream to the Spo0A and participate in the transcription of *spo0A*. Hence, SpoVG is an important regulator of Spo0A and play important role in regulatory mechanism. The study of the same can enlighten us with various vital information

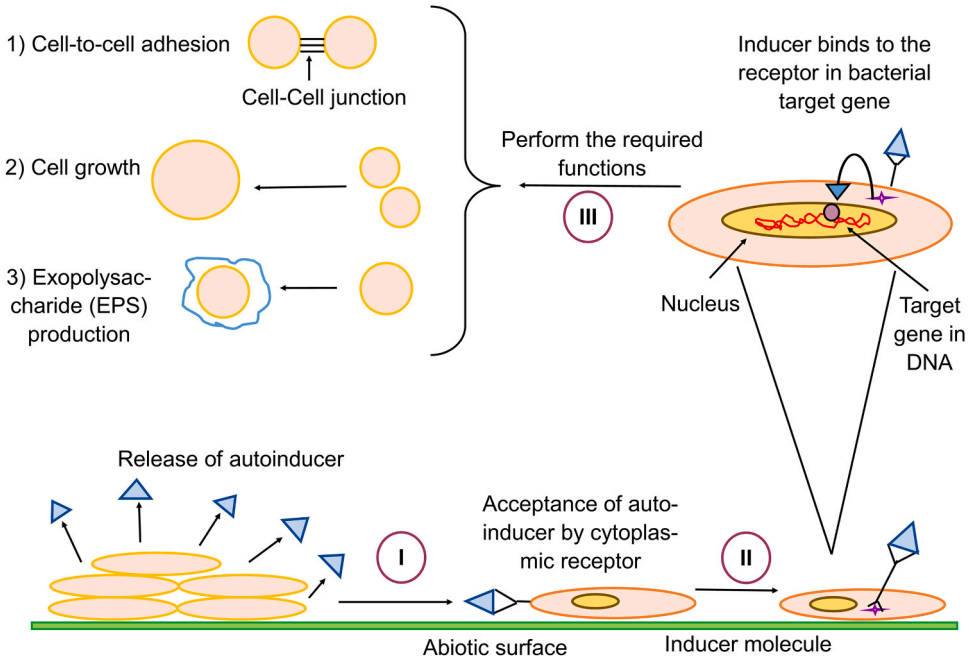

Fig. 3. The schematic representation of quorum sensing among the bacterial cells.

useful to understand and apply for the control of biofilm and spore formation (Huang et al., 2021a). In *ΔspoVG* the genes *sipW* and *calY* is downregulated. When the double mutants were created by deletion of *abrB* and *sinR*, higher quantity of biofilm was produced though the phenotype of the double mutant were significantly correlated with the properties of the single mutants of *sipW* and *calY*. This proves that *ΔspoVG* is presents in the upstream of *abrB*, *sinR* and *spo0A* and has vital role in biofilm production and sporulation (Huang et al., 2021a). According to previous studies by Zhang et al. in 2020, the SinI/R pathway and the CalY protein identified in *B. cereus* may be involved in biofilm development. Both *calY* and *sipW* were negatively regulated when the *spoVG* gene was eliminated, and the activity of the *calY* and *sipW* promoters was reduced. These findings suggested that SpoVG could influence *B. cereus* biofilm formation via the SinI/R pathway (Zhang et al., 2020) as shown in Fig. 2.

According to Yan et al., 2016, spo0A is necessary for biofilm formation in *B. cereus* (Yan et al., 2016). It is a transcription regulator belonging to the response regulators family that regulates biofilm formation in *B. subtilis* and *B. cereus* (Xu et al., 2017). Spo0A promotes biofilm formation in *B. cereus* via increasing SinI production. The regulatory relationship between SpoVG and Spo0A was investigated, and the results show that SpoVG may regulate Spo0A and hence play a role in the development of *B. cereus* biofilms (Huang et al., 2021a). AbrB, like Spo0A, is a transcription factor that impacts *B. cereus* biofilm formation and acts as a cereulide synthesis repressor (Lücking et al., 2009). RpoN - Sigma 54 controls several functions in *B. cereus*, with a deletion mutation indicating problems with sporulation, enterotoxin production, and biofilm formation. However, the exact roles of genes like *spo0A* and *abrB* in *B. cereus* biofilm formation are unknown.

4.3. Quorum sensing

Quorum sensing (QS) refers to the chemical signaling pathways that bacteria use to communicate with one another. Nutrition absorption, toxin synthesis, competence development, biofilm formation, and spore germination are just a few of the physiological functions that this mechanism is responsible for (Rocha-Estrada et al., 2010; Jimenez and Federle, 2014). The communication of the bacterial cells depends over the density of the cells and determines the virulence and pathogenicity of the cells. The expression of the virulence of the bacteria is determined by the concentration of the autoinducers (AIs) and signal molecules (Kachhadia et al., 2022). All approved QS systems are based on three key principles. To begin, community members generate signaling molecules known as autoinducers (AIs). Second, AIs are detected by cytoplasmic and membrane receptors. Finally, AIs are synthesized when they have been identified. An inducer molecule and a receptor protein make up most of the QS circuit (Rocha-Estrada et al., 2010; Jimenez and Federle, 2014). Fig. 3 represents the quorum sensing among the bacterial cells.

The signal transduction pathway of the bacteria determines the QS of the same. This includes binding of the signal molecules to the associated receptor resulting into change in its structure and making the DNA molecule more accessible and producing more diversity of the gene expression which effect the virulence of the bacterial cells. Hence, the knowledge of the QS networks can be used to exploit and degrade the same for controlling virulence or pathogenicity of the bacterium. *B. cereus* is not only an opportunistic pathogen for human but is also biocontrol agent in plants. One of the beneficial effects of *B. cereus* RC1 is, it can control soft rot of the carrot, potato, and cucumber by 91.22 %, 97.59 %, and 88.78 % respectively as reported by the Kachhadia et al. in 2022. Other than this, *B. cereus* is closely related to *B. thuringiensis* (entomopathogen), *B. subtilis* and *B. anthracis* which are pathogenic to human. The QS system PlcR–PapR is responsible for controlling the virulence factor of the *B. cereus*. This QS system includes enzyme degradation, enterotoxins and hemolysins in both *B. cereus* and *B. thuringiensis*. The PlcR active quorum sensor activated the transcription of virulent genes. The PapR is a pro-peptide which is signaling molecule and binds with the PlcR to activate it. The extracellular processing of PapR results into formation of active heptapeptide which is transferred inside the bacterial cell by oligopeptide permease system. The interaction of PapR and PlcR inside the cell created a complex which consist of 45 genes (Huillet and Gohar, 2016). The transcription factor PlcR, which binds to the intracellular autoinducing peptides (AIP) is produced by the PapR protein and regulates quorum sensing in *B. cereus*. PapR is encoded by seventy base pairs downstream of *plcR*. An amino-terminal (N-terminus) signal sequence on PapR, which is 48 amino acids long, targets the secretory pathway. After leaving the cell, the PapR pro-AIP is processed with neutral protease B, or NprB, to form functional AIP (Pomerantsev et al., 2009). AIP coupled to PlcR triggers

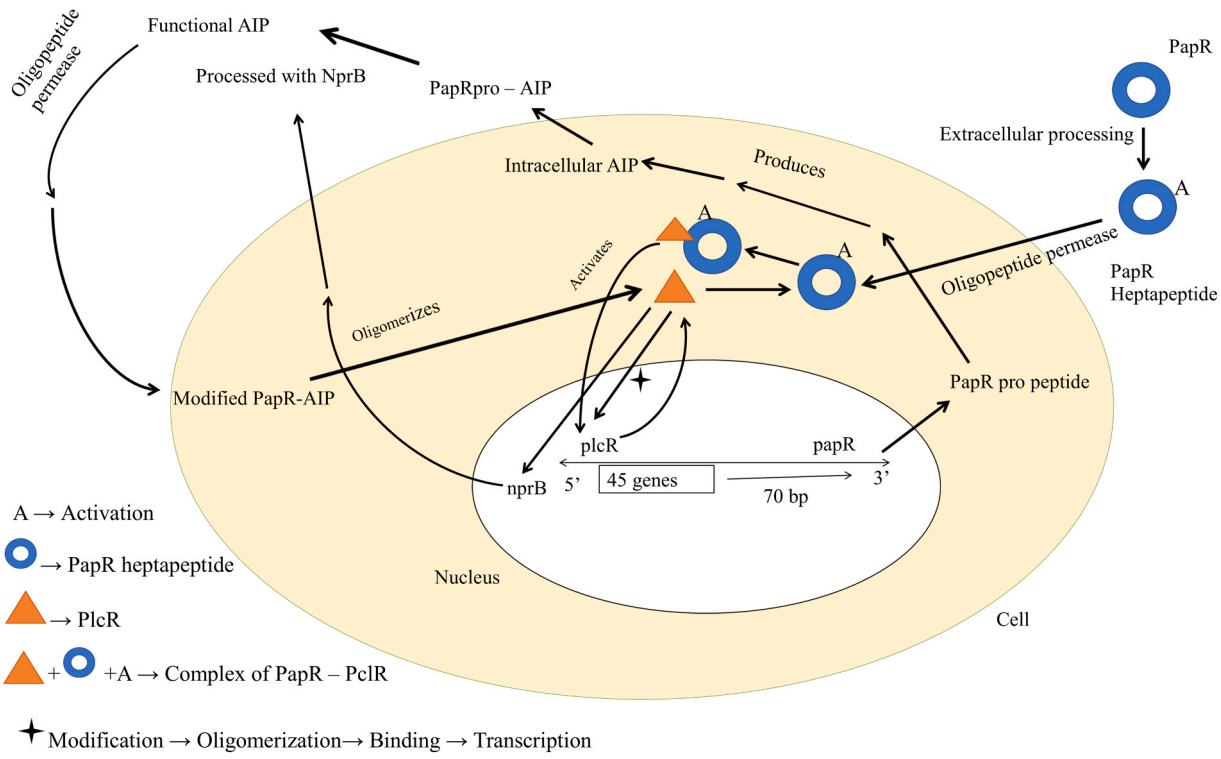

Fig. 4. Schematic diagram of PlcR-PapR QS system.

the expression of *nprB*, which is not encoded in the same way as *plcR*. PlcR activity is induced by only two peptides: pentapeptide and heptapeptide; however, the heptapeptide produces the most activation and is significantly more common in vivo (Bouillaut et al., 2008). The oligopeptide permease process imports the modified PapR AIP to the cell, where it interacts with the transcription factor PlcR, generating structural modifications in the PlcR DNA-binding domain, allowing PlcR to oligomerize, regulate transcription, and bind DNA. When PlcR links with PapR AIP and oligomerizes, it attaches to PlcR regions to control gene transcription as shown in Fig. 4.

The majority of PlcR encode extracellular proteins such as proteases, endotoxins, and phospholipases. Furthermore, the heptapeptide compound PlcRa was found to be crucial in the regulation of cystine genes and oxidative stress-related genes during the stationary growth phase (Huillet et al., 2012). Since PlcR-PapR QS play significant role in expression of the virulence property of *B. cereus* therefore, inhibiting this QS system can be helpful to control the virulence of the bacteria. But anti- PlcR-PapR QS was not available earlier (Rutherford and Bassler, 2012). Recently many studies have reported a synthetic $PapR_7$ which is peptide derivative of 7-mer PapR to inhibit PlcR-PapR QS system (Yehuda et al., 2018, 2019).

A new central regulatory quorum sensor, the RNPP family was identified while elucidating the structure of PlcR which is exclusively available in gram positive bacteria. The QS included in this family are PlcRa and NprR, PrgX, and RAP phosphatases from *B. cereus, Enterococcus faecalis,* and *B. subtilis* respectively. When internal signaling peptide controls the QS system then Rap-Rgg-NprR-PrgX-PlcR (RRNPP) is activated. Recently, the RNPP family is elucidated as RRNPP family (Neiditch et al., 2017a; Declerck et al., 2007). All these QS are activated by the interaction of the tetratricopeptide repeats (TPR) activation domain with the secreted signaling peptides (Huillet and Gohar, 2016). Other than the presence of the helix-turn-helix (HTH) DNA-binding domain also confirms the RRNPP QS system in the bacterial cells (Shi et al., 2005). The conjugation is regulated by the PrgX – cCF10 whereas, competition and sporulation are governed by the Rap phosphatases-Phr peptides system in *E. faecalis* and *B. subtilis* respectively (Perego, 2013).

The virulence and necrotrophism is controlled by the transcriptional regulator or peptide of PlcR – PapR and NprR – NprX in case of *B. cereus (*Grenha et al., 2013; Dubois et al., 2012). Other than this, the infectious and competence of the *Streptococcus salivarius* and various mutants of *streptococci* is controlled by the ComR, an ancient type transcriptional regulator of the RRgg family (Fontaine et al., 2015; Mignolet et al., 2018). Hence, this can be concluded that the RRNPP family is significant for the adaptation and virulence of the bacterial cells to the environment (Neiditch et al., 2017b).

4.4. *Influence of iron and manganese*

Iron (Fe) and Manganese (Mn) are very crucial micronutrients for the biofilm formation of *B. cereus.* Tomter in 2012, have observed that Fe^{2+} and Mn^{2+} can increase the activity of the RNR R2F in *B. cereus*. The rate limiting catalysis of the DNA synthesis is done by ribonucleotide reductase (RNR) where reduction of ribonucleotide to corresponding deoxyribonucleotides happens. The two homodimer subunits R1E and R2E belongs to class Ib RNRs. R1E contains the active site and R2E contains the metallo cofactor and a tyrosyl radical which is responsible for reduction reaction of ribonucleotides. The reconstitute of R2E with Mn^{2+} and Fe resulted in higher activity of the enzyme but the Mn^{2+} increased the activity of the enzyme to 8 times. Therefore, Fe and Mn^{2+} is vital for the DNA synthesis in *B. cereus*(Tomter et al., 2012).

Ferritin is a Fe storage and transporter molecule found in almost all biological organisms. Fe is isolated from host sources in two ways by pathogenic organisms. *B. cereus* produces two types of siderophores: petrobactin (PB) and bacillibactin (BB). The siderophores are ejected into the environment when iron binds, and they are imported into the cell via ABC transporters (Hotta et al., 2010). Bacillibactin (BB) appears to be more significant in *B. cereus* pathogenicity than petrobactin (PB) (Segond et al., 2014). The creation of specialized cell surface receptors that interact directly with host sources of iron is the second method of iron transmission. IlsA, a type of surface receptor, was recently discovered in *B. cereus* (Daou et al., 2009) as part of the Isd system, and had previously been identified in *Streptococcus aureus* and *B. anthracis*

Table 1
Effect of *B. cereus* in the Food industry.

Sr. No	Title	Inference	Author and Reference
1	*Bacillus cereus* in Infant Foods: Prevalence Study and Distribution of Enterotoxigenic Virulence Factors in Isfahan Province, Iran	The prevalence of *B. cereus* enterotoxigenic genes was found in significant concentrations in baby foods in Isfahan, Iran. Over 200 baby food was screened among which 42 % was found contaminated with *B. cereus* spores. The highest enterotoxin gene found was *entFM* (61.90 %) whereas the lowest was found to be *hblA* (13.09 %). The study of *25.5 %* of the *B. cereus* strains shows *bceT* gene as expected endotoxin gene. This study reveals that baby food are the major source of *B. cereus* endotoxin gene and is the major cause of outbreak of the diseases.	(Rahimi et al., 2013)
2	*Bacillus cereus* hazard and control in industrial dairy processing environment	Due to its exceptional capacity to attach to stainless steel surfaces of dairy plants and produce biofilm, *B. cereus* can cause major hygienic issues as well as economic loss owing to spoiling of dairy products and apparatus damage. These biofilms can be a cause of repeated contamination after pasteurization in pasteurizers and storage tanks. *B. cereus* biofilms have been shown to be inconsistently removed by cleaning-in-place (CIP) protocols, which are frequently employed in the dairy industry. The elimination of *B. cereus* biofilm cells is greatly increased by optimizing alkali-based CIP in comparison to the reference CIP that is often utilized in the dairy field. Because these could result in higher-quality products and processes, the dairy sector therefore has to optimize its current cleaning procedures and develop new, successful solutions.	(Kumari and Sarkar, 2016)
3	Chapter 20 - *Bacillus cereus* Food Poisoning	*B. cereus* produces different toxins that causes two types of foodborne illness: (i) diarrheal syndrome and (ii) emetic syndrome. Production of toxins in gut leads to diarrheal illness whereas synthesis of heat stable toxins in foods causes emetic illness. Meat, dairy items and desserts are the common mode of diarrheal illness while emetic illness is commonly transferred through rice. The food sector may be more concerned about the *B. cereus* group in the future due to the advent of psychrotrophic and thermophilic species.	(Griffiths and Schraft, 2017)
4	Preconditioning of the stainless steel surface affects the adhesion of *Bacillus cereus* spores	The spore of *B. cereus* isolated from the dairy industry has greater hydrophobicity than the reference strain ATCC 14579 of *B. cereus*. Conditioning the stainless steel with whole milk allowed better adherence of the spores than the without conditioning the stainless steel. The fat of the whole milk conditioned over the stainless steel has more affinity towards the microbes and allows better adherence. Whereas, in case of without pre-conditioning of the stainless steel, the spores present in the whole milk and fat which are both hydrophobic competes for binding site over the stainless steel have less affinity towards the steel. The Spore adhesion is enhanced in an inadequately cleaned processing line. Controlling spore adherence and formation of biofilm in the dairy sector may be linked to regular washing and sanitization.	(Ribeiro et al., 2017)
5	A Study on Prevalence and Characterization of *Bacillus cereus* in Ready-to-Eat Foods in China	*B. cereus* is found in Ready-to-Eat (RTE) foods. Among the 35 % isolates of *B. cereus*, gene clusters encoding enterotoxin *hblACD* is 39 % and *nheABC* is 83 %. Whereas, 68 % expressed *cytK* gene and 7 % consist of *cesB* an emetic toxin-encoding gene. All the *B. cereus* expressed *entFM* gene. These strains are mostly resistance to *β-lactam* and *rifamycin* antibiotics. 192 distinct sequence types (STs), including 93 novel STs, were identified among the 368 isolates using multi locus sequence typing (MLST). ST26 was the most common ST. These foods contain high number of *B. cereus* as they are not usually heat treated before consumption and may increase the risk of foodborne infections.	(Yu et al., 2020)
6	Incidence and characterisation of aerobic spore-forming bacteria originating from dairy milk in Tunisia. Detection of presumptive *Bacillus cereus* in the Irish dairy farm environment	*B. cereus* was recently discovered in unpasteurized milk at levels of 12.86 %, 23 %, 47.5 %, and 90.0 % in Ethiopia, Ireland, Tunisia, and Turkey, respectively. In Irish dairy industry, from 63 farms, samples of large quantities tank milk, grass, soil, milk sediment filter, rinse water from milking equipment, and tap water were taken. Aside from that, swabs were obtained from the teats of the cows before they were milked, as well as at the beginning and finish of the milking process. 81 isolates out of 98 that underwent sequencing were considered to be probable cases of *B. cereus s.l.* This is considered to be the major cause of spoilage in dairy industry.	(Aouadhi et al., 2014; O'Connell et al., 2016)
7	Occurrence and antibiotic resistance of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in raw milk and dairy products in Turkey. Evaluation of psychrotrophic behavior and lipolytic and proteolytic activity of *Bacillus cereus* isolated from refrigerated dairy products	In a survey in Ankara dairy processing plant, 150 sample of raw milk, white cheese and ice cream were studied. Among which 90 % in raw milk, 70 % in white cheese and 20 % in ice cream, *B. cereus* contamination was found. The isolates of *B. cereus* were shown to be susceptible to cefotaxime, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, and tetracycline, but resistant to ampicillin, penicillin, and trimethoprim/sulfamethoxazole. Of the 85 samples, 15 (17.6 %) included *B. cereus*, and 12 (80.6 %) of those samples were allowed to develop for seven days at 10°C. Four strains (26.7 %) developed after ten days of incubation at 7°C. At 30°C, all identified *B. cereus* strains shown proteolytic activity, and 5 (33 %) of them also displayed lipolytic activity. Six (40 %) and one (6.7 %) of the strains exhibited lipolytic activity during the seven days at 10°C. The	(Gundogan and Avci, 2014; Maike Taís Maziero and Luciano dos Santos, 2012)

(*continued on next page*)

Table 1 (*continued*)

Sr. No	Title	Inference	Author and Reference
		strains' ability to produce lipases and proteases was suppressed by a 7°C temperature for ten days. According to the study, dairy products contain *B. cereus* strains that have lipolytic, proteolytic, and psychrotrophic activities. This could be dangerous for items that are refrigerated.	

(Hayrapetyan, 2017). In another study, Hussain et al., 2018 reported the impact of heme and manganese over biofilm formation of two strains of *B. cereus* ATCC 10987 and ATCC 14579. When the combination of Fe and Mn^{2+} was introduced to BHI growth media, all the strains showed biofilm formation, but it was noticed that the effect of high concentration of Mn^{2+} has greater impact over biofilm formation than heme group. These micronutrients not only increased the cell density and swarming ability of the *B. cereus* cells present in the biofilm but increase sporulation and its resistance towards benzalkonium chloride (Hussain et al., 2018). Other than this, the combination of glycerol and manganese (Mn^{2+}) has been reported to promote *B. cereus* biofilm growth by converting Luria Bertani Broth (LB) a biofilm inert media to strongly biofilm inducing media (Yan et al., 2017). Thus, the micronutrients iron and manganese are very important for biofilm formation and various other cellular activities in *B. cereus*.

5. Principle of resistance

5.1. Antibiotic resistance

The opportunistic bacteria *B. cereus* causes food poising and various nosocomial diseases in immunosuppressed humans. The major enterotoxin produced by *B. cereus* is Nbl and Nhe (Stenfors Arnesen et al., 2008; Bottone, 2010). Mostly the genes involved in the release of the enterotoxins as reported in *B. cereus* are diarrheal such as Cytotoxin K, enterotoxin FM, hemolysin BL and non-hemolytic enterotoxin encoded by *cytk* gene, *entFM, hbl operon and nhe genes* respectively (Granum and Lindbäck, 2012; Walker-York-Moore et al., 2017). The properties of bacteria such as its metabolic activity, motility, biofilm production, virulence and antibiotic resistance are intrinsically linked together (Osman et al., 2018). Most of the bacteria from *B. cereus sensu lato* group shows resistance against the β-lactam antibiotics such as penicillin G and cefotaxime (100 %) as well as combination of amoxicillin/clavulanic acid and ampicillin (99.3 %) but susceptibility to ciprofloxacin (99.3 %), chloramphenicol (98.6 %), amikacin (98.0 %), imipenem (93.9 %), erythromycin (91.8 %), gentamicin (88.4 %), tetracycline (76.2 %) and combination of trimethoprim/sulfamethoxazole (52.4 %) respectively (Fiedler et al., 2019). Savić et al. in 2016 has reported regarding the β- lactamase production and antimicrobial susceptibility. The study reported regarding susceptibility towards imipenem, vancomycin, erythromycin and ciprofloxacin whereas sensitive towards tetracycline and trimethoprim-sulphamethoxazole (Savić et al., 2016). Torkar and Bedenić in 2018 has reported the screening of *B. cereus* isolates from different environment for the antibiotic susceptibility, characterization of different β-lactamase enzymes such as metallo-β-lactamases, extended-spectrum β-lactamases, and carbapenemases and its interaction. They found most of the *B. cereus* isolates to be resistant to either or all the β-lactam antibiotics (Torkar and Bedenić, 2018).

5.2. Spores' heat and chemical resistance

Bacteria adapt to unfavourable environments primarily through sporulation and the production of biofilms (Huang et al., 2021a). Bacterial spores are resistant to environmental stress which their vegetative cells cannot tolerate. These spores are not only resistant to heat but are also resistant to chemical treatments which is major problem in food possessing industries. The unique protective multilayer and its unique composition of spore core results into the resistant properties of the spores (Bressuire-Isoard et al., 2018). One of the major factors that determines the heat resistant property of the spores are their temperature of formation. The higher the temperature of spore formation the higher the temperature of heat resistance (Palop et al., 2007). The properties of the *B. cereus*'s spore such as heat resistance, germination and its outgrowth are the major reason for the various gastrointestinal diseases. The heat resistance and dormancy of the spores are increased by the biofilm formation and swarming of the bacterial cells (Ramirez-Peralta et al., 2012). As *B. cereus* matures and ages, it can produce spores inside of the formed biofilms, but for the most part, these biofilms are made up of vegetative cells (Faille et al., 2014). According to certain research, the percentage of spores in the connected biofilms may range from 0.01 % to 10 % of all the cells, though this may differ according to the strain. Only 10 % of all *B. cereus* cells in the biofilm became spores after 6–12 days of incubation, as demonstrated by Ryu and Beuchat (2005) (Ryu and Beuchat, 2005a). In food processing lines, these interfaces could be a significant source of food contamination, as *B. cereus* has been observed to produce air-liquid biofilms that contain up to 90 % spores (Wijman et al., 2007). Another study demonstrates that submerged biofilms of various *B. cereus* strains on stainless steel slides exhibit high levels (more than 50 %) of sporulation within 48 hours (Faille et al., 2014). According to Hussain and Oh (2018) (Hussain and Oh, 2018), there is a linear correlation between the amount of spore in Brain heart infusion (BHI) medium after three days of incubation and the biofilm formation of food isolates of *B. cereus*. The high cell density and nutrition restriction in the biofilm may account for the increased sporulation efficiency in biofilms (van Gestel et al., 2012; Huang et al., 2020).

The spores are geminated by sensing mechanism. First the receptors responsible for the germination senses the nutrients in the environment and triggers the germination process. In this process, the spores regain its water and burst the outer protective coating and releases vegetative cells. It is seen mostly that the strains which produces emetic toxins can survive in high temperature and hence, their spores are also heat resistant (van der Voort and Abee, 2013a). According to Voort and Abee in 2012, five different conditions effect the sporulation. These conditions determine the size, heat resistance and germination capacity of the emetic toxin producing *B. cereus* strains (van der Voort and Abee, 2013b).

Thus, *B. cereus* spores are a problem because of their resistance to heat and chemical treatments. As known, the heat resistance of the spore is determined by several ecological elements like temperature, medium composition, oxygen concentration, spore germination, mineral availability, and geographical distribution. Other than this, environmental conditions and growth history also effects the sporulation and heat resistance of the *B. cereus*. When *B. cereus* ATCC 10987 and *B. cereus* NIZO 4080 were studied for the sporulation dynamics in different substrate and in wet and dry condition, it was found that spores produced in moist stainless-steel biofilms (SS) had better heat resistance, whereas spores formed in dry biofilms with less water activity (a_w) had lower heat resistance though the exposure to air or drying condition resulted into higher percentage of spore formation (Hayrapetyan et al., 2016). Activation of spores in the dairy sector is most likely to be triggered by heat (Watterson et al., 2014). Pasteurized milk may contain

Table 2
Effect of B. cereus in the clinical field.

Sr. No	Title	Inference	Author and Reference
1	Hot and steamy: outbreak of *Bacillus cereus* in Singapore associated with construction work and laundry practices	During a six-month interval when large-scale construction development adjacent to the hospital fostered extensive pollution of the air and surroundings with *Bacillus spp*., *B. cereus* was collected from 171 patients and 85.4 % of patients manifested with Bacteremia, necessitating lengthy therapeutic sessions. 95 % of the hospital linen kept in the porous canvas bags were contaminated compared to the linen kept in air tight bags. Upgrading the ventilation system, laundry process and bleaching the environment reduced the outbreak but relaxation resulted in to the resurge of the infection cases. For disinfecting linen sodium hypochlorite and acidified bleach is the most effective way, but sodium hypochlorite is active in pH range 6–8 whereas bleach gets inactivated in presence of organic materials. As well as acidified bleach is corrosive material and addition of weak acid to sodium hypochlorite releases chlorine which causes airway irritation. Hence use of these chemicals needs caution. To sum up, the *B. cereus* outbreak highlights the dangers associated with construction near hospitals, especially in areas where people with impaired immune systems reside. It also emphasizes how crucial it is to maintain proper linen storage and laundry procedures, as well as to make sure they are followed.	(Balm et al., 2012)
2	*Bacillus cereus*, a serious cause of nosocomial infections: Epidemiologic and genetic survey	Multiple hospital-cross-contaminations are observed, in which similar *B. cereus* isolates are obtained from various patients and hospital surroundings. Notably linked to food poisoning and eye infections, *B. cereus* also causes a host of other serious infections, including devastating central nervous system infections. Premature newborns were the majority of infected patients, followed by elderly individuals. Over periods of time up to two years, and from different hospital settings, strains with identical profiles were isolated from different patients and/or environment samples. This indicates unequivocally that the same strain of *B. cereus* is able to survive in a hospital setting in spite of regular cleaning protocols and can continue to be a cause of infection for patients in hospital, most likely because of its propensity to produce spores as well as biofilms. Toxins such HBL, NHE, CytK, or cereulide are thought to be the primary cause of *B. cereus* gastrointestinal pathogenesis. Merely 25 % of the strains exhibited significant production of both HBL and NHE, whereas 54 % of the strains shown low production of HBL. In order to get more understanding of *B. cereus*'s pathogenic potential during non-gastrointestinal infections or to determine whether these infections are solely opportunistic, it appears important to recognize other unknown virulence determinants. Notably, three blood samples from patient 18 tested positive for *B. cereus*. Rifampicin susceptibility was present in the first strains, but resistance to the drug was seen in the last strain. This example clearly indicates progressive acquired resistance to rifampicin. Antibiotic resistance profiles need to provide prompt adjustments in patient care and treatment. Insufficient focus may cause a delay in the start of the right treatment, raise the risk of serious infections, and result in poor outcomes.	(Glasset et al., 2018)
3	A multicomponent toxin from *Bacillus cereus* incites inflammation and shapes host outcome via the NLRP3 inflammasome	The ability of the host to recognize microbial components is crucial for mediating a successful immune response. To support replication, cytosolic bacteria need to gain admission into the host cytoplasm. Once inside, they release microbial ligands, which trigger the inflammasome and cytosolic innate immune sensors. The haemolysin BL (HBL), a multicomponent enterotoxin, activates the inflammasome. The *B. cereus* shares a high degree of conservation with this toxin. The lytic pore created by the linear order in which the three HBL subunits bind to the cell membrane causes the NLRP3 inflammasome to get activated, interleukin-1β and interleukin-18 to be secreted, causing pyroptosis. The NLRP3 inflammasome is activated and potassium is efluxed as a consequence of the HBL-induced pore. Moreover, B. cereus that produces HBL causes fast inflammasome-mediated death. MCC950, a pharmacological inhibitor of the NLRP3 inflammasome, stops B. cereus-induced death. Overall, the findings show that the innate immune system's ability to recognize infection is largely dependent on the cytosolic sense of a toxin. By therapeutically altering this route, the host's defenses against lethal bacterial infections are strengthened.	(Mathur et al., 2019)
4	The *Bacillus cereus* Food Infection as Multifactorial Process	*B. cereus* causes the emetic toxicity caused by food poisoning and the diarrheal type associated with food infection harboring enteropathogenic strains. The diarrheal form of food poisoning occurs when live bacteria in the human intestine produces enterotoxins. Essentially, though, a multitude of factors contribute to the disease's manifestation. These factors include the prevalence and survival of *B. cereus* in various foods, the stomach passage's ability to pass, spore germination, motility, and adhesion, and ultimately the intestine's ability to produce enterotoxins. Furthermore, the intestinal microbiota and food items that are consumed have an impact on all of these processes, which means that they must be taken into account in order to accurately forecast the potentially dangerous nature of infected foods.	(Jessberger et al., 2020)

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Table 2 (*continued*)

Sr. No	Title	Inference	Author and Reference
5	*Bacillus Cereus*	*B. cereus* is a rod-shaped, spore-forming, gram-positive bacteria that can thrive in a range of pH levels and temperatures. *B. cereus* illness is highly prevalent and causes bacteremia, septicemia, visual loss, cellulitis, and mortality, among other complications. The majority of serious and systemic disorders are triggered by *B. cereus* ocular infections. Upon penetration of trauma with foreign entity, ocular infections may easily destroy an infected eye.	(McDowell et al., 2021)
6	Inhibition of Collagenase Q1 of *Bacillus cereus* as a Novel Antivirulence Strategy for the Treatment of Skin-Wound Infections	*B. cereus* ColQ1 and *B. cereus csn* have collagenolytic potential and are compared to the biological impact of two small compounds that block collagenases action in *B. cereus* and other pathogens. Collagen existing in the skin is degraded due to collagenolytic activity of recombinantly generated ColQ1 from *B. cereus*, resulting in gaps between the tissue. This makes it easier for bacteria to spread between the gaps. The enzyme elastase LasB synthesized by *Pseudomonas aeruginosa* and the inhibitors of collagenase ColH synthesized by *Clostridium histoliticum* can be employed as inhibitors of *B. cereus* collagenase activity. This approach could allow for effective targeted treatment of *B. cereus*-infected wounds.	(Alhayek et al., 2022)
7	Concerted Action of Sphingomyelinase and Non-Hemolytic Enterotoxin in Pathogenic *Bacillus cereus*.	Several *B. cereus* isolates produce hemolysin BL (Hbl), non-hemolytic (Nhe) enterotoxin, cereulide, an emesis-causing toxin, and cytotoxin K (CytK), all of which are controlled by the transcription factor PlcR. Other virulence factors that have been linked to *B. cereus* toxicity include sphingomyelinase and exoproteases. Sphingomyelinase (SMase) from *B. cereus* is a potent stimulant of death of epithelial cells. By utilizing *sph*, the SMase gene, and *nheBC* deletion mutants in *B. cereus*, it is proved that SMase plays a significant role in the in vitro cytotoxicity and *in vivo* pathogenicity of *B. cereus*. *In vitro*, SMase significantly reduced Nhe-induced cytotoxicity. Additionally, in the insect model *Galleria mellonella*, SMase but not Nhe dramatically increased the larval death rate in vivo. The findings indicate that Nhe and SMase greatly enhance each other to produce complete *B. cereus* virulence and, consequently, disease formation, and that the significance of *B. cereus* SMase as an expressed pathogenic mediator in *in vivo* pathogenicity has been underappreciated.	(Doll et al., 2013)
8	Analysis of enterotoxigenic *Bacillus cereus* strains from dried foods using whole genome sequencing, multi-locus sequence analysis and toxin gene prevalence and distribution using endpoint PCR analysis.	Samples of powdered baby formula (PIF), medicinal fish feed, dietary supplements, and multinational brands of spices from Mexico, South East Asia, and India were among the dry foods from which *B. cereus* strains were recovered. A multiplex endpoint PCR assay for nonhemolytic enterotoxin, hemolysin BL, cytotoxin K, and enterotoxin FM toxin genes was used to assess the genetic diversity of 64 strains from spices and PIF. The strains of *B. cereus* showed 13 distinct toxigenic gene profiles. Bioinformatics techniques were used to sequence a random selection of *B. cereus* strains and compare them with reference Genomic Groups from National Center Biotechnology Information. Alleles from 25 known MLST genes were used to create a thorough multi-loci sequence analysis (MLSA) that was especially made to work with whole genome assemblies. The effectiveness of the 25-MLSA method for quick clustering and identification of Genome Groups was demonstrated using a collection of comparable genomes of strains from a few FDA-regulated commodities, such as medicated fish feed and dry foods. Based on their evolutionary relationships, the strains from dry meals, medicated fish feed, and dietary supplements were grouped together by the analysis. Additionally, 25-MLSA revealed a higher than previously known range of B. cereus strains from meals and feed.	(Carter et al., 2018)

heat-resistant spores that can survive pasteurization and continue to proliferate while stored. Pasteurized milk with spores has a lower organoleptic quality and a shorter shelf life than pasteurized milk without spores (Ghosh and Setlow, 2009; Kumari and Sarkar, 2016). Milk-derived spores appeared to be more heat resistant than spores isolated from the Tryptic Soy Broth (TSB) medium. This could be owing to the presence of milk proteins such as casein and whey protein, which may have attached to the spore surface and protected it from heat (Huang et al., 2021a; Anema, 2020; Huang et al., 2021b). Shehata and Collins in 1972 reported that *Bacillus*'s psychrophilic strains found in skimmed milk can be treated by two heating steps. Though vegetative cells of this stain are destroyed by heating at 65 ℃ but not all the spores. Most of the spores about 99 % is destroyed by heating milk at 87.8℃ for 20 sec and then store it at 37 ℃ for 4 hrs and again repeated heating at 76.7℃ for 20 sec. One step heating of the skimmed milk at 87.8℃ for 20 sec results in only 37 % destruction of the spores. Hence, two step heating provides better sterilization of the skimmed milk (Shehata and Collins, 1972). Similar kind of contamination and spore formation and resistance is also seen in other cooked foods such as cooked rice by the *B. cereus*. The spores isolated from the cooked rice were found to be resistant to 95℃. Different isolated showed different spore and heat resistant properties as reported by the Parry and Gilbert in 1980 (Parry and Gilbert, 1980).

Hence, methods are required to destroy spore or to suppress its germination. Ryu and Beuchat in 2005 reported that the high relative humidity which is equal to or greater than 97 % and exposure to air of *B. cereus* biofilm results into higher spore formation. Even the spore and vegetative cells embedded in biofilm is protected from the inactivation by sanitizers. But it was also reported that lowering the relative humidity decrease the spore formation. Even treatment of the stainless-steel coupons with biofilm dipped in tryptic soy broth (TSB) at 22 ℃ with peroxyacetic acid-based sanitizer (Tsunami 200, 40 microg/ml), chlorine dioxide (50 microg/ml), and a chlorine (50 microg/ml) for 5 minutes reduced the vegetative cells as well as spores. The spores which survived the treatment of chlorine dioxide were more heat labile. Hence, this study enlightens the new possibility of sanitization and

controlling of spores in *B. cereus* (Ryu and Beuchat, 2005b). Other than this, as sanitizers cannot be a choice to control spore formation in food therefore, to prevent *B. cereus* sporulation and proliferation, warm meals must be kept at temperatures over 60°C and cold foods at temperatures below 4°C (Pal et al., 2014).

6. Impact of *B. cereus* on the food industry

The presence of *B. cereus* in the food industry is a major topic of concern because it is an infectious and spoilage-causing bacteria and it is easily transmitted. Biofilm formation is a significant problem since biofilms are difficult to remove from attached surfaces (Halstead et al., 2015). *B. cereus* strains have been found in a variety of foods, including dairy and confectioneries, baby meals, seasonings, whole grain, cereals, vegetables, cocoa, chocolates, and ready-to-cook food (Pagedar and Singh, 2012; Tewari and Abdullah, 2015).

Their spores are hydrophobic and very resistant, adhering firmly to inert surfaces. *Bacillus* spores and biofilms in food-processing industries can contaminate any surface. Rubber, stainless steel, gaskets, conveyor belts, and plastic surfaces are examples of inert surfaces (Hussain and Oh, 2017; Harada and Nascimento, 2021). Depending on the species or strain, the surface of cold rooms as well as process line apparatus may become contaminated (Kumari and Sarkar, 2014a, 2014b). Some of the other food hazards caused by *B. cereus* is mentioned in Table 1.

The ability of *B. cereus* to generate biofilms at 25 or 30°C was investigated for food processing regions such as stainless steel (SS), PC, or glass slides (GS). When compared to SS, the amount of biofilm generated on plastic slides (PS) and glass slides (GS) was much lower ($p < 0.05$). These findings indicated that for *B. cereus* food isolates, biofilm formation on stainless steel was significantly higher than on plastic slides (PS). Furthermore, pathogens such as *Salmonella sp*. and *B. cereus* are commonly associated with low moisture foods (LMFs). Hygiene maintenance in LMF production facilities is required on a regular basis to minimize moisture buildup.

Ultraviolet (UV-C) radiation, gaseous ozone, dry heat, and alcohols are examples of dry treatments that do not leave dangerous residues. Fast evaporation, minimal leftovers, and penetrating impact are three of their main advantages (Thomas, 2012). When stainless steel and polypropylene were exposed to ethanol, a drop of roughly 1.0 log CFU/cm^2 in *B. cereus* biofilm was observed, (Kim et al., 2019), as well as a decrease of 2.1 log CFU/ml on stainless steel when exposed to Ultraviolet (UV-C) radiation (HA and HA, 2010). As a result, dry sanitization is a crucial part of ensuring food safety.

7. Impact of *B. cereus* on the dairy industry

Milk is perishable and susceptible to contamination by bacteria of the *Enterobacter*, *Streptococcus*, *Bacillus*, and *Pseudomonas species*. Manufacturing, handling, and processing are all methods for *B. cereus* to infiltrate the dairy system. Biofilm formation in the dairy industry is impacted by several factors. Equipment used in the processing of dairy products is exposed to a wide range of temperature and pH, and poorly treated equipment can serve as breeding grounds for biofilm communities. However, little is known about the effects of time, temperature, and pH on *B. cereus* adhesion to the surfaces of dairy equipment (Peña et al., 2014).

B. cereus was recently discovered in unpasteurized milk at levels of 12.86 %, 23 %, 47.5 %, and 90.0 % in Ethiopia, Ireland, Tunisia, and Turkey, respectively (Aouadhi et al., 2014; O'Connell et al., 2016). In United States, Turkey, and Brazil, the pathogen was discovered in 17.64 %, 20 %, and 24.23 % of dairy products, respectively (Gundogan and Avci, 2014; Maike Taís Maziero and Luciano dos Santos, 2012). Milking machinery, silos, and pasteurizers are the main sources of *B. cereus* infection on dairy farms. Research indicates that *B. cereus* spores retrieved from dairy silos after hot alkaline treatments may still be able to germinate and form biofilms (Pagedar and Singh, 2012; Shaheen et al., 2010). According to a recent study in ten local dairy farms in Beijing, a high incidence of *B. cereus* (9.8 %) was found in unpasteurized milk, (Cui et al., 2016). Furthermore, Lin et al. in 2017 reported that after the ultra-high temperature sterilization (UHT) treatment, only a small amount of *B. cereus* was identified in the finished products, and that their distribution in UHT dairy processing plants was uneven (Lin et al., 2017).

Biofilm contamination is common in dairy products. Due to their hydrophobicity and endurance to high temperatures, dryness, and cleaning chemicals, *B. cereus* endospores can attach to manufacturing equipment and withstand cleaning processes (Srey et al., 2013). This complicates the removal of *B. cereus* from the dairy industry. Hazard Analysis and Critical Control Points - HACCP – are crucial in dairy processing plants to reduce the preliminary load of *B. cereus* in final products (Kumari and Sarkar, 2016).

8. Impact of *B. cereus* in the clinical field

In the clinical setting, bacterial biofilm plays a critical role in a variety of illnesses and disorders. Bacterial biofilms, as previously stated, are resistant to all disinfectants and biocidal agents. It also aids in evading the defense mechanisms of the human body (Høiby et al., 2011).

B. cereus is mostly recognized for causing food poisoning and other disorders. Extra gastrointestinal infections, diarrhea, and emetic symptoms are all frequent gastrointestinal illnesses. Though this sickness has a low fatality rate, patients with immunodeficiency, babies in the neonatal stage, and intravenous drug addiction with contaminated needles or drugs are at risk. This illness can also infect the inner eye, resulting in visual loss. This is known as endophthalmitis. The signs of this eye infection include a corneal ring abscess with growing discomfort, retinal hemorrhage, perivasculitis, chemosis, and proptosis, as well as widespread malaise, fever, and leukocytosis. Bacteremia and endocarditis are two more infections to be aware of. Bacteremia is caused by intravenous medicines, catheters that are accessible from the central venous system, immunocompromised patients, and mucosal injuries caused by neutropenia. In the case of *B. cereus* bacteremia, involvement of secondary nervous system and altered awareness are common. External insertions such as prophetic valves or pacemakers, catheters, and the use of intravenous medications are all linked to *B. cereus* endocarditis. This infection has been shown to penetrate soft tissue, bones, and mucosal injuries. Other sites of infection include burn wounds, gunshot wounds, animal attacks, and open fractures. Chronic osteomyelitis, soft tissue necrotizing, and cellulitis infection are all caused by *B. cereus*. Keratitis and the usage of contact lenses can cause corneal abrasion (McDowell et al., 2021). Other impacts of *B. cereus* in clinical sector are mentioned in Table 2.

Even though numerous surgical procedures and preventive medications are available, infections caused by *B. cereus* in wounds remain a public health concern, (Alhayek et al. in 2022). Rather than targeting the bacteria itself, they believe that targeting the toxins generated by the bacteria has intriguing uses. A lot of research has been done on *B. cereus* exoenzymes, but not on the collagenase enzyme in the event of wound infection. As a result, collagenase has been isolated and characterized, and its collagenolytic activity has been reported. Collagen existing in the skin is degraded due to collagenolytic activity of recombinantly generated ColQ1 from *B. cereus*, resulting in gaps between the tissue. This makes it easier for bacteria to spread between the gaps. The enzyme elastase LasB synthesized by *Pseudomonas aeruginosa* and the inhibitors of collagenase ColH synthesized by *Clostridium histolyticum* can be employed as inhibitors of *B. cereus* collagenase activity. This approach could allow for effective targeted treatment of *B. cereus*-infected wounds. In-vivo research has yielded encouraging findings in this area (Alhayek et al., 2022).

Several *B. cereus* isolates produce hemolysin BL (Hbl), non-hemolytic (Nhe) enterotoxin, cereulide, an emesis-causing toxin, and cytotoxin K (CytK), all of which are controlled by the transcription factor PlcR. Other

Table 3
The beneficial aspects of B. cereus biofilm.

Sr. No.	Title	Inference	Author and Reference
1	Biological Control of Damping-Off of Alfalfa Seedlings with *Bacillus cereus* UW85	*B. cereus* UW85 has the potential to act as biocontrol agents for alfalfa damping-off and it is an advantage over conventional disease management measures. Filtrates of UW85 cultures with a majority of vegetative cells or endospores within the parent cell had minimal biocontrol activity; fully sporulated cultures with mostly released spores and sterile filtrates of such cultures were effective in preventing seedlings from damping-off. More biocontrol activity was shown by cultures growing in two semidefined media than in the multifaceted tryptic soy medium.	(Handelsman et al., 1990)
2	Properties of the *Bacillus Cereus* strain used in probiotic CenBiot	*B. cereus* CenBiot spores that suppressed *E. coli* and *Yersinia pseudotuberculosis* proved harmless for suckling and mature mice and it was not inhibited by antibiotics at trace levels. As a result, it is applied as a probiotic.	(Gil-Turnes et al., 1999)
3	Production and characterization of lipopeptide from *Bacillus cereus* SNAU01 under solid state fermentation and its potential application as anti-biofilm agent	Peanut oil cake was utilised like a novel substrate for *B. cereus* SNAU01 to develop lipopeptide biosurfactant during solid state fermentation, as well as its applicability as an anti-biofilm agent. Based on Response Surface Methodology (RSM) data, the optimal circumstances for generating the maximum quantity of biosurfactant compounds include 8.18 g of peanut oil sheet as the substrate, inoculum of 2.5 ml, and pH 7 at 30 °C. FT-IR, TLC, and GC-MS were used to describe the biosurfactant and verified that B. cereus SNAU01 lipopeptide was present. Confocal Laser Scanning Microscopy (CLSM) demonstrated that the SNAU01 lipopeptide effectively removed biofilm from the glass surface. At 250 μg/ml, the SNAU01 lipopeptide disrupted the biofilm more effectively against the chosen pathogenic strains.	(Nalini et al., 2016)
4	Chapter 10 - *Bacillus cereus* Biocontrol Properties	Zwittermicin A formed by *B. cereus*; has a variety of biological functions, including the inhibition of microbiological infections in plants and the enhancement of the insecticidal efficacy of *B. thuringiensis*. The *B. cereus* strain UW85's ability to inhibit plant diseases led to the discovery of zwittermicin A, a linear aminopolyol. Given that certain Gram-negative, Gram-positive, and fungal species may be inhibited, it has a broad spectrum of activity. It appears that zwittermicin A generally inhibits Gram-positive bacteria less than Gram-negative bacteria. Additionally, *B. cereus* strains that fail to generate the antibiotic are active in the *B. thuringiensis* protein toxin, which increases the mortality of insects that are normally resistant to killing, such as gypsy moths raised on willow leaves that are generally susceptible to it. Curiously, it increases the insecticidal effect as well.	(Savini, 2016)
5	Beneficial effects of bio-controlling agent *Bacillus cereus* IB311 on the agricultural crop production and its biomass optimization through response surface methodology	*B. cereus* IB311 can be employed as a bio-control agent to avoid plant infections and boost the agricultural productivity. In ground nut (Arachis hypogaea var. Koushal, G201) and sesame (Sesamum indicum var. Kanak), respectively, the results showed that *B. cereus* IB311 has increased the production by 20 % and 26 % in terms of average pod number per plant, average seed number per pod, and seed yield per experimental plot.	(Banerjee et al., 2017)
6	Microbial Lipid Accumulation through Bioremediation of Palm Oil Mill Wastewater by *Bacillus cereus*	In future, *B. cereus* may be a promising bacterium for lipid accumulation via bioremediation of palm oil mill effluent (POME). Microbial oil synthesis and concurrent remediation of POME by *B. cereus* would be an appealing approach for achieving the joint objective of renewable energy generation and ecological flexibility. Having a lipid content of 18.04 % (dry weight basis), the 50 % (v/v) POME was shown to have the greatest potential for biomass development (8.09 g/L) and lipid accumulation (1.46 g/L). Under comparable circumstances, *B. cereus* accumulated more biomass and lipid than other bacterial strains like Rhodococcus opacus and Pseudomonas aeruginosa. Conversely, the level of bioremediation was measured by calculating the Vigna radiata seed germination index (GI) and analyzing a number of effluent characteristics. Because POME treated with *B. cereus* significantly remedied the harmful organics present in the POME, the treated samples showed higher GI values than the samples that were untreated. This result was further supported by the significant decrease in pollutant load, namely in chemical and biochemical oxygen demand (COD and BOD) for 50 % POME, which showed effective removal rates of 79.35 % and 72.65 %, respectively. Thus, in conjunction with POME bioremediation, the results of this work indicate that *B. cereus* growing in POME may be a promising strategy for achieving increased biomass growth and lipid synthesis.	(Karim et al., 2019)
7	Production, characterization, evaluation and toxicity assessment of a *Bacillus cereus* UCP 1615 biosurfactant for marine oil spills bioremediation	As a biosurfactant and bioremediation strain, *B. cereus* UCP 1615 has the application in industrial production and oil spill-polluted aquatic areas. on order to create a biosurfactant, *B. cereus* was grown on a mineral medium containing 0.12 % peptone and 2 % frying oil in this investigation. The production process was expanded from flasks to 1.2-, 3.0-, and 50-liter bioreactors, achieving 28.7, 27.5, and 32 mN/m of surface tension and 4.3, 4.6, and 4.7 g/L of biosurfactant concentration, respectively. The biosurfactant was identified as anionic, but its lipopeptide origin was	(Durval et al., 2020)

(*continued on next page*)

Table 3 (*continued*)

Sr. No.	Title	Inference	Author and Reference
		shown by nuclear magnetic resonance, thin-layer chromatography, and gas chromatography investigations. The application of this biosurfactant in the depollution of the marine environment is suggested by the survival rates of the bivalve *Anomalocardia brasiliana* and the fish *Poecilia vivipara*, which were found to be more than 90 and 55 %, respectively, in toxicity tests. Furthermore, the biosurfactant promoted autochthonous microbe growth in seawater bioassays without requiring the inclusion of motor oil. These findings show the biosurfactant's biocompatibility, potential for large-scale synthesis, and potential for use in the bioremediation of the marine environment that has been contaminated by oil spills.	
8	Petroleum Depletion Property and Microbial Community Shift After Bioremediation Using *Bacillus halotolerans* T-04 and *Bacillus cereus* 1–1	*B. cereus* T-04 and *B. halotolerans* 1–1 are two crude-oil-degrading strains exhibiting significant crude oil bioremediation efficiency. Under different circumstances, the crude oil degradation of every strain was examined. The ideal pH, 15–20 g/L NaCl content, and 5–15 g/L initial oil concentration were found to be responsible for the best oil degradation rates for both strains. Following 20 days of therapy, the rate of crude oil depletion could increase to 60–80 %. Experiments simulating the bioremediation of crude oil showed that the bioremediation significantly accelerated the exhaustion of crude oil. After receiving treatment for 180 days, the inoculum group using the inorganic medium had the greatest crude oil depletion (97.5 %), whereas the control group's crude oil depletion was just 26.6 %.	(Deng et al., 2020)
9	SigB regulates stress resistance, glucose starvation, MnSOD production, biofilm formation, and root colonization in *Bacillus cereus* 905	*B. cereus* 905 growth on wheat roots is aided by sigB and sodA2. Under stressed conditions, SigB of *B. cereus* 905 leads rhizosphere survival and root colonisation. Furthermore, the sigB mutant also showed a deficiency in biofilm development under stressed conditions. The bacterium's capacity to produce biofilms and efficiently use carbon sources is what leads to root colonization. Additionally, the manganese-containing superoxide dismutase (MnSOD2), which is encoded by the sodA2 gene, is essential to *B. cereus* 905's survival in the wheat rhizosphere. According to this study, *B. cereus* 905's resistance to unfavorable climatic circumstances is partly explained by the sigB gene-encoded alternative sigma factor σ^B being activated. When the sigB mutant cells were subjected to ethanol, acid, heat, oxidative stress, or glucose starvation, their survival rate dropped dramatically. A partial, σ^B-dependent activation of the sodA2 gene was found following glucose starvation or paraquat treatment, according to analysis of the gene's transcription.	(Gao et al., 2021)
10	The *Bacillus cereus* Strain EC9 Primes the Plant Immune System for Superior Biocontrol of *Fusarium oxysporum*	Both antifungal and non-antifungal *Bacillus* strains, may defend the Kalanchoe plant from the soilborne fungus *Fusarium oxysporum*. According to whole-genome sequencing, the non-antifungal strain EC9 did not have any of the biosynthetic gene clusters linked to commonly used antimicrobial substances. Rather, this strain of bacteria appears to guard Kalanchoe plants by stimulating immunity since it causes marker genes for the salicylic acid and jasmonic acid defense pathways to express, but only after a pathogen assault. For the advancement of innovative biological crop protection products, we propose that one promising method of interacting with biological control agents (BCAs) is the induction of the plant immune system.	(Madriz-Ordeñana et al., 2022)
11	Quorum Sensing Inhibitory and Quenching Activity of *Bacillus cereus* RC1 Extracts on Soft Rot-Causing Bacteria Lelliottia amnigena.	One of the beneficial effects of *B. cereus* RC1 is, it can control soft rot of the carrot, potato, and cucumber by 91.22 %, 97.59 %, and 88.78 % respectively. The GC–MS evaluation of the *B. cereus* extract and the zone of diffused metabolites revealed diketopiperazine as the predominant metabolite. *B. cereus* RC1 release a variety of metabolites that function as quorum quenching molecules and have the ability to restrict biofilm development in the soft rot-causing pathogen *L. amnigena* RCE and also production of pyocyanin in the monitor strain *P. aeruginosa* MTCC2297. It's possible that metabolites from the agar well travel through the agar and changed the pathogen's development pattern. Several metabolites were shown to be downregulated in the extract even in the pathogen's liquid culture.	(Kachhadia et al., 2022).

virulence factors that have been linked to *B. cereus* toxicity include sphingomyelinase and exoproteases (Doll et al., 2013). *B. cereus* food-borne illnesses are caused by these toxins. Emesis and diarrhea are two symptoms caused by *B. cereus*, which are prompted by a variety of toxins. Cerulide, the emetic toxin, is a cyclic 1.2-kDa dodecadepsipeptide. The emetic toxin is not inhibited during food preparation or stomach passage due to its great resistance to thermal treatment, pH fluctuations, and proteolytic breakdown. Enterotoxins produced in the stomach, where cells and/or spores penetrate and proliferate after consuming infected foods, cause diarrhea (Ceuppens et al., 2012).

B. cereus and *B. subtilis* are the most common spoilage bacteria in the dairy business. They produce extracellular enzymes that degrade milk proteins and additives, shortening the shelf life of pasteurized milk and milk products. The risk of foodborne illness has been investigated by Kumari and Sarkar in 2016 in *B. cereus* isolates from Norwegian farms. None of the 39 isolates were particularly toxic when tested at 37°C, indicating that they pose a modest risk of diarrheal food poisoning (Kumari and Sarkar, 2016). When cultivated at 25°C or 32°C, however, some of the 39 isolates were slightly or highly cytotoxic. Heat-stable proteases and lipases are produced by *B. cereus* strains from diverse

milk products, survive even after pasteurization and may cause complications in finished goods (Kumari and Sarkar, 2014a, 2014b; Lücking et al., 2013). *B. cereus* possesses enterotoxigenic potential, according to a study by Carter et al. in 2018 (Carter et al., 2018). Enterotoxin gene expression and toxin production are strain-specific, and environmental factors such as temperature, carbon sources, and oxygen concentration all play a role (Réjasse et al., 2012). Furthermore, CodY, a global regulator, inhibits biofilm formation and may indirectly induce enterotoxin production in *B. cereus*, which is also a crucial player in *B. cereus* emetic pathophysiology (Lindbäck et al., 2012). *B. cereus* can produce biofilms and release metabolites, toxins, and enzymes within the biofilm matrix. When bacteria are stressed, biofilms provide a haven for them, which could also be a good place for toxins to accumulate. Toxins have the potential to play a significant role in the biofilm community. EntFM, a rarely studied enterotoxin produced by *B. cereus* and related with cell wall peptidases - CwpFM, was thought to be involved in biofilm production (Tran et al., 2010), but this has yet to be confirmed. *B. subtilis* can also produce YIT, a biofilm-associated toxin that can be used to target competitors in biofilm communities, (Kobayashi and Ikemoto in 2019). It's debatable if biofilms emit toxins or are associated with *B. cereus* biofilm populations (Kobayashi and Ikemoto, 2019).

B. cereus-based gastrointestinal infections are uncommon, and their severity can only occur in people who are immunocompromised. Septicemia, gangrene, cellulitis, mortality, and other serious symptoms can occur in people with an extraintestinal infection. This infection may be avoided if people were taught to practice excellent personal hygiene and hand washing (McDowell et al., 2021).

9. Biofilms of *B. cereus* as other prospects

As previously stated, B. cereus is primarily responsible for various diseases, food spoilage, and loss in the food industry. It also plays an important role in a variety of other fields such as biocontrol agents, anti-biofilm agents, bioremediation, and biosurfactants, among others. B. cereus as a biocontrol agent is one of these applications that has received a lot of attention. Some of these investigations are featured in this review. Table 3. lists the specifics of some of the beneficial prospects.

Savini in 2016, reported on Zwittermicin A, an antibiotic family generated by *B. cereus*, has several functions in plant biology, including regulating diseases caused by microorganisms or potential insects such as *B. thuringiensis*(Savini, 2016). Another beneficial application of *B. cereus* IB311 strain is its ability to act as a biocontrol agent and improve agricultural crop productivity. As the bacterium is an antagonist to plant pathogens, it increased agricultural yield (Banerjee et al., 2017). *B. cereus* has been explored as a biocontrol agent in recent years, and it was also well-known in the 1990s. For example, the infection of Alfalfa, which causes seedling death or weakness owing to pathogenic microbe invasion, was discussed by Handelsman et al.in 1990. This is most common in moist-chilly conditions. The presence of *B. cereus* has been revealed to lower mortality and improve plant health (Handelsman et al., 1990). Other applications of *B. cereus* include the study of biofilm formation and stress resistance in plants using *B. cereus* 905 root colonization (Gao et al., 2021), the biosynthesis of bio-surfactant using marine oil recovered from spills using *B. cereus* UCP 1615 as a bioremediation method (Durval et al., 2020), the degradation of petroleum using *Bacillus halotolerans* T-04 and *B. cereus* 1–1 (Deng et al., 2020), lipid accumulation from wastewater of palm oil mill using *B. cereus* (Karim et al., 2019), and enhancing the immune system of plants from infection of *Fusarium oxysporum* using *B. cereus* EC9 primes (Madriz-Ordeñana et al., 2022), and synthesis of lipo-peptide based anti-biofilm agent from *B. cereus* SNAU01 for application in fermentation technology (Nalini et al., 2016). As a result of this research, the potential for beneficial applications for human use has been investigated.

10. Conclusion and future perspective

B. cereus offers both advantages and disadvantages, which need to be investigated further with greater initiatives. *B. cereus* biofilm has several contributions to beneficial roles, food toxicity, and diseases, as stated above in Sections 4, 5 and 6. Several *B. cereus*-related pathogenic diseases in various organs have not been thoroughly studied despite frequent research in the food sector. As biofilm development plays a significant role in stress and antibiotic resistance, a complete investigation of *B. cereus* biofilm formation and its significance in pathophysiology of diseases is necessary. Biofilm is made up of EPS, which can be used to extract sugar molecules, lipids, and other polymer-synthesizing substances so that polymer-based composites can be utilized for industrial use and medical use. Moreover, EPS can accumulate antibiotics and metals, acting as a bioremediation tool by preventing their release into the environment. As a result, *B. cereus* biofilm plays an important role in bioremediation, which needs to be investigated further. The potential of *B. cereus* as a probiotic has received little attention, and more research is needed. Therefore, a substantial number of research opportunities remain unexplored which must be studied in future.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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