Germinants and Their Receptors in Clostridia

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Many anaerobic spore-forming clostridial species are pathogenic, and some are industrially useful. Although many are strict anaerobes, the bacteria persist under aerobic and growth-limiting conditions as multilayered metabolically dormant spores. For many pathogens, the spore form is what most commonly transmits the organism between hosts. After the spores are introduced into the host, certain proteins (germinant receptors) recognize specific signals (germinants), inducing spores to germinate and subsequently grow into metabolically active cells. Upon germination of the spore into the metabolically active vegetative form, the resulting bacteria can colonize the host and cause disease due to the secretion of toxins from the cell. Spores are resistant to many environmental stressors, which make them challenging to remove from clinical environments. Identifying the conditions and the mechanisms of germination in toxin-producing species could help develop affordable remedies for some infections by inhibiting germination of the spore form. Unrelated to infectious disease, spore formation in species used in the industrial production of chemicals hinders the optimum production of the chemicals due to the depletion of the vegetative cells from the population. Understanding spore germination in acetone-butanol-ethanol-producing species can help boost the production of chemicals, leading to cheaper ethanol-based fuels. Until recently, clostridial spore germination is assumed to be similar to that of Bacillus subtilis. However, recent studies in Clostridium difficile shed light on a mechanism of spore germination that has not been observed in any endospore-forming organisms to date. In this review, we focus on the germinants and the receptors recognizing these germinants in various clostridial species.

Historically, bacteria found in the genus Clostridium were classified by their bacillus-like shape, anaerobic growth requirements, and ability to form spores (1). However, with the advent of more sophisticated methods for taxonomy (e.g., multilocus sequence analysis), clostridia have recently undergone a diversification in genus. Although the names of several clostridial species have changed, the fact that these organisms cause public health threats or are industrially important has not.

Many clostridia generate industrially relevant organic compounds, and they play a crucial role in biodegradation and industrial production of a large set of metabolites (e.g., acetone, butanol, ethanol, butanediol, propanol, acetoin, butyrate, and acetate) (2). As examples, Clostridium acetobutylicum and Clostridium butyricum are well known to produce acetone-butanol-ethanol (ABE) products during industrial fermentation (3). Clostridium beijerinckii can be used to produce butanol (4), while Clostridium cellulolyticum can use cellulose as a carbon source and generate lactate, acetate, and ethanol as valuable end products (5). Finally, Clostridium pasteurianum converts algal biomass to commercially useful butanol, ethanol, and propanediol (6).

Apart from their value in chemical production, other clostridial species are known to cause major human, animal, and economic losses in a variety of industries (7, 8). Clostridium perfringens is well known to cause foodborne illnesses as a result of contamination of food sources (9, 10), and several other clostridia have been shown to spoil food (11–13). Other clostridia also are known for their roles as pathogens of humans and animals. For example, Clostridium botulinum produces the acutely lethal botulinum toxin and is considered a potential bioterror agent due to the potent activity of the neurotoxin, which causes a fatal neuro-paralytic illness (14). Moreover, Clostridium tetani secretes the potent tetanosasmin neurotoxin that elicits the primary symptoms of tetanus disease, leading to an estimated 500,000 worldwide fatalities per year (15). Apart from its ability to cause food spoilage, C. perfringens causes a range of diseases from myonecrosis (gas gangrene) to foodborne and nonfoodborne gastrointestinal illnesses in both humans and animals (16). Finally, the Centers for Disease Control and Prevention listed Clostridium difficile (recently proposed to be renamed as Peptoclostridium difficile [17]) as an immediate and urgent threat to the public, due to it causing ~500,000 infections/year, approximately 29,000 deaths, and nearly $4.8 billion in treatment-associated costs (18).

Although many clostridia are important human pathogens, some may have potential for treating or controlling human diseases. Clostridium novyi, a soil-dwelling organism incriminated in wound associated gangrene and infections in intravenous (i.v.) drug users (19), was shown to be important in treating tumors (20–23). Moreover, Clostridium bifermens subsp. malaysia is active against a host of mosquito genera, especially Anopheles, a carrier of malarial parasites (24, 25).

Many clostridia survive in the environment in the dormant spore form. Spores are metabolically dormant forms of bacteria and, for many spore-forming pathogens, the spore is the infectious form (26). The majority of the sporulation process is conserved across species of endospore-forming bacteria. Bacillus subtilis has served as a model spore former for decades, and most of the processes of spore formation and germination were elucidated in this organism. Although there are certainly differences between...
**Minireview**

**FIG 1** Representation of Ger- and Csp-type receptors in the spore. (A) GerAA, GerAB, and GerAC are located within or on the inner spore membrane, and their location in clostridia is based mostly on what is observed in *B. subtilis*. The topology of Ger-type germinant receptors for clostridia is unknown. (B) The *C. difficile* germinant receptor complex contains the bile acid germinant receptor CspC and the CspB protease CspA and may be localized to the outer membrane by lipidated GerS.

*Bacillus* and *Clostridium* spore formation and germination, the spore form itself is largely conserved (27). Sporulation begins with the phosphorylation of the master sporulation transcription regulator Spo0A. Subsequently, the vegetative cell then divides asymmetrically into a smaller forespore and larger mother cell. Through coordinated gene expression between the forespore and the mother cell, the mother cell engulfs the forespore, and the smaller compartment matures into a metabolically dormant spore (28, 29). The spore form is composed of a partially dehydrated DNA-containing core surrounded by an inner cell membrane. In the core, much of the water is replaced with Ca²⁺-dipicolinic acid (DPA), which helps maintain spore dormancy and protect the spore from UV radiation and heat (30). The core is also packed with small acid-soluble proteins (SASPs) that bind and protect the DNA from radiation, heat, and genotoxic chemicals (30, 31). Surrounding the inner membrane is a germ cell wall composed of the typical N-acetylmuramic acid (NAG)- and N-acetylmuramiduramic acid (NAM)-containing peptidoglycan (Fig. 1). The germ cell wall is surrounded by a peptidoglycan-like cortex layer. The cortex is a specialized peptidoglycan, where much of the NAM residues are converted to muramic-δ-lactam (MAL) residues that are recognized by spore cortex lytic enzymes (SCLEs) during the process of germination. Surrounding the cortex layer is an outer membrane and a thick proteinaceous spore coat (Fig. 1). In some spore-forming bacteria, the coat is surrounded by an exosporium (31, 32).

During sporulation, the factors required to initiate the transition from a metabolically dormant to a metabolically active state, germination, are built into the spore (28). Germination is stimulated when the spore responds to an environmental signal (germinant) (33). The signals that stimulate germination can be a variety of small molecules, and these germinants activate germination by binding to specific germinant receptors (33). In most spore-forming organisms studied to date, the activated germinant receptor then propagates a signal to a channel that is important for releasing the DPA from the core (27, 33, 34). Subsequently, an SCLE is activated, which then degrades the cortex. Cortex degradation leads to swelling and further hydration of the core. Finally, a vegetative cell grows out from the germinated spore (35).

Germination has been studied, largely, in bacilli. With the development of new genetic tools, the mechanisms of germination in clostridia are being examined in detail (36–40). Here, we review the germinants and receptors that have been identified in clostridia.

**GERMINANTS**

Although dormant, spores respond to germinants that stimulate the return to vegetative growth using proteins specific to the signal (germinant receptors). Commonly, germinants are low-molecular-weight biomolecules found in the environment where growth of the organism is favored. Germinants, most commonly, are amino acids, but other molecules (e.g., cholesterol-based compounds, organic acids, nucleosides, peptidoglycan fragments, etc.) have been identified (41–46). Although germination of some spores can be triggered by a single germinant, some spores require the presence of more than one type of molecule to stimulate germination (35). Table 1 summarizes the known germinants, the combinations required to stimulate germination, and inhibitors of germination of the discussed clostridia. Below, we discuss the germinants and inhibitors of germination in the context of potential uses for the germinated organisms.

**CELL WALL PRECURSORS**

Similar to what is observed in bacilli, the most common germinant in clostridia is L-alanine (32). The reason(s) behind L-alanine functioning as the favored germinant across many spore-forming organisms is not known but may be linked to the conservation of peptidoglycan structure (47). Peptidoglycan is composed of alternating NAG and NAM residues, and each NAM includes a stem peptide that is composed of 5 amino acids. In many organisms, this structure is NAM-L-alanine-D-glutamic acid-diaminopimelic acid (DAP)-D-alanine-D-alanine. During transpeptidation, the DAP is cross-linked to the 4th amino acid (D-alanine) of the neighboring stem peptide, resulting in the cleavage of the terminal D-alanine (47). Thus, 3 alanines are required to synthesize each cell wall stem peptide (2 of the alanyl residues are converted to D-alanine by an alanine racemase). *C. botulinum* (except group IV type G isolates), *C. sporogenes*, *C. frigidicarnis*, *C. bifermantans*, *C. roseum*, *C. sordellii*, *C. difficile*, and *C. perfringens* spores derived from nonfoodborne isolates germinate in response to L-alanine (commonly in combination with other factors) (Table 1). Additionally, some strains of *C. botulinum*, *C. sporogenes*, *C. bifermantans*, and *C. frigidicarnis* germinate in response to L-lactate (Table 1). Even though L-lactate is not normally found in peptidoglycan.
D-lactate is found in some vancomycin resistance mechanisms, l-lactate can be converted to l-alanine in two enzymatic steps: lactate dehydrogenase converts l-lactate to pyruvate, which is then transaminated to l-alanine (48, 49).

In addition to l-alanine, C. botulinum, C. frigidicarnis, and C. perfringens type A isolate spores germinate in response to l-serine (Table 1). Again, and similar to l-lactate, l-serine is not normally found in peptidoglycan, except in certain vancomycin resistance mechanisms. However, l-serine dehydratase converts l-serine to pyruvate. From this precursor, l-alanine can be synthesized in one step (as described above).

Finally, glycine can act as a germinant for C. frigidicarnis and a co-germinant for C. difficile spores (and is most widely used as a co-germinant to stimulate C. difficile spore germination in combination with taurocholate, a bile salt [see below]) (Table 1). Glycine is not normally found in the cell walls of bacteria. However, Peltier et al. (50) demonstrated that the C. difficile cell wall has several uncommon features (the structure of the C. frigidicarnis peptidoglycan is not known) (50). Significantly, the authors found that in some stem peptides, glycine was present in the 4th position (in substitution for alanine) (50). Thus, although glycine is not normally found as a germinant among spore-forming organisms, this correlates with glycine being found as a component of the C. difficile peptidoglycan (in addition to its potential role as a nutrient; see below).

### GROWTH-PROMOTING GERMINANTS

The germinants listed above were within 2 biochemical steps of alanine or could be directly incorporated into the peptidoglycan. Importantly, though, that is not to ignore the fact that the amino acids listed above are clearly involved in protein synthesis and important from that perspective as well. Some clostridial spores germinate in response to certain amino acids that are not closely tied to cell wall synthesis, and thus, we have categorized them as growth promoting. In this section, we discuss the potential use(s) for the germinants using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (48, 49). If an organism’s genome was not present in KEGG, a closely related organism was used as a surrogate (e.g., C. difficile was substituted for C. sordellii).

### TABLE 1 List of clostridial species with their identified germinants and their respective inhibitors

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Germinant(s) (reference[s])</th>
<th>Inhibitor(s) (reference[s])</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. bifermentans</td>
<td>l-Alanine, l-arginine, l-phenylalanine (44, 93–95)</td>
<td>K⁺, Na⁺ (93)</td>
</tr>
<tr>
<td>C. botulinum, proteolytic</td>
<td>l-Alanine (96)</td>
<td>Sodium bicarbonate, exogenous DPA (77, 96, 97)</td>
</tr>
<tr>
<td>C. botulinum, nonproteolytic</td>
<td>l-Alanine, l-cysteine, l-serine (97)</td>
<td>l-Lactate (96, 97)</td>
</tr>
<tr>
<td>C. botulinum group IV type G</td>
<td>l-Cysteine (77)</td>
<td>Sodium thioglycolate, sodium bicarbonate (77)</td>
</tr>
<tr>
<td>C. butyricum</td>
<td>l-Cysteine (98)</td>
<td>Cholic acid and related bile acids (43, 59, 60)</td>
</tr>
<tr>
<td>C. difficile</td>
<td>Glycine, l-alanine, l-cysteine, l-norvaline, l-2-amino butyric acid, l-phenylalanine, l-arginine (43, 58)</td>
<td>l-Lactate, sodium bicarbonate (57)</td>
</tr>
<tr>
<td>C. frigidicarnis</td>
<td>l-Valine, l-cysteine, l-norvaline, l-threonine, glycine, l-serine, l-alanine (57)</td>
<td>NaHPO₄ (57)</td>
</tr>
<tr>
<td>C. pasteurianum</td>
<td>l-Asparagine, l-glutamine, l-cysteine, l-threonine, l-serine (34, 100, 101)</td>
<td>Exogenous DPA (99)</td>
</tr>
<tr>
<td>C. perfringens FP isolates</td>
<td>l-Alanine, l-valine, l-asparagine, l-cysteine, l-threonine, l-serine (34, 101)</td>
<td>KCl, Na⁺, Pᵢ (34, 102)</td>
</tr>
<tr>
<td>C. perfringens NFB isolates</td>
<td>l-Alanine, l-valine, l-asparagine, l-cysteine, l-threonine, l-serine (34, 101)</td>
<td>KCl (34)</td>
</tr>
<tr>
<td>C. roseum</td>
<td>l-Alanine, l-arginine, l-phenylalanine (103)</td>
<td></td>
</tr>
<tr>
<td>C. sordellii</td>
<td>l-Alanine, l-arginine, l-phenylalanine (62)</td>
<td>Sodium bicarbonate, progesterone, and related derivatives (45, 62)</td>
</tr>
<tr>
<td>C. sporogenes</td>
<td>l-Alanine (65, 77)</td>
<td>l-Lactate, sodium bicarbonate (65, 77)</td>
</tr>
<tr>
<td>C. tetani</td>
<td>Methionine (104)</td>
<td>D-Alanine (65)</td>
</tr>
</tbody>
</table>

**a** FP, food poisoning; NFB, nonfoodborne.

**b** Pᵢ, inorganic phosphate.

(D-lactate is found in some vancomycin resistance mechanisms), l-lactate can be converted to l-alanine in two enzymatic steps: lactate dehydrogenase converts l-lactate to pyruvate, which is then transaminated to l-alanine (48, 49).

In addition to l-alanine, C. botulinum, C. frigidicarnis, and C. perfringens type A isolate spores germinate in response to l-serine (Table 1). Again, and similar to l-lactate, l-serine is not normally found in peptidoglycan, except in certain vancomycin resistance mechanisms. However, l-serine dehydratase converts l-serine to pyruvate. From this precursor, l-alanine can be synthesized in one step (as described above).

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Despite the fact that glycine is found in the C. difficile pepti-
doglycan, glycine also is important for optimal growth of the organism (50, 51). C. difficile can oxidatively deaminate and decarboxylate amino acids in Stickland metabolism to generate ATP and NADH (52). In this manner, NADH accumulates and must be reduced to NAD⁺. This reduction is accomplished using proline or glycine and proline reductase or glycine reductase, respectively (52). In this reductive branch of Stickland metabolism, glycine reductase generates NAD⁺ and generates acetate, ammonium ion, and ATP, thus providing important biomolecules for growth (52). Lawley and colleagues (53) have shown that both proline reductase (PrdB) and glycine reductase (GrdA) are present within C. difficile spores, suggesting that glycine (or proline) availability could be important for Stickland metabolism after the spores have germinated (either for outgrowth or for the vegetative cell) (53, 54).

Certain C. botulinum strains, C. difficile, C. frigidicarnis, certain C. perfringens isolates, and C. butyricum germinate in response to L-cysteine (Table 1). L-Cysteine is a known reducing agent and thus could be a good indicator of an anaerobic (reducing) growth-promoting environment. Importantly, though, C. botulinum, C. difficile, C. perfringens, and C. butyricum all encode cysteine desulfurase (nifS). NifS catalyzes the removal of sulfur from L-cysteine, generating L-alanine as a by-product (which could be used for peptidoglycan synthesis) (55). The sulfur is then incorporated into Fe–S clusters, which are required for C. botulinum growth (55, 56).

During growth, cells must elongate in order to preserve cell size after division, and this process requires membrane (lipid) synthesis. Toward this requirement, C. frigidicarnis and spores derived from nonfoodborne C. perfringens strains germinate in response to the branched-chain amino acid L-valine (norvaline could also be used by C. frigidicarnis and C. difficile) (34, 57, 58). L-Valine is a precursor to lipid biosynthesis, and thus, L-valine could be used as a metric to sense the ability to produce cell membrane from the surrounding environment.

Many other germinants in clostridia are not tied to particular metabolic pathways but are important for growth nonetheless. For example, L-arginine and L-phenylalanine are precursors for multiple compounds within the cell. Moreover, L-threonine can be metabolized to glycine and acetyl coenzyme A (acetyl-CoA), while L-methionine is important for S-adenosylmethionine synthesis. Finally, nicotinamide is important for NAD⁺ synthesis and L-glutamine–L-asparagine are important for ammonia generation (Table 1) (48, 49).

**HOST-DERIVED GERMINANTS**

Some clostridial spores germinate in response to host-derived germinants. C. difficile spores germinate in response to a combination of certain bile acids and glycine (Table 1) (43, 59, 60). Bile acids are cholesterol-derived small amphipathic molecules synthesized by the liver (61). In humans, the liver synthesizes two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), which are then conjugated with glycine (glycocholic acid or glycchenodeoxycholic acid) or taurine (taurocholic acid or taurochenodeoxycholic acid). During passage through the intestinal tract, these bile acids are absorbed and recycled back to the liver (61). However, a small portion escapes this enterohepatic recirculation and enters the large intestine, where they become a substrate for modification by the normal intestinal microbiota. Bile acids are rapidly deconjugated to cholic acid and chenodeoxy-

cholic acid and then modified further through 7α-dehydroxylation (61). This enzymatic reaction converts primary bile acids (e.g., cholic acid) to secondary bile acids (e.g., deoxycholic acid). C. difficile spore germination is activated by CA derivatives, including deoxycholic acid, a molecule that is growth inhibitory to C. difficile vegetative cells (43, 61).

Although C. sordellii germinates in response to growth-promoting germinants (Table 1), C. sordellii spore germination also is enhanced by progesterone and progesterone-based steroids (cholesterol derivatives), as well as certain bile acids (45, 62). This pathogen causes serious infection in postpartum women or in medically induced abortions using mifepristone (63). The hormonal changes that occur in postpartum women, or mifepristone itself, are hypothesized to prime or enhance germination by C. sordellii spores and thus initiate colonization of the host (45).

**INHIBITORS OF SPORE GERMINATION**

Not only can germination be stimulated by germinants, but other structurally related small molecules can inhibit spore germination. For example, D-alanine, the stereoisomer of L-alanine, was shown approximately 30 years ago to competitively inhibit B. subtilis spore germination (64). Similarly, D-alanine, D-serine, D-cysteine, D-phenylalanine, and D-methionine can inhibit spore germination of C. sporogenes and some strains of C. botulinum and seem to cross-inhibit germination by other amino acids (e.g., D-serine inhibits germination by L-cysteine, L-methionine, L-phenylalanine, and L-serine), possibly suggesting that the germinant receptors have a relaxed specificity for germinant recognition (65). Importantly, though, not all organisms whose spores are activated by L-alanine are inhibited by D-alanine. C. sordellii and C. difficile germination is activated by L-alanine, but D-alanine does not inhibit germination in these organisms (62). Germination by some strains of C. botulinum is strongly inhibited by sorbate (66). Finally, C. difficile germination is competitively inhibited by CDCA and its derivatives (67, 68). Progesterone and its analogs also had an inhibitory effect on C. difficile germination (45). Importantly, inhibiting germination by C. difficile spores has been used to prevent C. difficile infection in a mouse model of disease, suggesting that this strategy could be used for the treatment of C. difficile infection (69).

**CLOSTRIDIAL GERMINANT RECEPTORS**

During germination, germinant molecules interact with specific receptors, leading to the release of DPA and ions from the spore core and degradation of the spore cortex (33). After germinant recognition by the receptor, the spore becomes committed to the germination process. Generally, the first easily measured step in germination is the release of DPA from the core, followed by cortex degradation and, eventually, outgrowth (27, 35). In B. subtilis, DPA activates SCLEs, and germination can be directly stimulated by exogenous DPA. Similarly, C. pasteuriunum, some strains of C. botulinum, and C. perfringens spores derived from food-poisoning-causing isolates germinate in response to DPA. The most widely used germinant receptors are the Ger-type receptors, the orthologues of which are found in most of the Gram-positive, endospore-forming bacteria identified to date (35). However, C. difficile does not encode orthologues of the Ger-type germinant receptors, and a novel mechanism was reported for germination by C. difficile spores (70–72). Figure 1 depicts the two classes of
germinant receptors and their hypothesized location within the spore (70, 72–75).

ger-type germinant receptors

The ger receptors have been most studied in *B. subtilis* (32). Here, L-alanine binds to the GerA germinant receptor (which is composed of the GerAA-GerAB-GerAC proteins) to initiate germination, while the mixture of L-asparagine, D-glucose, D-fructose, and potassium ions (AGFK) is recognized by the synergistic effects of GerB (composed of GerBA-GerBB-GerBC) and GerK (composed of GerKA-GerKB-GerKC) receptors (32). These Ger-type germinant receptors are thought to be located in or on the inner spore membrane, where they transmit the germinant signal to downstream proteins (35, 74–76).

In almost all clostridia, ger receptors are conserved. *C. botulinum* contains an operon with homology to the gerA operon, which is essential for *C. botulinum* spore germination (77). The *C. botulinum* GerAB-homologous protein was shown to be located in the inner spore membrane, and the *C. botulinum* GerAA- and GerAC-homologous proteins are suggested to localize to the same region (77, 78). *C. acetobutylicum* also contains genes with homology to gerAA, gerAB, and gerAC (77). In *C. pasteurianum*, the gerA-homologous genes code for receptors that do not respond to common germinants (i.e., alanine or AGFK), by which many Ger-type receptors are activated, suggesting that homology alone is not sufficient to predict which germinants are recognized by germinant receptors (27, 32, 77). *Clostridium* strains *C. asparagiforme*, *C. beijerinckii*, *C. butyricum*, *C. hathewayi*, *C. nexile*, *C. leptum*, *C. thermocellum*, *C. novyi*, *C. tetani*, and *C. scindens* also encode GerA-homologous proteins, but the requirements for spore germination in many of these organisms are currently unknown (35).

*C. sporogenes*, a close relative of *C. botulinum* and whose spores respond to similar signals to trigger spore germination (Table 1), also encodes ger-type germinant receptors that resemble those found in *C. botulinum* (77). *C. sporogenes* strain ATCC 15579 encodes three tricistronic germinant receptor operons and one tetracistronic germinant receptor operon (gerXA1, gerXA2, gerXA3, and gerXA4) (65). Mutational analyses revealed differences in germination profiles between site-directed mutants in each of the *C. sporogenes* and *C. botulinum* gerXA germinant receptors. Brunt and colleagues found *C. sporogenes* gerXA1 to be important for germination in tryptone yeast (TY) medium supplemented with lactate but less important for germination in buffer supplemented with certain amino acids (65). However, gerXA3 was important for germination under all tested conditions, and gerXA3 alone permitted germination in a strain where the three other germinant receptors were mutated (65). In *C. botulinum*, gerXA1 and gerXA3 were required for spore germination, but gerXA2 was not (65). Thus, despite the close similarity between these two organisms, there are clear differences between their germinant receptor requirements.

*C. perfringens* does not encode a gerA orthologue, but other germinant receptors have been identified by homology searches and functional analyses. The *C. perfringens* GerKA and GerKC gene products are localized to the inner membrane of the spore and were shown to be necessary for activation of SCLEs and for DPA release, which then activate downstream germination events (34, 79). However, unlike what is observed in most spore-forming bacteria, germination by *C. perfringens* spores seems to vary considerably between strains/isolates. It will be interesting to determine how the different germinant receptors among all studied strains vary and contribute to germinant recognition.

csp-type germinant receptors

The ger-type receptors mostly respond to amino acid-based germinants. Typically, those clostridia that do not contain any genes with homology to known ger-type receptors do not respond to canonical germinants. For example, *C. difficile* and *Clostridium barletti* (recently renamed *Intestinibacter barletti*) do not encode orthologues of ger-type receptors (35, 72).

In *C. difficile*, the germination-specific protease locus (*csp*) is a bicistronic operon containing *cspBA* and *cspC* (35), where *cspBA* produces a fusion protein of CspB and CspA proteins. These proteins are cleaved to the CspB and CspA proteins, likely by the YabG protease (80). CspC is encoded downstream of *cspBA* and is the receptor for the bile acid germinants (70). Most of our knowledge of the Csp proteases has come from studies in *C. perfringens*. In *C. perfringens*, the subtilisin-like proteases CspA, CspB, and CspC are predicted to be catalytically active (81, 82). Much work has been done on the subtilase family of proteases, and prior work has led to the identification of the residues important for catalysis, and the identification of these residues is based on surrounding sequence motifs (83, 84). Subtilases are commonly produced as a proenzyme in which the prodomain is autocatalytically removed to activate the protease (83, 85–88). In *C. perfringens*, these three proteins are predicted activate the SCLE SleC. In *C. difficile*, the residues important for catalysis are mutated in CspA and CspC, but the catalytic triad characteristic of subtilisin-like proteases is maintained in CspB (88). Adams et al. demonstrated that CspB cleaves the pro-SleC zymogen to an active form (88). Since the catalytic triads are mutated in CspA and CspC, these proteins have alternative functions in *C. difficile*, where CspC is the bile acid germinant receptor (70) and CspA controls the levels of CspC in the spore (80).

Interestingly, it appears that only members of *Peptostreptococcaceae* (e.g., *C. difficile*) use catalytically dead versions of the Csp proteins for spore germination (80). Recently, Kevorkian and colleagues analyzed the Csp loci from several bacteria and found that *Clostridiaceae* (e.g., *C. perfringens*) and *Lachnospiraceae* (e.g., *C. phytofermentans*) maintained catalytically active CspA and CspC proteases (80). The authors suggest that *Peptostreptococcaceae* family members are under selective pressure to diversify their Csp loci, while *Clostridiaceae* and *Lachnospiraceae* are under selective pressure to maintain Csp enzymatic activity (80).

Although the signals that stimulate germination by *C. difficile* spores are not as variable as what is observed for germination by *C. perfringens* spores, there are differences between strains/ribotypes (89–91). Some *C. difficile* strains are more primed to germinate in response to the taurocholic acid germinant than others (91). However, these differences do not appear to be linked to differences in the CspBA or CspC protein sequences, because any differences in protein sequence were ribotype specific, but the observed differences in germination were not (91).

Recently, another germination regulator was identified (GerS) and is hypothesized to regulate germination by an unknown mechanism (73). Interestingly, a gerS mutation in *C. difficile* resulted in cleavage of pro-SleC to an active form in response to germinants; however, it appears that SleC was unable to hydrolyze cortex (presumably either by being held in an inactive state or due to modifications of the cortex that prevented SleC activity) (73). It
is possible that the C. difficile CspB, CspA, CspC, GerS, and pro-SleC are all part of a “germinosome” that functions to recognize germinants and transmit the germination signal to CspB to activate pro-SleC to initiate cortex degradation (73).

Although this new type of Csp germinant receptor has not been studied as well as the ger receptors, and many aspects of the mechanism of spore germination are not understood, the Csp proteins have not been shown to be a receptor in any species other than C. difficile. It is possible that other clostridial species that do not encode orthologues of the ger receptors initiate spore germination using a similar mechanism or use both the Csp-type germinant receptor in combination with the Ger-type germinant receptor.

CONCLUSIONS AND FUTURE PERSPECTIVES

Spores germinate in response to germinants, and this begins the transformation of a spore into a vegetative cell. These growing cells can produce toxins and cause physiological symptoms associated with certain diseases (92). The vegetative cells eventually sporulate to escape into the environment to either be taken up by other hosts or remain in the current host. If germination were to be blocked before the transformation into a vegetative cell and toxin production, hosts would not develop infections or diseases caused by these bacteria (69). From this perspective, germination is an attractive target for potential therapeutics.

Identifying the signals that trigger germination and the mechanisms by which germination occurs is necessary for understanding how pathogenic bacterial causes disease and how others can lead to food spoilage. Historically, due to the lack of genetic systems, our understanding of the mechanisms of spore germination in clostridia has lagged far behind that of bacilli. However, recent advances in genetics have made such studies feasible (36–40). In clostridia, the germinant signals are commonly amino acids, and their cognate receptors seem to be homologous to the Ger-type receptors identified in B. subtilis. Certain clostridia require more than mixtures of amino acids to germinate. As examples, C. difficile utilizes cholesterol derivatives (bile acids), and C. sordellii recognizes other cholesterol derivatives (progesterone and bile acids) as germinants (41, 43, 45, 59). Although the information on germination is not complete, and some pathogenic strains need to be studied further, genome sequences are becoming more and more available for many different clostridia (not just pathogenic clostridia). These genomes will yield important information about the modes of germination (i.e., whether the organism encodes the classical germinant receptors or potentially germinate using novel mechanisms or both).

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