Appendix 7.4 *In silico* proteomic analysis of coat and exosporium proteins

Alanine racemase 1, Alr. Alanine racemase 1 (WP_003234284.1) is an enzymatic component of the *Bacillus subtilis (B. subtilis)* and *Bacillus anthracis* (*B. anthracis)* coat—potentially responsible for preventing germination of spores under unfavorable conditions or during sporulation. It functions through the conversion of L-alanine, a germinant, to D-alanine, a germination inhibitor and component of the peptidoglycan layer of cell walls (1). Given its absence in *Homo sapiens* and conservation across bacterial species, it is considered an optimal target for therapeutics (1, 2). Previous research has established the conservation of Alr in 22 *Clostridia* species. However, the *in-silico* proteomic analysis performed in **Chapter 3** showed that it is also conserved in 10 other species.

Coat protein, BAX60_21285. Newly established by this proteomic analysis, there were 18 homologues of BAX60_21285 identified in *Clostridia* proteomes. Further research will need to investigate the localization and role of this protein.

Collagen-like protein, BclA. The BclA protein is localized to the exosporium layer of *B. cereus* (WP_042515567.1) (1). This collagen-like glycoprotein is the predominant component of the hair-like filaments on the exosporium of *B. anthracis* spores, contributing to spore adhesion (1, 3). While its role in spores is not clear, it may prevent early germination of spores, like alanine racemase (1). This species is encoded by *Clostridioides difficile (C. difficile)* and two other species, *Clostridium arbusti (C. arbusti)* and *Clostridium pasteurianum (C. pasteuranium).*

Spore coat glycoprotein, BclB. The BclB protein (WP_076537677.1)—a variant of BclA—is another glycoprotein found in the exosporium of *B. cereus* (1). Research has shown that while BclB is exposed on the exosporium, it may be partially covered by the BclA protein (1). It has been hypothesized as a key structural component of the exosporium that dictates the proper arrangement of proteins in this spore layer, though further research is needed to verify this (1).

There were 3 novel* BclB homologues discovered through this proteomic analysis across the studied species. This notably includes *C. difficile* as well as *Clostridium baratii (C. baratii)* and *Clostridium jeddahense (C. jeddahense).* The filament-like projections on *C. difficile*'s exosporium are responsible for their adherence to gut epithelial cells, a key step to pathogenicity in AAD (4); BclA and BclB may be the main cofactors of binding, and their presence within this proteome may contribute to infection within humans.

Diglucosyl diacylglycerol synthase, BetA. The exosporium protein, BetA, is found in the *Bacillus cereus (B. cereus)* group as well as in the crust of *B. subtilis* (WP_003246153.1), localized beneath the BclA layer with a similar composition (1). Research has shown BetA is present on the spore surface around 5 hours into sporulation in *B. anthracis*, though its exosporium localization in this species is still unverified (1). In *Clostridia*, this protein is well conserved across the class, and all homologues except for one of the 92 are novel.

Peroxiredoxin, CD630_14330. As previously discussed, the "CotE"—renamed CD630_14330—protein identified in *C. difficile* is non-homologous to the morphogenetic version of CotE in *B. subtilis*. However, research has shown the presence of this protein on the spore coat surface and exosporium, and it is hypothesized that this protein is involved with adhesion of spores to the mucosal layer of the intestines (13, 21). As such, this CotE protein is putatively relabeled as CD630_14330 (WP_011861211.1), given its non-homology to other *B. subtilis* coat proteins but clear existence in the spore coat structure of *C. difficile*. There are 65 species of *Clostridia* with CD630_14330 homologues.

DUF2935 domain-containing protein, CD630_15110. The CD630_15110 protein created from the mislabeled "CotB" of Sebaihia, et al. was shown to have 69 homologues throughout the *Clostridia* class (12). From the limited research conducted by Permpoonpattana, et al. on CD630_15110, it is hypothesized that this protein localizes to the exosporium of *C. difficile*, but its function remains unknown (13).

Spore coat assembly protein, CD630_16130. The coat assembly protein, CD630_16130 (CAJ68478.1), was re-labelled from "CotA" in Sebaihia, et al. given this protein is non-

homologous to CotA of *B. subtilis* (12). From the limited research conducted on CD630_16130, Permpoonpattana, et al. suggests that this protein is involved in the outer coat assembly of *C. difficile* spores (13). Mutant spores had reduced density in their coats and were less resistant to lysozyme and ethanol treatments (13). Researchers believe that it localizes to the outer coat, but it is unlikely to be surface-exposed (13). The proteomic analysis conducted here found CD630_16130 in *C. difficile* only.

MATE family efflux transporter, CdeA. CdeA is a multidrug and toxic compound extrusion (MATE) family protein of *C. difficile* (WP_009902537.1). This protein causes ethidium bromide energy dependent efflux from spores of *C. difficile* (5). As such, this protein is known to convey resistance to ethidium bromide, acriflavine, and fluoroquinolones (5). Reliant upon the CdeC protein of *C. difficile*, this protein localizes to the exosporium where it is surface exposed (4). In addition to the established protein in *C. difficile*, CdeA homologues were newly discovered in 32 species of *Clostridia*.

Exosporium morphogenetic protein, CdeC. CdeC is a well-studied, morphogenetic protein in the exosporium of *C. difficile* (WP_003418933.1) (6). Null mutants of *cdeC* express a disheveled exosporium and thin spore coat potentially due to its role in crosslinking proteins; the effect of this mutation, however, varies in magnitude depending on the strain studied (6). In addition to its role in exosporium assembly, CdeC also conveys resistance to ethanol, temperature, lysozyme, and macrophages while increasing adhesion properties of *C. difficile* spores (6). Proteins CdeA, CD630_15110, and BclA are dependent on CdeC, and given that null mutants express permeable coats, CdeC may localize both to the surface of the exosporium as well as the interface between this layer and the outer coat (6). CdeC is dependent on CdeM (6). Strains of *C. difficile* with higher amounts of CdeC were shown to be more pathogenic and more likely to cause recurrent infection in humans (6). Only four species in the *Clostridia* class other than *C.* difficile were shown to express this protein.

Exosporium morphogenetic protein, CdeM. As with CdeC, the exosporium morphogenetic protein, CdeM, of *C. difficile* (WP_003436550.1) is involved in the assembly of this outermost layer, though to a lesser degree (6). Unlike CdeC, it does not convey any resistance properties

but does increase adhesion to mucosa in vitro (6). Assembly of proteins CD630_16130, CgeB, and BclA into the coat and exosporium were all dependent on CdeM which is surface exposed on the exosporium (6). No species of *Clostridia* besides *C. difficile* had CdeM homologues.

Spore maturation protein, CgeB. The spore coat protein, CgeB, in *B. subtilis* (WP_004399421.1) is not well defined. This glycosyltransferase, however, has been shown to have morphogenetic properties in the outer coat, as null mutants of *cgeB* do not possess an external crust (7). Furthermore, CgeB may be involved in enzyme modification of the spore envelope polysaccharides during glycosylation activities of CgeA (8). While previous literature has shown CgeB in the proteomes of *Clostridium acetobutylicum (C. acetobutylicum)* and *Clostridium fimetarium (C. fimetarium)*, the MCL clustering produced disparate clusters of these homologues, and thus they were determined to be non-homologous to CgeB in *B. subtilis*.

Spore maturation glycosyltransferase, CgeD. The spore maturation glycosyltransferase, CgeD, is well studied in *B. subtilis* (WP_003230828.1) (7). CgeD, like other Cge proteins, conveys hydrophilicity to spores of *B. subtilis* (7). Null mutants of *cgeD* have outer coats with reduced density (7); these coats are splotchy and lamellar like null mutants of *cgeA*, which implies CgeD contributes to the assembly of the polysaccharide crust (7). Four species of *Clostridia* possess homologues of CgeD.

Spore maturation N-acetyltransferase, CgeE. A putative acetyltransferase of the spore crust—identified as CgeE (WP_003230830.1)—is found in *B. subtilis* (7). Protein CgeE plays a role in the assembly of the polysaccharide crust, though it does not convey hydrophobicity nor density to this layer, unlike CgeD (7). The CgeE proteins of *Clostridium sulfidigenes (C. sulfidigenes)* and *Clostridium tagluense (C. tagluense)* were determined to be true homologues to that in *B. subtilis*.

Outer spore coat copper-dependent laccase, CotA. The outer spore coat protein CotA of *B. subtilis* (WP_003243170.1) has previously been shown to serve limited purpose beyond conveying the brown pigment that signifies the late stages of sporulation (9). While it was previously hypothesized that CotA's similarity to multicopper oxidases signifies it is involved with the mechanisms of SodA and CotJC, further research has shown that CotA is not responsible

for this because it is a laccase (9, 10). This protein's purpose may instead be UV light protection, as the brown pigment produced by this protein has melanin-like properties (10). Forming a ring around the spore, this protein has been shown to be CotE dependent and CotH independent during coat assembly (11). Of the 31 CotA homologues identified in *Clostridia*, 25 were novel to this study.

Spore coat protein, CotB. In *B. subtilis* (WP_003242882.1), CotB is a surface-exposed coat protein reliant upon expression of both CotG and CotH (14). It has been shown to undergo modification from a 46 to a 66 kDa protein immediately after synthesis by these two proteins, but it does not require their presence to express at the lower molecular mass (14). Forming a ring around the spore coat, CotB is partially dependent on CotE as well as CotH, the latter due to its stabilizing function on CotG (11, 14). The function of this protein, however, remains unclear. While functionally important in *Bacillus*, there were no homologues of CotB identified in the *Clostridia* proteomes. That which was previously identified in *Clostridium aceticum (C. aceticum)* was non-homologous as a result of nomenclature and should be correctly viewed as such hereafter.

Inner spore coat protein, CotD. The inner coat protein CotD of *B. subtilis* (WP_010886551.1) is one of the few coat proteins in this species to be CotE-independent (11). Beyond this, little is known of CotD's structure, assembly, or function. No CotD homologues in the *Clostridia* species were discovered here, and that which was previously identified in *C. difficile* was shown to be non-homologous to the query control.

Outer spore coat protein, CotE. CotE is a morphogenetic protein found in a ring around *B. subtilis* (WP_003221022.1) (11). It has a key role in the structural formation of the exosporium in the *B. cereus* family as well as for the outer coat in *B. subtilis* (11). Localized in the outer coat of the latter, it is hypothesized that CotE bridges the coat and exosporium during formation of the latter; it is then expected to cleave, producing the interspace between coat and exosporium (1). This assembly is at least partially dependent on CotH, but independent of Tgl (11). While CotE is well conserved across the *Bacilli* class, no species in *Clostridia* were shown to possess this protein. The CotE previously reported in *C. difficile* literature was shown to be non-homologous

to that in *Bacilli*. This validates the previous finding that "CotE" in *C. difficile* is not morphogenetic as it is in *Bacilli* (15).

Outer spore coat protein, CotF. While CotF may be an inner spore coat component like CotD in *B. subtilis* (WP_003243364.1), research has shown its reliance on CotE for assembly (16). This coat F family protein is structurally similar to the forespore proteins YraE, YraG, YraF, and YraD—the former two with similar N-terminal sequences while the latter have C-terminals that resemble CotF (17). This protein does not affect the structure of the spores nor sporulation (9). It is predicted to assemble around the shell of CotE within the spore coat (9). Fifteen CotF homologues were previously established in the *Clostridia* class, all of which were missed in this analysis. However, six of these were shown to be non-homologous to CotF in *B. subtilis.*

Spore coat protein, CotG. Hydrophilic protein CotG localizes as a ring in the outer spore coat of *B. subtilis* (WP_010886627.1) (11). Highly similar to CotB, these two proteins do not convey resistance or germination properties to the mature spore; however, *cotG* null mutants show a dispersed outer coat layer, implying that CotG and CotB are highly prevalent and/or structurally important in the outer coat (9). A further study into this matter showed that superoxide dismutase, SodA, in *B. subtilis* may crosslink CotG into the outer coat matrix, thus increasing the density of the coat (9). It is hypothesized that SodA produces hydrogen peroxide for a peroxidase within the spore coat; this is then used to crosslink CotG with itself or other proteins (9). As CotG is dependent on CotE for assembly but only partially on CotH and not on Tgl, this hypothesis seems valid (11). While previous researchers have hypothesized that the peroxidase in this mechanism is CotE, it is more likely to be CD630_14330 in *Clostridia* since no true CotE homologues were found.

The MCL clustering showed that the putative CotG protein in *C. difficile* was non-homologous. Otherwise, no homologues of CotG were found in the *Clostridia* species. However, given that *Clostridia* species typically express thick and dense coats, a similar protein likely exists as a functional homologue. Literature has shown that the crosslinking of CotG via a peroxidase and superoxide dismutase—both found in *Clostridia* proteomes—conveys density that *Clostridia* spores often exhibit (9). As such, further research into the CotG-replacement in

Clostridia should be pursued, as the presence of the other factors in this mechanism imply its presence in the proteomes of *Clostridia*.

Inner spore coat protein, CotH. Localizing to the inner coat, the CotH protein is conserved across many spore-forming bacterial species, including *B. subtilis* (WP_003227854.1) (9). It is predicted to be assembled via CotE sometime between the middle and end of sporulation, but it does not convey resistance properties (9, 11). Morphogenetic to the spore coat, it directs the assembly of at least nine other coat proteins in *B. subtilis*, specifically CotG, and without it, the outer coat loses its integrity (14, 18). Furthermore, the CotH protein kinase directly effects the ease of germination by phosphorylating CotB and CotG (18). Null mutants of this protein do not present the outer coat proteins CotB and CotG, and there is a decreased number of CotC proteins as well (9). For this reason, CotH is predicted to work similarly and alongside CotE in the construction of the outer coat (9).

The proteomic analysis found four novel CotH homologues in *Clostridia.* Six other homologues were missed by the proteomic analysis but validated via clustering, and the homologue in *C. aceticum* was found and validated here. Given the relative importance of CotH in constructing the outer coat of *B. subtilis* spores, there is likely another protein serving this role for *Clostridia* species. This is not unexpected given that the other important protein, CotE, is rarely found in *Clostridia*. As such, either a different mechanism to that described in *B. subtilis* is used to assemble the outer coat of *Clostridia* spores, or there are replacements to CotE and CotH in these species.

Spore coat kinase, CotI. Spore coat protein kinase in *B. subtilis* (WP_010886600.1), CotI, belongs to the CotS family of proteins (19). Similar to YutH and CotS, mutants of this protein have reduced levels of CotS and CotSA, which implies that these proteins interact in synthesis or assembly (11). Furthermore, this means that CotI is marginally morphogenetic in the *B. subtilis* spore (11). Research has yet to investigate the direct role of CotI, but it is found to be dependent on CotE assembly (11).

The proteomic analysis conducted here identified 91 (57 novel, 34 validated) homologous proteins in the *Clostridia* species in addition to the one missed in the analysis. Many of these

were wrongly labelled "CotS". While this is not surprising given the sequence similarity, special attention towards this distinction needs to be practiced in the future.

Spore coat associated proteins, CotJA and VT28_37200, CD630_05960, and C7U56_00100. The CotJA protein of *B. subtilis* (WP_003219489.1) has a reciprocal dependence on CotJC in this species (11). Localizing to the precoat, CotJA is a putative component of the matrix built by CotE at this stage, potentially heterodimerizing with CotJC (11). It is unlikely to be a component of the inner or outer spore coat layers given its presence in *cotE* and *gerE* null mutants; as such, it is likely in the undercoat or matrix in mature spores (9).

Previous literature had established 6 homologues of *B. subtilis*'s CotJA, but this proteomic analysis has shown that they were all more closely related to one of the novel CotJA-like proteins established here. When clustering the potential homologues for CotJA, none of those previously established or those discovered from the proteomic analysis clustered with CotJA of *B. subtilis.* Instead, these proteins formed distinct clusters with other CotJA-like proteins in the *Clostridia* species, all expressing minimal similarities with the query control CotJA and more so with each other. As such, new labels were created for each distinct cluster, decreasing in size, from VT28_37200 to CD630_05960 to C7U56_00100. Twenty-eight protein homologues of VT28_37200 were found in *Clostridia* with 14 for CD630_05960 and 6 for C7U56_00100.

It should be noted that *Clostridium cuniculi (C. cuniculi)* has a couple of these proteins whereas the remainder only possess one. As the CotJA protein is absent across the *Clostridia* class, these novel proteins may serve in its role, though further research will need to investigate the matter.

Inner spore coat protein, CotJB. Unlike CotJA and CotJC, little is known about the function of CotJB in *B. subtilis* (WP_003219491.1). It is shown to be abundant in spores, and it is encoded in the genome between CotJA and CotJC (9). This protein does not regulate the assembly of CotJC in spores, unlike CotJA (20). CotJB is one of the best conserved proteins throughout the *Clostridia* class. This proteomic analysis has found 57 other homologues, which adds to the previous total of 13 *Clostridia* homologues. There were also 2 proteins that were missed in the initial analysis but validated via clustering.

Inner spore coat protein, CotJC. Abundant spore coat protein CotJC of *B. subtilis* (WP_003233850.1) is an under or inner coat protein that is mutually reliant upon expression of CotJA (9, 11, 19-21). This protein has a composition similar to a peroxidase, leading to the hypothesis that it controls hydrogen peroxide levels via catalysis (19). The chemical might be produced by SodA to crosslink CotG into the outer coat via CotE (19). In either case, CotJC is independent of CotE and CotH, and it conveys no resistance or germination properties (11, 19). Only 14 of the studied *Clostridia* species do *not* have CotJC encoded into their proteomes, making it one of the best conserved. While better studied than CotJA and CotJB in *B. subtilis*, most of the results (78 of 93) are novel to this proteomic analysis.

Sporulation hydrolase, CotR. The patatin-like phospholipase family protein of *B. subtilis* (WP_003243674.1), CotR, is relatively well-studied, though its exact function remains unknown. CotR is hypothesized to directly interact with CotE, upon which it is reliant for assembly (11). This patatin-like protein is independent of the other morphogenetic proteins—CotG, CotS, yaaH, CotA, and CotH (9, 11). Research has shown that it does not affect structural stability, lysozyme resistance, correct coat composition, or germination rates (9). CotR is not well-conserved across *Clostridia*, but there are 5 homologues.

Spore coat protein, CotS. The spore coat protein, CotS, is among the best studied of the *B. subtilis* (WP_029727105.1) coat proteins. Localizing to the inner coat, it forms a ring around mature spores (9, 11). However, null mutant experiments have shown that this protein does not affect the final morphology of spores upon maturation (22). Nonetheless, CotSA is dependent on CotS for assembly (22). CotE is responsible for the inclusion of CotS in the inner coat near the cortex, but CotE assembly is also affected by the antagonistic relationship between CotG and CotH (10, 23). Beyond these proteins, CotS assembly is independent of CotA, CotB, CotC, CotD, CotF, CotT, and CotSA (10). It was hypothesized that CotS functions in the assembly of the matrix aligned between the shells of SpoIVA and CotE, although this has yet to be verified (9).

While the proteomic analysis invalidated one previously identified homologue, there were still 3 novel homologues in the *Clostridia* class, 4 were validated through MCL clustering, and a further 19 were missed. This was likely due to the similarity of CotS to CotI and the multiplicity of "CotS" replicates in the UniProt database.

Spore coat protein, CotSA. CotSA is a glycosyltransferase family 4 protein in *B. subtilis* (WP_003229028.1). Localizing to the inner coat, it is synthesized alongside CotS but assembled by this latter protein (9, 22). CotE, CotH, and CotO have also been implicated in assembly of this protein, but CotSA's function is still unknown (11, 24). This protein is marginally conserved with homologues in 23 of the species, of which 18 were novel findings and five were missed.

Exosporium protein, CsxA. The exosporium protein, CsxA, of *Clostridium sporogenes (C. sporogenes)* (WP_163226358.1) is a potentially morphogenetic protein that serves as the foremost structural component of the exosporium's basal layer (25). This protein self-assembles during sporulation, forming disulfide crosslinks that possess hexagonal symmetry on a molecular level (25). This symmetry is similar to that seen in *B. subtilis*'s CotY and *B. cereus*'s ExsY and CotY, so it is likely they serve similar roles in the spore (25). Forming the internal surface of the basal layer with BclA, other exosporium proteins attach to the CsxA sheet which is highly resistant to tearing during germination (25). This protein, in addition to its structural role, has also been shown to convey high levels of heat resistance as well as resistance to DTT (25).

As an abundant protein on the external-most layer of the spore, CsxA would be an optimal target for the engineering of diagnostics and therapeutics. It is conserved across 35 species of *Clostridia*, 33 of which are novel findings. While past CsxA homologues in *Clostridium felsineum (C. felsineum), Clostridium disporicum (C. disporicum),* and *Clostridium roseum (C. roseum)* were all proven to be non-homologous to that in *C. sporogenes*, a genuine homologue was validated in *Clostridium oryzae (C. oryzae)*.

Exosporium protein, CsxB. Compared to CsxA, the exosporium protein CsxB of *C. sporogenes* (WP_003486263.1) is not well-studied. Considered to be a homologue of CotJC in *B. subtilis*, this protein belongs to the CotS family (26). It is known to interact with BclA but not CsxA in the exosporium (26). Twenty-three novel homologues of the CsxB protein were established through this proteomic analysis.

Exosporium protein, CsxC. Beyond its localization to the exosporium, the CsxC protein of *C. sporogenes* (WP_045517358.1) has not been described by literature. Further research will need to

investigate this, as homologues of this protein were found in 49 species of *Clostridia*. Forty-eight of these results were novel.

Cell wall hydrolase, CwlJ. The CwlJ protein of *B. subtilis* (WP_000538153.1) is a cell wall hydrolase (27). This protein promotes germination in dormant spores and is redundant with SleB, as both depolymerize cortex peptidoglycans during Stage II of germination (27). CwlJ localizes to the inner spore coat, likely closer to the cortex than the outer coat (27). Despite this fact, CwlJ was shown to be dependent on CotE for assembly in Kim, et al. which implies it may be more dispersed within the coat; further research will need to investigate this discrepancy (11). There are 24 homologues of the CwlJ protein found throughout the *Clostridia* class, 7 of which are novel results, 7 were validated, and 10 were missed but validated by MCL clustering.

7-cyano-7-deazaguanine synthase, ExsB/QueC. The ExsB protein (WP_003245417.1) is partially responsible for the stability of the exosporium and its attachment to the coat in *B. cereus* and *B. anthracis* (1). Previous research has shown the localization of this protein to the inner coat and cortex within the mature spore (1). Unlike other exosporium proteins, ExsB was shown to be sensitive to pH and temperature, but under proper conditions, it conveys hydrophobicity and resistance to germination in mature spores (1). Fewer than half (50) of the *Clostridia* species were shown to possess this non-morphogenetic protein.

Exosporium protein, ExsE. As with ExsC and ExsD, little research has been conducted on the exosporium protein ExsE of *B.cereus* (WP_098536388.1). Localizing to the basal layer, it is processed from a larger precursor, and previous research has claimed it is only conserved within the *B. cereus* family (3). However, 4 species of *Clostridia* were shown to encode homologues of ExsE, all novel findings.

Exosporium protein, ExsH. The collagen-like glycoprotein, ExsH, is found in the proteome of *B. cereus* (WP_000183798.1) (1). It possesses the BclB C-terminal domain which is why BclB is considered "ExsH-like" (1). Research has pointed to ExsH's localization within the exosporium, but it does not appear to convey any adhesive properties like other exosporium proteins do (28).

The *Clostridia* species of *Clostridium botulinum (C. botulinum)* and *C. sporogenes* were the only two species that encoded this protein.

Ferrous iron transport protein A, FeoA, and ferrous iron transport protein B, FeoB. The ferrous iron transport proteins A and B—encoded by *feoA* and *feoB*—are iron uptake proteins, hypothesized to contribute to the colonization of the gut (29). Those in *C. difficile* (WP_003419941.1 and WP_009903695.1, respectively) are localized to the exosporium (30). In vegetative cells, FeoA is a small cytoplasm protein that is suspected to activate FeoB's GTPase activity, and FeoB is a transmembrane permease through which ferrous iron is transported into the bacterial cell (30). While their role in spores remains unknown, the activation of these proteins leads to an increase in bacterial population and translation of iron-dependent proteins (30). Through the proteomic analysis, FeoA and FeoB homologues were identified in 97 and 106 species of *Clostridia*—respectively. 65 of the FeoA homologues were novel, 29 were validated, two were missed, and one was nullified. For FeoB, 45 homologues were novel and 61 were validated.

Nucleoside hydrolases, IunA and IunH. Nucleoside hydrolase, IunA, has been documented in both *B. subtilis* (QBJ65612.1) and *B. anthracis*. However, limited research has investigated its distinction from the IunH protein and its role in the exosporium (1). IunA is known to localize to the surface of the exosporium in *B. anthracis*, but it is at least partially covered by the BclA layer (1). The IunH protein of *B. cereus* (WP_000029504.1), like IunA, is an inosine-uridine-preferring nucleoside hydrolase, though it is likely to localize to the interspace of the mature spore (1). Little is known about the function of IunH. There were 17 novel homologues of IunA and 7 of IunH found in *Clostridia*. Other IunH protein homologues were validated in *C. difficile* and *Clostridium sordellii (C. sordellii). Clostridium hydrogeniformans (C. hydrogeniformans*), *Clostridium neonatale (C. neonatale),* and *C. sordellii* were the only species to have both proteins.

Lipase, LipC. LipC is a lipolytic enzyme of *B. subtilis* (BAP19127.1) that localizes to the mother cell during sporulation and later to the basement layer of mature spores (31, 32). Forming a ring around the spore, this protein has been found to depend on CotE, SafA, and SpoVID for assembly

(31). Beyond promoting germination of spores in the presence of L-alanine, LipC has not been shown to convey resistance properties or otherwise affect sporulation or germination (31). This protein is believed to form a ring around SafA, at the junction between the cortex and inner spore coat (31). As such, LipC is suspected to hydrolyze and degrade phospholipids in the outer spore membrane during sporulation (33).

While research has previously established homologues of LipC in 7 species of *Clostridia*, the proteomic analysis here failed to validate these results. As such, there are no true *Clostridia* homologues of LipC.

Oxalate decarboxylase, OxdC. The cupin domain-containing protein, OxdC, of *B. subtilis* (WP_003243476.1) is a manganese-dependent enzyme (34). OxdC is responsible for the oxygen-dependent oxidation of oxalate to carbon dioxide and formate, alongside OxdD (34). This protein has also been implicated in catalyzing the oxidation of oxalate to carbon dioxide and hydrogen peroxide (35). As such, this protein is involved in the protection of vegetative cells in low pH environments by consuming protons (34). It's role in spores remains unknown, but OxdC likely localizes to the inner coat of spores alongside OxdD, although to a lesser magnitude (36). It is more abundant in vegetative cells (36). One previously established homologue in *C. sporogenes* was missed by the proteomic analysis but validated by MCL clustering.

Oxalate decarboxylase, OxdD. Oxalate decarboxylase, OxdD, of *B. subtilis* (WP_003231409.1) is SafA dependent for assembly and CotE dependent for maintenance (11). Null mutants of *cotE* expressed fewer OxdD proteins over time (36). This protein has been associated with the catalysis of oxalate into formate and carbon dioxide in bacterial spores which may explain why overexpression of this protein leads to degradation of oxalate (36, 37). While past literature has localized the OxdD protein to the inner spore coat (31), more recent studies have shown that OxdD is at least partially surface exposed (37, 38). Given successful use of OxdD for exogenous surface display, it is likely this protein is localized to the outer versus the inner coat (37). Previously established in *C. botulinum*, this homologue was missed by the proteomic analysis but validated by MCL clustering.

Serine/threonine protein kinase, PrkA. Protein kinase A, or PrkA, is a protein involved in the sporulation of *B. subtilis* (WP_003233433.1). This protein accelerates sporulation—specifically through sigma-K—by suppressing Hpr which is involved in homologous recombination (39). Null-mutants for *prkA* have shown delayed sporulation and decreased spore counts resulting from sporulation (40). PrkA localizes to the coat in the forespore, but its location in mature spores remains unknown (40). Given the importance of this protein, it is not surprising that there are 69 homologues of PrkA in *Clostridia.* Of these results, 58 were novel.

Spore coat protein, SipL. In addition to SpoIVA, the SipL protein of *C. difficile* (WP_009898760.1) is the major early morphogenetic protein involved in spore coat assembly (41). This protein is hypothesized to be the functional homologue of SpoVID (from *Bacilli*) given they both are involved in encasement of the spore core and cortex (41). In the mother cell of null mutants for *sipL*, the coats are dispersed throughout the cytoplasm, indicating the reliance of SpoIVA on SipL to assemble these layers (41). This reliance is mutual, as SipL requires multimerization with SpoIVA to localize to the forespore (41). These two proteins hypothetically assemble coat proteins and direct their encasement in the basement layer, moving from the poles towards the center (41). As such, it is unsurprising that SipL conveys heat resistance properties to mature spores though its localization at this stage is unknown (42). Given its reliance and multimerization with SpoIVA, however, it likely localizes between the cortex and inner coat with SpoIVA.

Given the morphogenetic role of SipL, it is unsurprising that this protein is conserved throughout the *Clostridia* class in all but 5 species; 99 of these homologues are novel to the *in-silico* proteomic analysis conducted here.

Spore cortex-lytic enzyme, SleC. SleC is a lytic transglucosylase that is required for bile-induced germination in *C. difficile* via cortex hydrolysis (43). It is hypothesized that SleC of *Clostridia* replicates the joint role of *Bacilli*'s SleB and YpeB which are involved in cortical hydrolysis during germination (44). The localization of SleC has been implicated to be within the coat, but further research will need to validate this hypothesis. The *in-silico* proteomic analysis conducted here has identified 67 homologues.

Cortical fragment-lytic enzyme, SleL/YaaH. In *B. subtilis* (AIC96667.1), the LysM peptidoglycan-binding protein, SleL, operates as a spore lytic enzyme during germination (45). The other lytic proteins, SleB and CwlJ, hydrolyze the full peptidoglycan layer of the cortex which SleL then further degrades (45). Given its early activation during sporulation, this protein is suspected to localize to the coat of the developing and mature spore. This proteomic analysis was able to establish 44 novel homologues of SleL.

Cortical-lytic enzyme, SleM. Just like SleL is involved in the further degradation of cortex peptidoglycans from SleB and CwlJ—it appears that SleM serves in the similar role but for SleC in *Clostridia* (9). SleM is a muramidase, although it is not necessary for cortex hydrolysis unlike SleC upon which it depends (9). This protein is suspected to localize to the area between the cortex and spore coat (9). There were 48 novel homologues established through this analysis in addition to that in *Clostridium perfringens (C. perfringens*) (WP_003450466.1).

Superoxide dismutase, SodA. SodA is a superoxide dismutase protein found in the outer coat of *B. subtilis* (WP_004398583.1). When studied in *B. anthracis*, it was shown to convey resistance to oxidation as well as to increase the virulence of the bacteria itself (1). It is involved with the localization and assembly of CotG in the outer coat, but it does not provide resistance to heat or lysozyme treatment (6). SodA provides hydrogen peroxide for outer coat crosslinking (9). Past research has shown that SodA is surface exposed in *C. difficile* (13). This protein is found in 66 *Clostridia* species. Six of these homologues were initially missed and twenty-one had been previously found but were also detected here.

Superoxide dismutase, SodC. The superoxide dismutase, SodC, in *B. cereus* (WP_095843219.1) differs from its family member SodA in its specificity to copper and zinc (46). Little research has been conducted to the exact role of this protein, but it has been shown to localize to the periplasmic space (47). Furthermore, this protein has been shown to increase the pathogenicity of spore-forming bacteria by conveying resistance to extracellular superoxide, macrophages, and neutrophils (46-48). Twenty-three novel homologues of SodC in *Clostridia* were identified by the proteomic analysis, contributing to the total of 43 homologues. One protein was missed, and the remainder were validated.

Stage IV sporulation protein A, SpoIVA. The stage IV sporulation protein A is a morphogenetic protein in *B. subtilis* (WP_134975396.1) that is responsible for attaching the forespore to the precoat during sporulation (9). While similar to CotE in its morphogenetic role, research has shown that SpoIVA completely surrounds the forespore and works to attach the precoat that CotE constructs (9). This is hypothesized to occur early in sporulation and at least prior to attachment of CotE to the forespore (9). In addition to its morphogenetic role in coat attachment, SpoIVA also had been shown to regulate the synthesis and assembly of inner layers; null *spoIVA* mutants typically possess no cortex (9). This is logical given that SpoIVA forms the ring between coat and cortex during and after precoat assembly (9). Through the *in-silico* proteomic analysis, SpoIVA was identified in every *Clostridia* species, of which 49 were novel results.

Stage V sporulation protein, SpoVM. The morphogenetic stage V sporulation, SpoVM, of *B. subtilis* (WP_003221545.1) forms the basement layer of the coat, affecting the assembly of all other coat and exosporium proteins (49). Binding to the convex membranes on the outer edge of the forespore, SpoVM essentially marks the forespore for assembly (49). SpoIVA is recruited and anchored to SpoVM, the former protein then going on to assemble the inner coat (49). There is a mutual dependence between SpoVM and SpoIVA, although their exact interactions are unknown (49).

Remarkably, there are no homologues for SpoVM in the *Clostridia* class. Six homologues were previously hypothesized in *Clostridia* species, but none clustered with *B. subtilis* SpoVM. Given the importance of this protein and the high conservation rate of SpoIVA in *Clostridia*, another protein is likely serving in this role. Further research will need to identify functional homologues of SpoVM.

Signal peptidase I, SpsB. The signal peptidase I, SpsB, of *B. subtilis* (QBJ66973.1) is a dTDP-glycosylphosphate transferase (50). Reliant on CotE but not on CotZ for assembly, this protein forms a ring around the spore in the outer coat (50). Its exact function remains unknown, but it has been implicated in the maturation of the coat surface (51). SpsB is conserved in all 107 *Clostridia* species; of these, 94 results were novel.

Spore coat polysaccharide biosynthesis protein, SpsC. SpsC is a glutamine-dependent transaminase of *B. subtilis* (WP_003243878.1) (50). Localized to the spore coat, this protein synthesizes polysaccharides on the coat surface, contributing to spore maturation and hydrophilicity in mature spore (50, 52). SpsC's exact localization remains unknown. This protein is well conserved across *Clostridia* with 75 species encoding SpsC homologues. Of these results, 68 homologues were novel, 6 were validated, and 1 was missed.

Spore coat polysaccharide biosynthesis protein, SpsE. There is limited information available on the SpsE coat biosynthesis protein of *B. subtilis* (WP_038429814.1). SpsE is a phosphoenolpyruvate-sugar pyruvyltransferase that likely localizes to the spore crust with others of the Sps family (50). In *Clostridia*, there are 76 homologues of SpsE across the species, 72 of which are novel.

Spore coat polysaccharide biosynthesis protein, SpsF. As with SpsE, little research has been conducted as to the localization or role of SpsF, a coat biosynthesis protein in *B. subtilis* (WP_003243421.1) (50). This protein is a glycosyltransferase that likely assembles in the spore crust and contributes to coat biosynthesis (50). Forty-nine homologues of this protein were identified in *Clostridia*, of which 45 were novel results.

Spore coat polysaccharide biosynthesis protein, SpsG. A glycosyltransferase in *B. subtilis* (WP_003244190.1), SpsG is involved in polysaccharide biosynthesis for the spore coat (50). Likely to localize with the other Sps proteins, more research needs to be conducted on its exact location within the mature spore. There were 26 novel homologues identified in the *Clostridia* class, adding to the total of 28.

Glucose-1-phosphate thymidylyltransferase, SpsI/RfbA. The *B. cereus* (WP_000676186.1) dTDP-glucose pyrophosphorylase, SpsI or RfbA, is also conserved in *B. subtilis* (50). In this latter species, SpsI has been shown to significantly contribute to the synthesis and assembly of the polysaccharide layer of the crust (7). This is likely due to its ability to produce rhamnose moieties, which "decorate" the surfaces of *Bacillus* spores (7). While this protein does not affect coat composition, it depends on CotE, CotX, CotY, and CotZ for its localization; it does not affect

the morphogenetic proteins of the crust (7, 53). Given its role in the synthesis of the polysaccharide layer, SpsI conveys hydrophilicity to spores. There are 86 homologues, and 77 of these results were novel.

dTDP-glucose 4,6-dehydratase, SpsJ. SpsJ is a dTDP-glucose 4,6-dehydratase in *B. subtilis* (WP_003244201.1) (50). As with others in its family, SpsJ conveys hydrophilicity to spores via synthesis of rhamnose on the crust (7). Likely to localize near those in its operon—SpsI, SpsK, and SpsL—little else is known on this protein (7). Better conserved than SpsI, 90 species of *Clostridia* have homologues of SpsJ. Of these, 85 were novel.

Spore coat polysaccharide biosynthesis protein, SpsK. The SpsK protein of *B. subtilis* (WP_003242585.1) is a dTDP-4-dehydrorhamnose reductase similarly involved in rhamnose production (7, 50). As with SpsI, this protein is involved in the polysaccharide synthesis on the forespore itself—versus the mother cell—during sporulation and maturation (7). Conveying hydrophilicity, this protein probably localizes with SpsI in the coat (7). In addition to the 4 homologues in *Clostridia* that were previously established, 77 other homologues were identified here alongside the one homologue that was missed.

Spore coat polysaccharide biosynthesis protein, SpsL. The last spore coat polysaccharide biosynthesis protein, SpsL, of *B. subtilis* (WP_003242881.1) is also involved in the rhamnose synthesis on the crust (7). This dTDP-4-dehydrorhamnose 3,5-epimerase conveys hydrophilicity to the spore, but it does not affect sporulation or germination rates in *Bacillus* (7, 50, 54). Conserved at a similar rate to other Sps proteins, all 58 homologues of SpsL were novel.

Stage IV sporulation protein H, StoA/SpoIVH. In *B. subtilis* (WP_077670684.1), the stage IV sporulation protein, StoA or SpoIVH, has been implicated in the synthesis of the protective peptidoglycan cortex layer during sporulation—though its localization in mature spores remain unknown (55). Specifically, this protein breaks the disulfide bonds in the SpoVD protein on the forespore envelope (56). This protein is a thiol-sulfide oxidoreductase, and it localizes to the intermembrane space during sporulation (57). StoA conveys heat, lysozyme, and chloroform resistance to spores, and it is particularly important in spore maturation (57). Null mutants of

stoA have a 100-fold decrease in sporulation rate, and while they possess a spore core, inner coat, and outer coat—null mutants have no cortex (57). Only two species in *Clostridia* encode a homologue of this protein; thus, it is likely there is functional homologue in *Clostridia*.

Protein-glutamine gamma-glutamyltransferase, Tgl. Tgl is a protein-glutamine gamma-glutamyltransferase in *B. subtilis* (WP_003243977.1). Its primary role is to crosslink GerQ during spore coat assembly, although it has also been implicated in mediating changes to coat proteins under temperature variation—such as during germination—with YabG (58, 59). The former activity occurs late in sporulation, and Tgl has been shown to crosslink itself to the proteins it is crosslinking (60, 61). Localized throughout the inner and outer coats of *B. subtilis*, Tgl conveys stability to the latter, although it is not a requisite component (58). Abundant enough to form a ring around the spore, this protein is believed to convey mechanical resistance properties (11, 58). It is dependent on CotE, but independent of CotH (11), and it has been shown *not* to crosslink CotB, CotC, CotE, CotG, CotM, CotX, CotY, or CotZ (11, 58).

Previous literature has claimed that no *Clostridia* species encode this protein (61). This has led authors to suggest that the oxidizing environment following mother cell lysis in aerobic species is requisite for GerQ crosslinking (61). However, the proteomic analysis conducted here has shown 23 homologues in *Clostridia*, of which 9 were previously established and 14 are novel.

Hypothetical protein, VT28_33540. The VT28_33540 protein described here is a novel, putative protein which was previously mislabeled as "CotS" in *C. sporogenes* (KRU24872.1). VT28_33540's localization and function are still unknown, but it appears to belong to the CotS family of proteins. As with another in this family—CotI—this protein is well conserved throughout the *Clostridia* class with 77 homologues identified here.

Sporulation-specific protease, YabG. The peptidase, YabG, of *B. subtilis* (WP_003243478.1) is a sporulation protein involved in the assembly but not the synthesis of the spore coat (62). This protein depends on SpoIVA but not CotE, which implies YabG localizes to the inner coat (62). While null mutants of *yabG* show no obvious change in phenotype, a structural difference can be detected on a molecular level (62). YabG is involved in the maturation of SpoIVA and YrbA via proteolysis as the spore enters dormancy (63). YabG is a highly conserved protein with 47

homologues identified, all of which were missed in this proteomic analysis; this was likely due to the similarly between YabG, YabP, and YabQ.

Spore protein, YabP. YabP is a protein found in *B. subtilis* spores (WP_003226714.1). This protein's effect on sporulation remains unclear, but it was shown *not* to effect germination (64, 65). Localization wise, YabQ recruits YabP which then forms a shell and later a ring around the forespore in *B. subtilis* sporulating cells, and it is hypothesized to be replaced by SpoIVA during later stages of sporulation (66). While past research has not detected this protein in mature spores, this is still not certain (66). In *Clostridia,* there are 100 species that encode a homologue of this protein. Eleven of these homologues were missed by this analysis, but 40 were validated and 49 were novel.

Spore cortex biosynthesis protein, YabQ. In *B. subtilis* (WP_003243260.1), YabQ is a protein involved in the synthesis of the spore cortex (65). During sporulation, this protein localizes to the forespore membrane (65). While its exact location in the dormant spore remains unknown, this protein is maintained in the coat upon maturation (65, 66). It does not require SpoIVA or CotE for assembly (65, 66). *YabQ* null mutants produce atypical spores with small or no cortices and with the coats partially detached (65). These mutants are sensitive to chloroform, lysozyme, and heat (65). This protein, like YabG, was missed by this proteomic analysis; there are 30 species of *Clostridia* that encode this protein.

Putative sporulation-specific glycosylase, YdhD. In *B. subtilis* (QBJ69211.1), the LysM peptidoglycan binding domain-containing protein, YdhD, is involved in sporulation and is retained in mature spores (67). Shown not to convey resistance properties, YdhD seemingly inhibits germination in response to L-alanine (67). However, in null mutants, YdhD was shown to have no effect on germination rates (68). YdhD is a cortex-lytic enzyme, but research has shown that is has no effect on cortex peptidoglycan synthesis during sporulation nor on its breakdown during germination (68). Of the 107 *Clostridia* species, 39 of them encoded YdhD in their proteomes: 26 novel results, 7 validated, 5 missed, and 1 false.

Spore coat F-like protein, YgzC. YgzC is an implicated spore coat protein in *B. subtilis* (WP_010886449.1). Little is known about this protein, but null-mutants for *ygzC* produce fewer spores, and they are less heat resistant (10). *C. homopropionicum* was the only species to encode a homologue of this protein.

Stress response protein, YhaX. The HAD-11B family hydrolase protein, YhaX, of *B. subtilis* (WP_003233277.1) has not been thoroughly studied by past researchers. Found by Kim, et al. to be independent of CotE, CotH, and Tgl, this protein is suspected to localize in the basement membrane or inner coat with SpoVID, SpoIVA, and SpoVM (11, 69). Only 2 species of *Clostridia* encoded a homologue to the YhaX protein, both of which were missed by the analysis.

Epoxyqueuosine reductase, YhbA/QueG. Yhba (or the synonymous, QueG) of *B. subtilis* (AFQ56810.1) is a tRNA epoxyqueuosine reductase that is hypothesized to play a role in sporulation (19). Cobalamin dependent, this iron-sulfur binding protein performs epoxide reduction (19, 70). In effect, YhbA catalyzes the final step in biosynthesis of queuosine, but its effect on sporulation remains unknown at this point (71). Four *Clostridia* species express homologues of YhbA, all of which were novel results.

Amidase domain-containing protein, YhbB. In *B. subtilis* (WP_010886452.1), the amidase domain-containing protein, YhbB, is another suspected contributor to sporulation (19). Given it is independent of CotE and Tgl and only dependent on CotH at certain temperatures, this protein is likely to localize to the inner coat or outer membrane (11). Beyond this, little is known about YhbB. There are 54 protein homologues of YhbB in the *Clostridia* class, all of which were novel.

Sporulation lipoprotein, YhcN. YhcN is an abundant lipoprotein involved in the sporulation of *B. subtilis* (WP_003245125.1 and WP_003232269.1) as well as in other species of *Firmicutes* (72). Localizing mainly to the inner spore membrane between the core and cortex, this protein has also been found in the outer coat layers (63, 72, 73). YhcN conveys heat resistance and promotes germination in the presence of L-alanine; as such, null mutants had reduced colony growth due to delays in germination (72). This protein also contributes to the function of SleB in cortex hydrolysis (72).

It was previously hypothesized that YhcN is only encoded in proteomes that also encode SleB and CwlJ—the foremost cortex hydrolysis proteins—but not those encoding SleC (72). This *in-silico* proteomic analysis disproves that hypothesis: three species encoding YhcN also encoded SleC. As such, more research needs to be conducted on the co-dependencies of YhcN to CwlJ/SleB versus SleC in their respective pathways. In total, there are 10 homologues of YhcN in *Clostridia*, of which 2 were novel results, 1 was validated, and 7 were missed.

Spore coat F-like protein, YhcQ. Spore coat protein, YhcQ, of *B. subtilis* (WP_009966881.1) has not been extensively studied. With a high sequence similarity to CotF, this protein is part of the spore coat and has been detected during germination (74). It is dependent on CotH for assembly, but its role and exact localization remains unknown (74). In *Clostridia*, 7 novel homologues of YhcQ were identified.

Endospore coat-associated protein, YheC. The putative endospore coat-associated protein, YheC, in *B. subtilis* (WP_003245845.1) has not been extensively studied. Known to be synthesized in the mother cell and later assembled into the coat, its exact localization in dormant spores is undetermined (63). This protein belongs to the ATP-grasp amidoligase superfamily, but its function is similarly obscure (75). *Clostridium thermarum* (*C. thermarum)* was the only species to encode this protein.

Endospore coat-associated protein, YheD. Unlike the upstream-encoded YheC, the spore coat protein YheD of *B. subtilis* (WP_003245015.1) has been well studied, at least localization wise. Independent of CotE, CotH, and Tgl, this protein was shown to only rely upon SpoIVA which anchors the coat to the forespore (11, 66). During sporulation, this protein forms two distinct rings around the forespore before forming a shell around the developing spore (66). YheD is found in the basement layer beneath the inner coat in mature spores (69). And while YheD was previously believed to not exist in *Clostridia*, there are 6 homologues—one previously established—encoded in the class.

Four-helix bundle copper-binding protein, YhjQ. The copper-binding protein, YhjQ, of *B. subtilis* (WP_009966950.1) has not been thoroughly studied. This protein prevents copper

toxicity in bacterial cells through binding activity, allowing for proper copper storage in the cytosol (76, 77). Its role and localization in spores are unknown but implicated. There are 39 species of *Clostridia* that encoded this protein, all of which—other than the homologue in *C. botulinum*—are novel.

Alpha/beta hydrolase, YisY. The alpha/beta hydrolase YisY of *B. subtilis* (WP_003245141.1) is a suspected chloride peroxidase whose role likely involves protection or germination of spores (19). Specifically, oxidases have been shown to crosslink coat proteins, detoxify environmental contaminants, and symbiose with microorganisms in the environment; YisY may serve in any of these roles (19). While the exact location of YisY remains unknown, it has been shown to form a ring around the spore and assemble independently of CotE, CotH, and Tgl (11). Kuwana, et al. hypothesizes that this protein is synthesized within the forespore and localizes to the spore core (63). At the very least, YisY is unlikely to localize within or beyond the outer coat given its independence from CotE (19).

While the exact role of this protein remains unclear, the fact that YisY is an oxidase implies its importance to the functioning of the *Bacillus* spore. It is thus unsurprising that this protein is very well conserved across *Clostridia* with 77 homologues of which 73 are novel.

Oxidoreductase, YjgC. Very little is known about YjgC of *B. subtilis* (WP_003245240.1) beyond its identity as a formate dehydrogenase and implication towards involvement in the spore stress response system (78). There are 34 homologues of YjgC in *Clostridia,* all of which are novel results.

Manganese catalase family protein, YjqC. YjqC is a well-studied protein in *B. altitudinis,* though the query protein used here was that in *B. subtilis* (WP_003245071.1). In the former species, YjqC is a structural protein in the outer coat, responsible for conveying resistance to hydrogen peroxide and heat (79). This manganese catalase is exposed on the spore surface and is involved in the sporulation process (79). Specifically, YjqC is responsible for laccase and peroxidase activities (79). In *Clostridia,* there are 6 species that encode this protein, all of which are novel.

L,D-transpeptidase family protein, YkuD. YkuD is a protein in *B. subtilis* (WP_010886500.1) that is upregulated by sigma-K and potentially downregulated by *gerE* (67). Overexpression of this protein does not affect cellular growth, L-alanine germination, or resistance to chloroform, lysozyme, or heat (67). Likely synthesized in the mother cell during sporulation, this protein has been detected in mature spores, but its exact localization and function remains unknown (67). It is hypothesized to locate to the membrane between the cortex and inner coat (67). There are 60 homologues of YkuD in *Clostridia;* 42 of these results were novel, 17 were validated, and 1 was missed by the analysis.

Spore protein, YkvP. The *B. subtilis* (WP_003245384.1) protein, YkvP, possesses a cell-wall binding motif (63). This LysM domain is hypothesized to bind sugar moieties or peptidoglycans to the cortex during sporulation; it may also attach spore crust polysaccharides (8). Like YkuD, this protein was shown *not* to affect resistance, germination, or outgrowth of vegetative cells (67). It was detected in mature spores and may localize to the basement layer alongside YkuD (67). YkvP is probably synthesized in the mother cell, and its expression is positively regulated by both sigma-K and *gerE* (67). In *Clostridia,* 18 novel homologues of YkvP were identified in addition to the 5 validated and 1 missed.

Sporulation-specific glycosylase, YkvQ. Little is known about the coat protein YkvQ of *B. subtilis* (AYF10890.1). This protein is a glycosylase, but its localization and purpose within the spore assembly remains unclear (76). Two species of *Clostridia* have novel YkvQ homologues.

Spore coat F-like protein, YraD. YraD of *B. subtilis* (WP_003246006.1) is a spore coat protein that is very similar to CotF in their C-terminal sequences and YraF in their N-terminal sequences (9). The function and localization of this protein remains unknown. In *Clostridia*, 2 homologues of YraD had already been identified, but this *in-silico* proteomic analysis found 14 more.

Spore coat F-like protein, YraF. Spore coat protein, YraF, of *B. subtilis* (WP_009967868.1) has not been extensively studied, so its localization and function remain unknown. Similar to CotF in its C-terminal, this protein is also similar to YraD at the N-terminal (9). There were 10

novel homologues of YraF identified in the *Clostridia* species, totaling 12 with previously established proteins.

Spore coat F-like protein, YraG. In *B. subtilis*(WP_003229845.1), the Yra spore coat protein, YraG, is unstudied like the remainder of its family. This protein is not conserved within *B. cereus*, but it is similar to CotF at the N-terminal (9, 80). Of the 12 homologues of YraG in *Clostridia*, 11 of these results were novel to this analysis.

Transcriptional regulatory protein, YrbC. The only known information on the YrbC protein of *B. subtilis* (WP_004398802.1) is that it is somehow involved in sporulation (81). However, this protein is likely to be important given it is conserved across all *Clostridia* species; only two of these results were previously established.

Methionine-binding lipoprotein, YusA. YusA, or MetQ in *Escherichia coli (E. coli)*, is a spore coat protein that is involved in transport in *B. subtilis* cells (WP_003228595.1) (82). Forming a ring around the spore, this protein is dependent on CotE, CotG, and partially CotH for assembly and localization; as such, YusA is likely an outer coat or crust protein (11). Specifically, YusA is an ABS transporter substrate-binding protein that conducts L-methionine, and potentially D-methionine, transport (19, 82). This protein is very well conserved across *Clostridia* with 97 homologues, all of which are novel results.

Spore coat protein, YvdP. It remains uncertain whether CotQ and YvdP (WP_003228217.1) of *B. subtilis* are the same protein, but the fact they clustered separately in this analysis implies their differential identities. The literature, however, refers to the two proteins as one, reporting that YvdP/CotQ is synthesized late in sporulation, partially dependent on CotE, CotH, and CotG, and that it forms a ring around the spore (11, 19). This literature also hypothesizes that YvdP is regulated by sigma-G and synthesized in the forespore (63). While CotQ is not conserved in *Clostridia,* there are 48 homologues of YvdP, providing further evidence of their differential identities.

Putative transporter permease subunit protein, YybL. YybL is a putative transporter permease protein of *B. subtilis* (WP_003243512.1). Independent of CotE, CotH, and Tgl, it is unlikely to localize beyond the inner coat (11). This protein is hypothesized to span membranes as an ABC transporter (83). Fifty-six homologues were detected in the *Clostridia* proteomes.

* "novel" does not mean newly identified; it means that previous literature has not reported on it. All results have been previously identified (given sequences on NCBI), but majority of the proteins were not published results.

1. Stewart GC. 2015. The Exosporium Layer of Bacterial Spores: a Connection to the Environment and the Infected Host. Microbiology and Molecular Biology Reviews 79:437-457.
2. Im H, Sharpe ML, Strych U, Davlieva M, Krause KL. 2011. The crystal structure of alanine racemase from *Streptococcus pneumoniae,* a target for structure-based drug design. BMC Microbiology 11:116.
3. Todd SJ, Moir AJG, Johnson MJ, Moir A. 2003. Genes of *Bacillus cereus* and *Bacillus anthracis* encoding proteins of the exosporium. Journal of Bacteriology 185:3373-3378.
4. Barra-Carrasco J, Olguín-Araneda V, Plaza-Garrido A, Miranda-Cárdenas C, Cofré-Araneda G, Pizarro-Guajardo M, Sarker MR, Paredes-Sabja D. 2013. The *Clostridium difficile* exosporium cysteine (CdeC)-rich protein is required for exosporium morphogenesis and coat assembly. Journal of Bacteriology 195:3863-75.
5. Anonymous. 2004. CdeA of *Clostridium difficile*, a New Multidrug Efflux Transporter of the MATE Family. Microbial Drug Resistance 10:191-196.
6. Calderón-Romero P, Castro-Córdova P, Reyes-Ramírez R, Milano-Céspedes M, Guerrero-Araya E, Pizarro-Guajardo M, Olguín-Araneda V, Gil F, Paredes-Sabja D. 2018. *Clostridium difficile* exosporium cysteine-rich proteins are essential for the morphogenesis of the exosporium layer, spore resistance, and affect *C. difficile* pathogenesis. PLOS Pathogens 14:e1007199.
7. Shuster B, Khemmani M, Abe K, Huang X, Nakaya Y, Maryn N, Buttar S, Gonzalez AN, Driks A, Sato T, Eichenberger P. 2019. Contributions of crust proteins to spore surface properties in *Bacillus subtilis*. Molecular Microbiology 111:825-843.
8. Bartels J, Blüher A, López Castellanos S, Richter M, Günther M, Mascher T. 2019. The *Bacillus subtilis* endospore crust: protein interaction network, architecture and glycosylation state of a potential glycoprotein layer. Molecular Microbiology 112:1576-1592.
9. Driks A. 1999. *Bacillus subtilis* Spore Coat. Microbiology and Molecular Biology Reviews 63:1-20.
10. Hullo M-FO, Moszer I, Danchin A, Martin-Verstraete I. 2001. CotA of *Bacillus subtilis* Is a Copper-Dependent Laccase. Journal of Bacteriology 183:5426-5430.

11. Kim H, Hahn M, Grabowski P, McPherson DC, Otte MM, Wang R, Ferguson CC, Eichenberger P, Driks A. 2006. The *Bacillus subtilis* spore coat protein interaction network. Molecular Microbiology 59:487-502.
12. Sebaihia M, Wren BW, Mullany P, Fairweather NF, Minton N, Stabler R, Thomson NR, Roberts AP, Cerdeño-Tárraga AM, Wang H, Holden MT, Wright A, Churcher C, Quail MA, Baker S, Bason N, Brooks K, Chillingworth T, Cronin A, Davis P, Dowd L, Fraser A, Feltwell T, Hance Z, Holroyd S, Jagels K, Moule S, Mungall K, Price C, Rabbinowitsch E, Sharp S, Simmonds M, Stevens K, Unwin L, Whithead S, Dupuy B, Dougan G, Barrell B, Parkhill J. 2006. The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome. Nature Genetics 38:779-786.
13. Permpoonpattana P, Tolls EH, Nadem R, Tan S, Brisson A, Cutting SM. 2011. Surface Layers of *Clostridium difficile* Endospores. Journal of Bacteriology 193:6461-6470.
14. ZilhãO R, Serrano MN, Isticato R, Ricca E, Moran CP, Henriques AO. 2004. Interactions among CotB, CotG, and CotH during Assembly of the *Bacillus subtilis* Spore Coat. Journal of Bacteriology 186:1110-1119.
15. Paredes-Sabja D, Shen A, Sorg JA. 2014. *Clostridium difficile* spore biology: sporulation, germination, and spore structural proteins. Trends in Microbiology 22:406-416.
16. Imamura D, Kuwana R, Takamatsu H, Watabe K. 2010. Localization of Proteins to Different Layers and Regions of *Bacillus subtilis* Spore Coats. Journal of Bacteriology 192:518-524.
17. Ramos-Silva P, Serrano M, Henriques AO. 2019. From Root to Tips: Sporulation Evolution and Specialization in *Bacillus subtilis* and the Intestinal Pathogen *Clostridioides difficile*. Molecular Biology and Evolution 36:2714-2736.
18. Nguyen KB, Sreelatha A, Durrant ES, Lopez-Garrido J, Muszewska A, Dudkiewicz M, Grynberg M, Yee S, Pogliano K, Tomchick DR, Pawłowski K, Dixon JE, Tagliabracci VS. 2016. Phosphorylation of spore coat proteins by a family of atypical protein kinases. Proceedings of the National Academy of Sciences 113:E3482-E3491.
19. Lai E-M, Phadke ND, Kachman M, Giorno R, Vazquez S, Vazquez JA, Maddock JR, Driks A. 2003. Proteomic Analysis of the Spore Coats of *Bacillus subtilis* and *Bacillus anthracis*. Journal of Bacteriology 185:1443 - 1454.

20. Seyler Jr RW, Henriques AO, Ozin AJ, Moran Jr CP. 1997. Assembly and interactions of cotJ-encoded proteins, constituents of the inner layers of the *Bacillus subtilis* spore coat. Molecular Microbiology 25:955-966.
21. Whittingham JL, Hanai S, Brannigan JA, Ferreira WT, Dodson EJ, Turkenburg JP, Cartwright J, Cutting SM, Wilkinson AJ. 2020. Crystal structures of the GH18 domain of the bifunctional peroxiredoxin-chitinase CotE from *Clostridium difficile*. Acta crystallographica Section F, Structural biology communications 76:241-249.
22. Takamatsu H, Chikahiro Y, Kodama T, Koide H, Kozuka S, Tochikubo K, Watabe K. 1998. A spore coat protein, CotS, of *Bacillus subtilis* is synthesized under the regulation of sigmaK and GerE during development and is located in the inner coat layer of spores. Journal of Bacteriology 180:2968-2974.
23. Saggese A, Scamardella V, Sirec T, Cangiano G, Isticato R, Pane F, Amoresano A, Ricca E, Baccigalupi L. 2014. Antagonistic Role of CotG and CotH on Spore Germination and Coat Formation in *Bacillus subtilis*. PLOS ONE 9:e104900.
24. McPherson DC, Kim H, Hahn M, Wang R, Grabowski P, Eichenberger P, Driks A. 2005. Characterization of the *Bacillus subtilis* spore morphogenetic coat protein CotO. Journal of Bacteriology 187:8278-90.
25. Janganan TK, Mullin N, Dafis-Sagarmendi A, Brunt J, Tzokov SB, Stringer S, Moir A, Chaudhuri RR, Fagan RP, Hobbs JK, Bullough PA. 2020. Architecture and Self-Assembly of *Clostridium sporogenes* and *Clostridium botulinum* Spore Surfaces Illustrate a General Protective Strategy across Spore Formers. mSphere 5.
26. Janganan TK, Mullin N, Tzokov SB, Stringer SC, Fagan RP, Hobbs JK, Moir A, Bullough PA. 2016. Characterization of the spore surface and exosporium proteins of *Clostridium sporogenes*; implications for *Clostridium botulinum* group I strains. Food Microbiology 59:205 - 212.
27. Bagyan I, Setlow P. 2002. Localization of the cortex lytic enzyme CwlJ in spores of *Bacillus subtilis*. Journal of Bacteriology 184:1219-24.
28. Lequette Y, Garénaux E, Tauveron G, Dumez S, Perchat S, Slomianny C, Lereclus D, Guérardel Y, Faille C. 2011. Role Played by Exosporium Glycoproteins in the Surface Properties of *Bacillus cereus* Spores and in Their Adhesion to Stainless Steel. Applied and Environmental Microbiology 77:4905-4911.

29. Janoir C, Denève C, Bouttier S, Barbut F, Hoys S, Caleechum L, Chapetón-Montes D, Pereira FC, Henriques AO, Collignon A, Monot M, Dupuy B. 2013. Adaptive strategies and pathogenesis of *Clostridium difficile* from in vivo transcriptomics. Infection and Immunity 81:3757-69.
30. Díaz-González F, Milano M, Olguin-Araneda V, Pizarro-Cerda J, Castro-Córdova P, Tzeng S-C, Maier CS, Sarker MR, Paredes-Sabja D. 2015. Protein composition of the outermost exosporium-like layer of *Clostridium difficile* 630 spores. Journal of Proteomics 123:1-13.
31. Masayama A, Kuwana R, Takamatsu H, Hemmi H, Yoshimura T, Watabe K, Moriyama R. 2007. A novel lipolytic enzyme, YcsK (LipC), located in the spore coat of *Bacillus subtilis*, is involved in spore germination. Journal of Bacteriology 189:2369-2375.
32. McKenney PT, Eichenberger P. 2012. Dynamics of spore coat morphogenesis in *Bacillus subtilis*. Molecular Microbiology 83:245-60.
33. Masayama A, Kato S, Terashima T, Mølgaard A, Hemmi H, Yoshimura T, Moriyama R. 2010. *Bacillus subtilis* Spore Coat Protein LipC Is a Phospholipase B. Bioscience, Biotechnology, and Biochemistry 74:24-30.
34. Conter C, Oppici E, Dindo M, Rossi L, Magnani M, Cellini B. 2019. Biochemical properties and oxalate-degrading activity of oxalate decarboxylase from *Bacillus subtilis* at neutral pH. IUBMB Life 71:917-927.
35. Anand R, Dorrestein PC, Kinsland C, Begley TP, Ealick SE. 2002. Structure of oxalate decarboxylase from *Bacillus subtilis* at 1.75 A resolution. Biochemistry 41:7659-69.
36. Costa T, Steil L, Martins LO, Völker U, Henriques AO. 2004. Assembly of an oxalate decarboxylase produced under sigmaK control into the *Bacillus subtilis* spore coat. Journal of Bacteriology 186:1462-74.
37. Lin P, Yuan H, Du J, Liu K, Liu H, Wang T. 2020. Progress in research and application development of surface display technology using *Bacillus subtilis* spores. Applied Microbiology and Biotechnology 104:2319-2331.
38. Potot S, Serra CR, Henriques AO, Schyns G. 2010. Display of recombinant proteins on *Bacillus subtilis* spores, using a coat-associated enzyme as the carrier. Applied and Environmental Microbiology 76:5926-33.

39. Yan J, Zou W, Fang J, Huang X, Gao F, He Z, Zhang K, Zhao N. 2015. Eukaryote-like Ser/Thr protein kinase PrkA modulates sporulation via regulating the transcriptional factor σ(K) in *Bacillus subtilis*. Frontiers in Microbiology 6:382.
40. Pompeo F, Foulquier E, Galinier A. 2016. Impact of Serine/Threonine Protein Kinases on the Regulation of Sporulation in *Bacillus subtilis*. Frontiers in Microbiology 7:568-568.
41. Putnam EE, Nock AM, Lawley TD, Shen A. 2013. SpoIVA and SipL are *Clostridium difficile* spore morphogenetic proteins. Journal of Bacteriology 195:1214-25.
42. Touchette MH, Benito de la Puebla H, Ravichandran P, Shen A. 2019. SpoIVA-SipL Complex Formation Is Essential for *Clostridioides difficile* Spore Assembly. Journal of Bacteriology 201.
43. Burns DA, Heap JT, Minton NP. 2010. SleC is essential for germination of *Clostridium difficile* spores in nutrient-rich medium supplemented with the bile salt taurocholate. Journal of Bacteriology 192:657-64.
44. Bernhards CB, Popham DL. 2014. Role of YpeB in cortex hydrolysis during germination of *Bacillus anthracis* spores. Journal of Bacteriology 196:3399-3409.
45. Lambert EA, Sherry N, Popham DL. 2012. In vitro and in vivo analyses of the *Bacillus anthracis* spore cortex lytic protein SleL. Microbiology (Reading) 158:1359-1368.
46. Passalacqua KD, Bergman NH, Herring-Palmer A, Hanna P. 2006. The Superoxide Dismutases of *Bacillus anthracis* Do Not Cooperatively Protect against Endogenous Superoxide Stress. Journal of Bacteriology 188:3837-3848.
47. Vanaporn M, Wand M, Michell SL, Sarkar-Tyson M, Ireland P, Goldman S, Kewcharoenwong C, Rinchai D, Lertmemongkolchai G, Titball RW. 2011. Superoxide dismutase C is required for intracellular survival and virulence of *Burkholderia pseudomallei*. Microbiology (Reading) 157:2392-2400.
48. Keith KE, Valvano MA. 2007. Characterization of SodC, a periplasmic superoxide dismutase from *Burkholderia cenocepacia*. Infection and Immunity 75:2451-60.
49. Peluso EA, Updegrove TB, Chen J, Shroff H, Ramamurthi KS. 2019. A 2-dimensional ratchet model describes assembly initiation of a specialized bacterial cell surface. Proceedings of the National Academy of Sciences 116:21789-21799.
50. Cangiano G, Sirec T, Panarella C, Isticato R, Baccigalupi L, Felice MD, Ricca E, Parales RE. 2014. The *Sps* Gene Products Affect the Germination, Hydrophobicity, and Protein

Adsorption of *Bacillus subtilis* Spores. Applied and Environmental Microbiology 80:7293-7302.

51. Dubois T, Krzewinski F, Yamakawa N, Lemy C, Hamiot A, Brunet L, Lacoste AS, Knirel Y, Guerardel Y, Faille C. 2020. The *Sps* Genes Encode an Original Legionaminic Acid Pathway Required for Crust Assembly in *Bacillus subtilis*. mBio 11.
52. Knurr J, Benedek O, Heslop J, Vinson RB, Boydston JA, McAndrew J, Kearney JF, Turnbough CL, Jr. 2003. Peptide ligands that bind selectively to spores of *Bacillus subtilis* and closely related species. Applied and Environmental Microbiology 69:6841-7.
53. Arrieta-Ortiz ML, Hafemeister C, Bate AR, Chu T, Greenfield A, Shuster B, Barry SN, Gallitto M, Liu B, Kacmarczyk T, Santoriello F, Chen J, Rodrigues CD, Sato T, Rudner DZ, Driks A, Bonneau R, Eichenberger P. 2015. An experimentally supported model of the *Bacillus subtilis* global transcriptional regulatory network. Molecular Systems Biology 11:839.
54. Ragkousi K, Eichenberger P, Ooij Cv, Setlow P. 2003. Identification of a New Gene Essential for Germinationof *Bacillus subtilis* Spores with Ca^{2+}-Dipicolinate. Journal of Bacteriology 185:2315-2329.
55. Crow A, Liu Y, Möller MC, Le Brun NE, Hederstedt L. 2009. Structure and functional properties of *Bacillus subtilis* endospore biogenesis factor StoA. The Journal of Biological Chemistry 284:10056-10066.
56. Liu Y, Carlsson Möller M, Petersen L, Söderberg CA, Hederstedt L. 2010. Penicillin-binding protein SpoVD disulphide is a target for StoA in *Bacillus subtilis* forespores. Molecular Microbiology 75:46-60.
57. Erlendsson LS, Möller M, Hederstedt L. 2004. *Bacillus subtilis* StoA Is a thiol-disulfide oxidoreductase important for spore cortex synthesis. Journal of Bacteriology 186:6230-8.
58. Zilhão R, Isticato R, Martins LO, Steil L, Völker U, Ricca E, Moran CP, Henriques AO. 2005. Assembly and Function of a Spore Coat-Associated Transglutaminase of *Bacillus subtilis*. Journal of Bacteriology 187:7753-7764.
59. Kuwana R, Okuda N, Takamatsu H, Watabe K. 2006. Modification of GerQ reveals a functional relationship between Tgl and YabG in the coat of *Bacillus subtilis* spores. Journal of Biochemistry 139:887-901.

60. Ragkousi K, Setlow P. 2004. Transglutaminase-mediated cross-linking of GerQ in the coats of *Bacillus subtilis* spores. Journal of Bacteriology 186:5567-75.
61. Fernandes CG, Martins D, Hernandez G, Sousa AL, Freitas C, Tranfield EM, Cordeiro TN, Serrano M, Moran CP, Jr., Henriques AO. 2019. Temporal and spatial regulation of protein cross-linking by the pre-assembled substrates of a *Bacillus subtilis* spore coat transglutaminase. PLoS Genetics 15:e1007912.
62. Takamatsu H, Kodama T, Imamura A, Asai K, Kobayashi K, Nakayama T, Ogasawara N, Watabe K. 2000. The *Bacillus subtilis YabG* gene is transcribed by SigK RNA polymerase during sporulation, and *YabG* mutant spores have altered coat protein composition. Journal of Bacteriology 182:1883-1888.
63. Kuwana R, Kasahara Y, Fujibayashi M, Takamatsu H, Ogasawara N, Watabe K. 2002. Proteomics characterization of novel spore proteins of *Bacillus subtilis*. Microbiology 148 Pt 12:3971-82.
64. Fawcett P, Eichenberger P, Losick R, Youngman P. 2000. The transcriptional profile of early to middle sporulation in *Bacillus subtilis*. Proceedings of the National Academy of Sciences U S A 97:8063-8.
65. Asai K, Takamatsu H, Iwano M, Kodama T, Watabe K, Ogasawara N. 2001. The *Bacillus subtilis YabQ* gene is essential for formation of the spore cortex. Microbiology (Reading) 147:919-927.
66. Ooij Cv, Eichenberger P, Losick R. 2004. Dynamic Patterns of Subcellular Protein Localization during Spore Coat Morphogenesis in *Bacillus subtilis*. Journal of Bacteriology 186:4441-4448.
67. Kodama T, Takamatsu H, Asai K, Ogasawara N, Sadaie Y, Watabe K. 2000. Synthesis and characterization of the spore proteins of *Bacillus subtilis* YdhD, YkuD, and YkvP, which carry a motif conserved among cell wall binding proteins. Journal of Biochemistry 128:655-63.
68. Chirakkal H, O'Rourke M, Atrih A, Foster SJ, Moir A. 2002. Analysis of spore cortex lytic enzymes and related proteins in *Bacillus subtilis* endospore germination. Microbiology 148:2383-2392.

69. McKenney PT, Driks A, Eskandarian HA, Grabowski P, Guberman J, Wang KH, Gitai Z, Eichenberger P. 2010. A distance-weighted interaction map reveals a previously uncharacterized layer of the *Bacillus subtilis* spore coat. Current Biology 20:934-8.
70. Dowling DP, Miles ZD, Köhrer C, Maiocco SJ, Elliott SJ, Bandarian V, Drennan CL. 2016. Molecular basis of cobalamin-dependent RNA modification. Nucleic Acids Research 44:9965-9976.
71. Payne KAP, Fisher K, Sjuts H, Dunstan MS, Bellina B, Johannissen L, Barran P, Hay S, Rigby SEJ, Leys D. 2015. Epoxyqueuosine Reductase Structure Suggests a Mechanism for Cobalamin-dependent tRNA Modification *. Journal of Biological Chemistry 290:27572-27581.
72. Johnson MJ, Todd SJ, Ball DA, Shepherd AM, Sylvestre P, Moir A. 2006. ExsY and CotY Are Required for the Correct Assembly of the Exosporium and Spore Coat of *Bacillus cereus*. Journal of Bacteriology 188:7905-7913.
73. Bagyan I, Noback M, Bron S, Paidhungat M, Setlow P. 1998. Characterization of YhcN, a new forespore-specific gene of *Bacillus subtilis*. Gene 212:179-88.
74. Freitas C, Plannic J, Isticato R, Pelosi A, Zilhão R, Serrano M, Baccigalupi L, Ricca E, Elsholz AKW, Losick R, O. Henriques A. 2020. A protein phosphorylation module patterns the *Bacillus subtilis* spore outer coat. Molecular Microbiology 114:934-951.
75. Iiyama K, Otao M, Mori K, Mon H, Lee JM, Kusakabe T, Tashiro K, Asano S-I, Yasunaga-Aoki C. 2014. Phylogenetic relationship of *Paenibacillus* species based on putative replication origin regions and analysis of an YheCD-like sequence found in this region. Bioscience, Biotechnology, and Biochemistry 78:891-897.
76. Secaira-Morocho H, Castillo JA, Driks A. 2020. Diversity and evolutionary dynamics of spore-coat proteins in spore-forming species of *Bacillales*. Microbial Genomics 6.
77. Vita N, Landolfi G, Baslé A, Platsaki S, Lee J, Waldron KJ, Dennison C. 2016. Bacterial cytosolic proteins with a high capacity for Cu(I) that protect against copper toxicity. Scientific Reports 6:39065.
78. Petersohn A, Brigulla M, Haas S, Hoheisel JD, Völker U, Hecker M. 2001. Global analysis of the general stress response of *Bacillus subtilis*. Journal of Bacteriology 183:5617-31.

79. Zhang Y, Li X, Hao Z, Xi R, Cai Y, Liao X. 2016. Hydrogen Peroxide-Resistant CotA and YjqC of *Bacillus altitudinis* Spores Are a Promising Biocatalyst for Catalyzing Reduction of Sinapic Acid and Sinapine in Rapeseed Meal. PLOS ONE 11:e0158351.
80. Galperin MY, Mekhedov SL, Puigbo P, Smirnov S, Wolf YI, Rigden DJ. 2012. Genomic determinants of sporulation in *Bacilli* and *Clostridia*: towards the minimal set of sporulation-specific genes. Environmental Microbiology 14:2870-90.
81. Omony J, de Jong A, Krawczyk AO, Eijlander RT, Kuipers OP. 2018. Dynamic sporulation gene co-expression networks for *Bacillus subtilis* 168 and the food-borne isolate *Bacillus amyloliquefaciens*: a transcriptomic model. Microbial Genomics 4.
82. Hullo MF, Auger S, Dassa E, Danchin A, Martin-Verstraete I. 2004. The metNPQ operon of *Bacillus subtilis* encodes an ABC permease transporting methionine sulfoxide, D- and L-methionine. Research in Microbiology 155:80-6.
83. Albano M, Smits WK, Ho LT, Kraigher B, Mandic-Mulec I, Kuipers OP, Dubnau D. 2005. The Rok protein of *Bacillus subtilis* represses genes for cell surface and extracellular functions. Journal of Bacteriology 187:2010-9.