Research Workshop on Bacillus in Clinical and Agricultural Environments

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Research Workshop on *Bacillus* in Clinical and Agricultural Environments

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Abstract

The This event was inspired by the 2023 European Spores Conference and BACT Conference. These events bring many research groups together on spores and the common causative species, primarily *Bacillus*. However, to bring the latest knowledge to solve "real-world" problems brought by spores and *Bacillus* species, we wanted a dedicated event that facilitates knowledge transfer. Thus, the idea for this workshop was born. The focus of this event is to exchange knowledge between academia and other sectors, like agriculture or biosafety, where *Bacillus* species are a problem with substantial costs. We have speakers covering topics from *Bacillus* genomics to spore structure and opportunities in industry to decontamination. We hope this event will help communication and collaboration between universities and industries. We are thankful for those who have made the trip here and hope you enjoyed the meeting and the wintry aesthetic of the city as a whole.

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About Umeå University

Formally established in 1965, Umeå University is a comprehensive university covering research and education in medicine, science and technology, social sciences, arts and humanities, and educational sciences. Our cohesive campus environment enables a dynamic and open culture with a strong sense of community. Here, we have over 35000 students and over 2000 researchers that help to promote factfulness and contribute to scientific progress. In 2020, Emmanuelle Charpentier was awarded the Nobel Prize in Chemistry for her discovery of the gene-editing tool CRISPR-Cas9. A discovery she made during her time as a researcher at Umeå University. The prize was awarded jointly to Emmanuelle Charpentier and the American researcher Jennifer A. Doudna. Emmanuelle is an honorary doctor at Umeå University, a former visiting researcher at the Umeå Centre for Microbial Research, UCMR, and a former research leader at the Laboratory of Molecular Infection Medicine Sweden, MIMS.

Organizing Committee

Dmitry Malyshev (Umeå University, Sweden)

Magnus Andersson (Umeå University, Sweden)

Graham Christie (University of Cambridge, UK)

Laura Carroll (Umeå University, Sweden)

Les Baillie (Cardiff University, UK)

Daniel Nilsson (Umeå University, Sweden)

Sessions

1. Spore germination
2. Bacillus spore and cellular architecture
3. Bacillus in food & industry
4. Environmental decontamination of Bacillus

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Genotype-informed forecasting of *Bacillus cereus* group phenotypic traits

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Abstract

The *Bacillus cereus* group comprises strains with the potential to cause human illness and food spoilage, among other effects. The genetic determinants supporting the ability of individual strains to cause diseases such as anthrax and emetic intoxication are well understood. However, there is a notable gap in understanding the genotype-phenotype relationship concerning diarrheal foodborne illness. Most isolates of the B. cereus group possess at least one gene encoding enterotoxins. The presence of the enterotoxin genes *nhe*, *hbl*, and *cytK* serves as a sensitive, though non-specific, predictor of cytotoxicity. Currently, the classification into phylogenetic groups is one of the most accurate methods for predicting an isolate's cytotoxicity towards CaCo-2 cells. To improve strain-based predictions of cytotoxic potential, single nucleotide polymorphisms have been identified that offer greater predictive accuracy than phylogenetic grouping. These genetic markers require further investigation and validation to determine their usefulness in risk assessment. The assessment of food safety risks related to exposure to cytotoxic strains of the B. cereus group through food has been hampered by a lack of predictive tools. This gap has begun to be addressed through the experimental quantification and modeling of the growth of various cytotoxic B. cereus group genotypes representing six phylogenetic groups. Secondary growth models have facilitated the development of an exposure assessment model using HTST milk as a food model. This model simulates a five-stage supply chain and allows for up to 35 days of consumer storage, providing insights into the exposure of HTST milk consumers to different cytotoxic B. cereus group genotypes. This talk will highlight recent advancements in comprehending the associations between genotypes and phenotypes and will showcase an example of how genotype-phenotype information can be applied in exposure and risk assessment.

Keywords: *Bacillus cereus*, food spoilage, genotyping, phenotyping

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Bacillus anthracis: The bad boy of the *Bacillus cereus* group

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Abstract

Classical anthrax is a disease caused by the spore-forming *bacterium Bacillus anthracis*, a member of the *Bacillus cereus* group. Differentiating *B. anthracis* from other members of the group is complicated by the ability to transfer the pathogen's plasmid-borne virulence factors, a tripartite toxin (pXO1) and a protein-based anti-phagocytic capsule (pXO2), between species. This has led to the emergence of new pathogenic variants, such as the recently described *B. cereus* biovar *B. anthracis* in West and Central Africa. Anthrax primarily affects domestic livestock such as cattle, sheep, and goats, with humans contracting the disease through contact with contaminated animal products and environments or as a consequence of malicious intent. Death is often sudden and is due to a combination of massive bacteraemia and toxin production. Studies suggest that limited replication and gene exchange can occur outside of a host, provided the environment is permissive. Factors identified as being important include calcium and organic-rich soil, a pH above 6.0, ambient temperatures above 15.50°C, vegetation coverage, and water content. It has been proposed that climate change is driving an increase in the incidence of anthrax outbreaks, as seen in reindeer in northern Russia in 2016 and the recent outbreak in Africa in 2023. The spread of the Zambia outbreak to neighboring countries is thought to have been facilitated by limited community knowledge regarding anthrax transmission, food insecurity, unrestricted cross-border movement of infected animals, ineffective disposal of infected carcasses, and a shortage of effective vaccines. Increased cases in more prosperous northern latitudes may also occur but are likely to be limited by access to healthcare and veterinary services. Changes in the climate could also create opportunities for the emergence of more cross-over strains capable of causing anthrax-like infections.

Keywords: Anthrax, *B. anthracis*, *Bacillus cereus*, tripartite toxin (pXO1), anti-phagocytic capsule (pXO2).

Citation. Baillie, L. 2024. *Bacillus anthracis*: The bad boy of the *Bacillus cereus* group. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb – 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp.3. https://doi.org/10.51585/gtop.2024.1.0036

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S-and L-Enas: Novel types of extremely resilient Pili expressed on endospores of *B. cereus spp.*

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Abstract

The ability to form endospores is a remarkable survival strategy used by a large group of Gram-positive bacterial species. Endospores withstand extreme physical and chemical stressors, ensuring the long-term survival of bacteria under conditions that do not permit growth. Spores of species belonging to the *B. cereus* group are decorated with multiple micrometers long and a few nanometers wide endospore appendages (Enas). Enas resemble pili of Gram-negative and Gram-positive bacteria, which serve a diverse range of biological functions, for instance, adherence to inorganic and organic surfaces. Despite being observed for more than 50 years, all efforts to characterize Enas in detail have failed until recently, largely due to their extraordinary resilience towards solubilization and proteolytic digestion. By combining resources and expertise between collaborating teams at the Norwegian University of Life Sciences (NMBU) and Vrije Universiteit Brussel (VIB), we have recently identified the protein subunits that build Ena fibers and the genes encoding them. The Enas vary in number and morphology between endospores of different B. cereus group strains and species, and they are represented by at least two distinct families of proteinaceous nanofibers (S and L-Enas) with structural properties and self-assembly mechanisms that have not been described before. The genes encoding the Enas are localized in gene clusters, and bioinformatic analysis of more than 700 *Bacillus* spp. genomes revealed that ena-genes are ubiquitous (95% occurrence) among *B. cereus* group spp. species of all eco- and pathotypes, suggesting that they play an important biological role. Our structural and genetic studies have forced a breakthrough in the knowledge of Enas and laid a foundation for studies of Enas biophysical properties and biological functions.

Keywords: *Bacillus cereus*, endospore appendages (Enas), pili, bioinformatic

Citation. Aspholm, M. 2024. S-and L-Enas: Novel types of extremely resilient Pili expressed on endospores of *B. cereus* spp. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb – 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 4. https://doi.org/10.51585/gtop.2024.1.0036

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Assembly of the *Bacillus subtilis* spore surface layers: Lessons for spore display

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Abstract

In *Bacillus subtilis*, the surface of the spore is formed by proteins that self-organize into an inner coat, an outer coat, and a crust. These sub-layers cover a more internal peptidoglycan layer, the cortex. The proteins that form the spore surface layers are first targeted to the surface of the developing spore, and then, in a process called encasement, they encircle the spore. The SpoIVA ATPase, which self-assembles into cables in an ATP-dependent process, is involved in the first targeting step. SpoIVA recruits the main organizers of the inner coat, outer coat, and crust assembly, SafA, CotE, and CotZ, respectively, which then recruit their client proteins. Encasement, in turn, relies mainly on the SpoVID protein, which functions as a non-competitive hub coordinating the assembly of the SafA, CotE, and CotZ modules. The assembly of the spore surface layers further involves direct protein-protein interactions and protein post-translational modifications. Recent work has begun to elucidate the assembly of the various coat sub-layers. For instance, SafA is processed by a coat-associated cysteine protease, YabG, and self-assembles into large complexes, which are then cross-linked by a transglutaminase, Tgl, to cement the cortex-inner coat interface. Thus, a cascade of protein posttranslational modifications intervenes in the assembly of the inner coat. In another example, the CotH kinase phosphorylates the outer CotG and CotB proteins, and the CotH/CotG/CotB module patterns this coat sub-layer. Knowledge of the molecular and structural details of the assembly of the spore surface proteins is thus emerging and is essential to guide the construction of fusions between coat/crust-carrier proteins and foreign passenger proteins. Spore display has a wide range of applications in biotechnology and biomedicine. We will show how work on the fundamental aspects of spore coat/crust assembly can guide the display of biologically active foreign proteins at high density while minimizing perturbations to the spore structure, resistance and functional properties such as germination.

Keywords: *Bacillus subtilis,* spores, SpoIVA ATPase, SpoVID protein

Citation. Silva, D., Neves, C., Amara, K., Serrano, M. and Henriques, A. O. 2024. Assembly of the Bacillus subtilis spore surface layers: Lessons for spore display. Research Workshop on Bacillus in Clinical and Agricultural Environments 29th Feb–1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 5. https://doi.org/10.51585/gtop.2024.1.0036

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High-pressure processing for mild bacterial spore control in industry and research

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Abstract

High-pressure (HP) processing can enable mild bacterial spore control. Established sterilization processes, such as thermal treatment, are not universally applicable for heat-sensitive products. The industry thus requires processes that effectively inactivate spores while preserving sensitive components, such as vitamins, the food color, or the food texture. This is in line with the consumer demand for healthy, minimally processed products. Current industrial non-thermal HP processing at 400–600 MPa and temperatures commonly ≤25°C can increase the shelf-life o heat sensitive products. However, bacterial spores are not inactivated and must be controlled by the product formulation or storage conditions. Inactivation of dormant spores is achieved through a combination of HP with high temperatures (>90°C), which is emerging in industry. To inactivate spores by HP while keeping the thermal input as low as possible, HP processing at a moderate temperature (<90°C) is under research. HP and moderate temperatures can trigger germination, which makes the spores sensitive to mild inactivation by HP or heat. The limitation of an HP germination-inactivation strategy is that not all spores could be triggered to germinate so far. Hence, our study evaluated strategies to increase the HP germination efficacy of *Bacillus* spores. HP treatments (150 MPa, 37°C, 5 min or 550 MPa, 60°C, 2.5 min) were combined with other potential germination-promoting factors, such as nutrient germinants, nisin or incubation at 37°C and atmospheric pressure. The most effective combination led to -8.0±0.1 or -2.0±0.1 $\log_{10}$ units (n=3) germinated *Bacillus subtilis* or *Bacillus amyloliquefaciens* spores, respectively, as analyzed by plate count. Complete germination could be achieved for neither species. In conclusion, HP treatment at moderate temperature seems only to ensure effective mild bacterial spore inactivation in combination with additional germination promotors or preservation strategies. Our study provides future research directions and contributes to the efficient development of mild spore inactivation strategies.

Keywords: High-pressure, bacterial spores, *Bacillus subtilis, Bacillus amyloliquefaciens*

Citation. Heydenreich, R., Delbrück, A. I., Trunet, C. and Mathys, A. 2024. High-pressure processing for mild bacterial spore control in industry and research. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 6. https://doi.org/10.51585/gtop.2024.1.0036

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Co-evolution at protein-protein interfaces guides inference of stoichiometry of spore germination protein complexes by de novo structure prediction

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Abstract

The quaternary structure with specific stoichiometry is pivotal to the functions of protein complexes. However, determining these structures experimentally remains a major bottleneck. For instance, endospore protein complexes with critical roles in spore physiology remain structurally uncharacterized. Structural bioinformatics approaches, such as the deep learning algorithm Alphafold2-multimer (AF2-multimer), leverage the co-evolution of amino acids and sequence structure relationships for accurate de novo structure of and contact prediction in protein complexes. Pseudo-likelihood maximization direct coupling analysis (plmDCA) has been used to detect co-evolving residue pairs at intermolecular interfaces in complexes by statistical modeling. I present evidence that both methods synergize for de novo prediction of the quaternary structure and stoichiometry of protein complexes. This is achieved by augmenting the existing AF2-multimer confidence metrics with an interpretable score to identify the complex with an optimum of native contacts of co-evolving residue pairs at intermolecular interfaces. I illustrate this strategy using enzymatic complexes of known quaternary structure and explore predictions of *Bacillus subtilis* spore germination protein complexes with currently unknown structures, such as the SpoVA dipicolinic acid channel and others. This approach may enable new studies of many structurally uncharacterized protein complexes in bacteria.

Keywords: Endospore, spore germination, AF2-multimer, SpoVA

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The distribution of anthrax in Europe with a focus on Ukraine

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Abstract

Anthrax is a highly infectious disease caused by *Bacillus anthracis* that affects mostly grazing ruminants like cows, sheep, goats, and wildlife. It is a serious and notifiable zoonotic disease. Data from the World Organization of Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO) were used to analyze the spatiotemporal distribution of registered anthrax cases in animals in Europe from 2005 to 2022. The study identified 267 anthrax cases in European animals, with 251 cases in domestic animals and 16 in wildlife. The highest number of cases was reported in 2005 and 2016, followed by 2008. Anthrax was registered in 25 countries in Europe over the last 18 years, with Albania, Russia, and Italy having the highest number of cases, followed by Romania, France, and Moldova. Ukraine reported sporadic infections of anthrax. From 1999-2020, there were 35 cases of human anthrax, with most cases reported in the Kherson region, followed by Kyiv and Odesa. In non-human sources, 28 notifications were reported since 2007, with isolates mainly from soil samples and cattle. The largest number of positive anthrax samples was reported in 2018, and Odesa, which is close to Moldova, had the highest number of cases, followed by the Cherkasy region. However, the data extracted from WOAH and FAO databases did not match, indicating the need for collaboration between these organizations to ensure consistency and harmonization of data. Genetic analysis of isolates, investigation of susceptibility to antimicrobial compounds, and determination of virulence and pathogenicity factors are required for awareness raising and preparedness.

Keywords: Anthrax, *Bacillus anthracis*, Ukraine, Europe, WOAH, epidemiology

Citation. Wareth, G., Kozytska, T. and Neubauer, H. 2024. The distribution of Anthrax in Europe with a focus on Ukraine. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 8. https://doi.org/10.51585/gtop.2024.1.0036

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Molecular architecture of the *Bacillus cereus* group exosporium

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Abstract

Most of the spores found in the *Bacillus cereus* family are engulfed by a paracrystalline saccule called the exosporium. This balloon-shaped structure is an ultra-robust, semi-permeable, multilayered, protein-carbohydrate ultrastructure. As the outermost layer, it serves as the primary point of contact between the spore and the host and is a major source of immunogenic surface antigens. Owing to its tightly packed structure, it functions as a molecular sieve, fending off antagonistic proteins such as proteases while remaining permeable to germinants. The composition and general architecture of the exosporium are fairly well understood, yet a molecular and ultrastructural understanding of the exosporium are still missing. Considering that notable pathogens *Bacillus anthracis* and *Bacillus cereus* but also the bio-insecticidal *Bacillus thuringiensis*- all share a common exosporium architecture, gaining further structural insights into this enigmatic S-layer could contribute to the development of next-generation vaccines or increasing the efficacy of Bt-based pesticides. Here, we employ single-particle cryogenic transmission electron microscopy (cryoEM) on ex vivo and recombinantly derived exosporium sheets to propose a molecular model of the complete exosporium structure, i.e., basal layer and hairy nap. We present a 2.9˚A cryoEM reconstruction of the ExsY basal layer and a 3.1˚A volume of the composite ExsY-ExsF double layer and discuss structural transitions between the two models. In addition, we present a 1.9 ˚A X-ray structure of the ExsF trimer in complex with a peptide comprising the BclA exosporium attachment motif (residues 21-35). We show that our molecular models derived through recombinant means are in agreement with cryoEM class averages and 3D reconstructions from exosporium fragments of *Bacillus thuringiensis* serovar kurstaki and *Bacillus paranthracis*. Through the integration of the available cryoEM and X-ray data, we propose a comprehensive atomic model of the ExsY-ExsF-BclA ultrastructure that rationalizes how the exosporium manages to marry astonishing physicochemical stability with remarkable mesoscale flexibility and controlled permeability.

Keywords: *Bacillus cereus, Bacillus thuringiensis*, electron microscopy, molecular models

Citation. Sleutel, M., Sogues, A., Molle, I. V., Gerven, N. V. and Remaut, H. 2024. Molecular architecture of the *Bacillus cereus* group exosporium. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 9. https://doi.org/10.51585/gtop.2024.1.0036

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Structure and function of the EA1 surface layer of *Bacillus anthracis*

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Abstract

The Gram-positive and spore-forming bacterium *Bacillus anthracis* is the etiological agent of anthrax, a deadly disease that today affects mostly wildlife and livestock but remains a concern for human health due to the high fatality rate and its potential use in bioterrorism. Its vegetative cells are covered in a surface layer or S-layer composed of two mutually excluding proteins, Sap and EA1, present in the exponential and stationary growth phases, respectively. Whilst the Sap S-layer was shown to represent an important virulence factor and cell envelope support structure, little is known about the EA1 S-layer structure and function. Here we generated and selected nanobodies that halt EA1 self-assembly, maintaining it monomeric and allowing us to obtain diffracting crystals. We report the X-ray structure of the EA1 assembly domain at 1.8 ˚A resolution and reveal its native lattice contacts based on its docking into 3D cryo-EM maps derived from the in vitro reconstituted EA1 S-layer. We show the EA1 assembly domain consists of a beads-on-a-string architecture of 6 immunoglobulin-like domains, where calcium binding structures interdomain contact loops and allow the protomers to adopt their assembly-competent conformation. We further show that the selected nanobodies can depolymerize the EA1 S-layer in vivo, leading to a compromised cell surface. Nanobody-induced depolymerization of EA1 results in membrane blebbing and cell lysis under hypotonic conditions, suggesting a crystalline S-layer confers additional mechanical stability to the bacterial cell wall and is required to withstand high turgor pressure. Taken together, we provide a complete structure of the EA1 S-layer and present a set of nanobodies that could have potential therapeutic effects.

Keywords: *Bacillus anthracis*, EA1 S-layer, Potential therapeutic

Citation. Sogues, A., Fioravanti, A., Jonckheere, W., Pardon, E., Steyaert, J. and Remaut, H. 2024. Structure and function of the EA1 surface layer of *Bacillus anthracis*. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 10. https://doi.org/10.51585/gtop.2024.1.0036

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Quorum sensing peptidic inhibitor rescue host immune system eradication: A novel bacterial infection strategy

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Abstract

Subverting the host immune system is a major task for any given pathogen to assure its survival and proliferation. For the opportunistic human pathogen *Bacillus cereus* (Bc), immune evasion enables the establishment of potent infections. In various species of the Bc group, the pleiotropic regulator PlcR and its cognate cell–cell signaling peptide PapR7 regulates virulence genes expression in response to fluctuations in population density, i.e., a quorum-sensing (QS) system. However, how QS exerts its effects during infections and whether PlcR confers the immune evading ability remains unclear. In my seminar, I will share our insights on how the interception of the QS communication in Bc obliterates the ability to control the host immune system. We have designed a peptide-based QS inhibitor that suppresses PlcR-dependent virulence factor expression and attenuates Bc infectivity in mouse models. We have demonstrated that the QS peptidic inhibitor blocks host immune system-mediated eradication by reducing the expression of PlcR-regulated major toxins. Our findings provide the first evidence that Bc infectivity is regulated by QS circuit-mediated destruction of the host immunity, thus revealing a new strategy to limit Bc virulence and enhance host defense. This peptidic quorum-quenching agent constitutes a readily accessible chemical tool for studying how other QS systems modulate host immunity and forms a basis for the development of anti-infective therapeutics.

Keywords: *Bacillus cereus,* Quorum-sensing (QS), Host immunity, Therapeutics

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Evaluation of manual sampling methods for the recovery of *Bacillus thuringiensis* cry- spores from a variety of surface types

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Abstract

The potential public health and economic consequences from the deliberate release of hazardous and persistent *Bacillus anthracis* (*B. anthracis*) spores into the urban environment are enormous. Beyond the immediate challenges of managing the consequences of such an event, technical options are required to characterize the extent of contamination on urban surfaces pre and post decontamination. The UK Government's Department for Environment, Food and Rural Affairs (Defra) is responsible for remediation following a homeland CBR event. Defra has partnered with Dstl to establish a National Technical Advisory Group for Recovery (NTAG-R), which is developing an integrated sampling to analysis strategy. To this end Dstl has performed a comprehensive market survey of manual sampling methods (swabs and wipes) to select technology options for evaluation in the laboratory and field settings. Laboratory methods were optimized to recover *Bacillus thuringiensis* cryospores from these sampling technologies. These were then applied to different surface types nebulized with defined spore densities to evaluate sampling efficiencies and repeatability. Dstl has also assessed the practicalities associated with the use of the identified technologies in field conditions, which includes packaging and transport, as well as the durability of the samplers on surfaces. Analysis of recovered spores from field samples was performed using plate culture, including methods to differentiate background flora from target spores. A wide area release is expected to generate a large number of samples for testing, necessitating the requirement for high throughput methods for their analysis. For post-decontamination assurance this requires methods that can rapidly identify residual viable spores. Rapid viability-PCR-based methods have been investigated, and the impact of residual decon on this assay is discussed.

Keywords: *Bacillus thuringiensis*, Sampling methods, decontamination

Citation. Padgen, D., Jordan, J., Gazi, E., Dwarampudi, V., Stein, A., Clough, D., Casey, S., Govan, N. and Mitchell, S. 2024. Evaluation of manual sampling methods for the recovery of *Bacillus thuringiensis* cry- spores from a variety of surface types. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 12. https://doi.org/10.51585/gtop.2024.1.0036

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Developing logistically viable approaches for wide area decontamination of *Bacillus anthracis* using surrogate spores

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Abstract

The potential public health and economic consequences from the deliberate release of hazardous and persistent *Bacillus anthracis* (*B. anthracis*) spores into the urban environment are enormous. Beyond the immediate challenges of managing the consequences of such an event, technical options are required that can be deployed at scale to decontaminate affected urban areas. The UK Government's Department for Environment, Food and Rural Affairs (Defra) is responsible for remediation following a homeland CBR event. Defra has partnered with Dstl to establish a National Technical Advisory Group for Recovery (NTAG-R), which is developing practical, low-volume (≤300 mL/m2) and scalable decontamination strategies by pulling through state-of-the-art equipment and processes used in the agricultural industry. We present the sporicidal efficacy of pH-adjusted peracetic acid (PAA) decontaminant through a combination of track-sprayer experiments using agricultural equipment and dose-response data collected under controlled meteorological conditions. We determined the lowest dose (volume and concentration) of decontaminant needed to achieve target levels of hazard reduction on porous and non-porous surfaces representative of urban materials. A vehicle-mounted sprayer system (VMSS), suitable in scale for operation on urban highways, was designed and manufactured with commercial-off-the-shelf components to deliver target doses onto spore-contaminated surfaces. Novel protocols were developed to determine dose-response on vertically orientated surfaces (while enabling surface run-off) using a further refined PAA decontaminant delivered in vertical spray mode by the VMSS. Furthermore, we discuss the technical risks of decontaminant preparation and its impact on spray delivery, as well as the challenges in delivering a uniform initial target dose on vertical surfaces. Finally, we present containment level 3 suspension tests, which confirmed that the developed decontaminant can achieve at least the measured level of hazard reduction when applying the same treatment conditions to fully virulent B. anthracis spores.

Keywords: *Bacillus anthracis*, Decontamination, surrogate spores

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Bacillus thuringiensis: Navigating the crossroads between sustainable agriculture and food safety

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Abstract

Bacillus thuringiensis (Bt) based biopesticides have been used in agriculture for nearly 80 years, with demand for these products growing in response to environmental and consumer-focused regulations on synthetic pesticides. Late-season applications with Bt products are common because they leave no chemical residue on produce. However, an inability to differentiate commercial Bt from native *Bacillus cereus* (Bc) sensu lato has led to misconceptions about the safety of these products. Because of the intersection between phenotype and agricultural use patterns, which associated Bt-based biopesticides with potentially pathogenic Bc strains, there is a dire need for clarity and scientific rigor in assessing the safety of commercial Bt in support of their continued use in Europe and the rest of the world. Despite their strong safety profile and long history of use, the safety of commercial Bt products has been called into question because of their close relation to Bc, where some strains can be opportunistic foodborne pathogens. This has led to a disproportionate application of the precautionary principle that fails to distinguish clearly defined, individual microbial strains with a long history of use from undefined, potentially harmful strains of Bc. The rationale for restricting commercial Bt use is based on limits established for the entirety of Bc strains found in nature, which encompasses harmless and pathogenic strains alike and does not account for the fact that commercial Bt strain has been extensively characterized. Biopesticides based on Bt exhibit selective activity against target pests with no impact on pollinators, are fast-acting, and have a nominal environmental impact. Robust scientific literature combined with decades of exposure by hundreds of agricultural workers and billions of consumers worldwide provides a weight of evidence that these strains can be excluded from food poisoning caused by Bt applications on fresh produce (fruits and vegetables).

Keywords: *Bacillus thuringiensis,* agriculture, food safety

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Industrial rest materials as biocide replacement in paper production

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Abstract

Microbial contamination causes environmental and costly problems in paper production (gas formation in stored pulp and biofilm/slime) and wastewater systems (biofilm formation causing corrosion of the pipes). Today, chemical-based biocides are used to control these problems. We have developed a biocide substitute consisting of industrial rest products: rye bran or spruce bark. Rye bran is a suitable nutrient for the growth of a selected strain of *Lactobacillus plantarum*. Previous studies have shown that fermented rye bran, as well as spruce bark extracts, contains bioactive metabolites with the power to limit virtually all bacterial growth. These products also contain fibers with the potential to be a supplement as raw material in the pulp. Our intention with this study was to investigate the potential of these rest materials as an antibacterial raw material in paper production. We will specifically determine the antibacterial effect on strains isolated from pulp, process water from the paper mill, and wastewater pipes. In addition, likewise analyse the antimicrobial activity of single metabolites discovered in the crude extracts. The results so far show that the products efficiently inhibit the growth of the bacterial strains isolated from wastewater and the paper mill. The pure metabolites also showed antibacterial properties but were less potent as compared to the crude products. The *Lactobacillus* strain used to ferment the rye bran in the present study was resistant to all the tested products. In conclusion, fermented rye bran and spruce bark extracts show properties indicating a potential to be used as a bioactive raw material, limiting the need to add synthetic biocides in paper production and wastewater systems.

Keywords: *Lactobacillus plantarum*, biocides, rye bran

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More genomes, more problems? *Bacillus cereus* group taxonomy in the genomic era

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Abstract

The *Bacillus cereus* group is a complex of closely related bacterial species, which vary in pathogenic potential. While some strains are considered to pose a low risk to humans, other strains can cause foodborne illness, severe non-gastrointestinal infections, or anthrax. Differentiating high-risk *B. cereus* group strains from their low-risk counterparts is essential from a public health perspective but remains challenging. Public databases currently housing *B. cereus* group genomes are plagued by taxonomic misclassifications, unstandardized metadata, and low-quality genomes. Using thousands of newly assembled *B. cereus* group genomes, we show how popular, state-of-the-art bioinformatic tools for bacterial species identification can lead to dangerous pathogen misidentifications when applied to the *B. cereus* group. We discuss what *B. cereus* group researchers and stakeholders can do to avoid such misidentifications, and we showcase how genomic data can be leveraged to gain insights into the evolution and epidemiology of illnesses caused by *B. cereus* group members.

Keywords: *Bacillus cereus,* genomics, bioinformatics, epidemiology

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Structure and function of a putative water channel in *Bacillus subtilis* germinant *receptor protein GerAB*

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Abstract

With no known water channels in the model spore-forming organism *Bacillus subtilis*, the molecular mechanism of spore water uptake during germination is unknown. Recent work showed that a subunit of the prototypical *B. subtilis* spore germinant receptor GerA, the integral inner membrane protein GerAB, initiates spore germination in response to L-alanine. Our previous work found that GerAB contains what appears to be a water channel. Using Molecular Dynamic (MD) simulation, we found water passing through the GerAB protein in silico. At the same time, utilizing Steered MD simulation, we now also pull single water molecules through the GerAB channel and calculate the free energy of water permeation. These computational methods, as well as the predicted GerAB structure, provided indications that GerAB residues Y97, L199, and F342 may be crucial in the water channel's function. Mutagenesis of the three residues to alanine corroborated the assumptions with in vivo data. The so-called triA mutant, where Y97, L199, and F342 were all mutated to alanine, showed virtually no germination anymore, measured as water uptake in a live-imaging experiment setting. In contrast, the response to the germinant mixture of Asparagine, Glucose, Fructose, and Potassium (AGFK) was maintained, though remarkable, only at 82% efficiency of the wild-type. Y97A showed 93% germination efficiency with AGFK but only 1.5% with L-alanine. Preliminary western-blot analyses indicate that the germination complex is likely present in Y97A, supporting the functional role of this residue in water passage through the *B. subtilis* spore protein. L199A and F342A showed similar germination patterns but the presence of a full germinsome was not sure in these mutants. Future experiments include kinetic analysis of spore germination events, including confirmation of B. cereus FRET and co-localization data of germinoma and spoVA channel components (see abstract Brul et al.) in *B. subtilis* germinoma and the SpoVA channel protein.

Keywords: *Bacillus subtilis*, germination, structure, receptors, GerAB

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Robust and highly sensitive detection of spore biomarker CaDPA using SERS

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Abstract

Spore forming bacteria are responsible for a vast number of infections and costly contaminations in agriculture, the food industry, and healthcare. To minimize the spread of spores and reduce damage, rapid field detection of spores is important. However, detection is difficult since spores are resistant to many of the bacterial disruption techniques used to bring out the biomarkers necessary for detection. Because of this, quick and effective spore disruption methods are desirable. Here we show a highly sensitive method of detecting the biomarker CaDPA from bacterial spores using surface-enhanced Raman spectroscopy (SERS), a method utilizing laser light to identify chemical compounds. We release CaDPA from spores using sonication and collect the liquid fraction of the sample. Then, using golden nanorods optimized for SERS, we can detect CaDPA in our sample. We can dilute a sample to the level of detecting single-spore quantities of CaDPA. Crucially, we work directly with a biological spore suspension with minimal sample treatment, unlike typical SERS measurements, which rely on highly purified samples or synthetized target chemicals. Thus, we conclude that this method is a promising option for the practical detection of spore contamination in a field setting.

Keywords: *Bacillus,* spores, germination, biomarkers, CaDPA

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Structure-function analysis of *Bacillus cereus* spore germination proteins

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Abstract

Spores of the *Bacillus cereus* sensu lato group are distinct in that they can be triggered to germinate in response to the nucleoside inosine. Previous work has established that the inosine germinative response in *Bacillus cereus* 10876 spores is mediated by the multi subunit GerI and GerQ germinant receptor complexes, although a precise delineation of how these receptors function in isolation and or cooperatively has remained elusive. We report here on structure led analyses of GerI and GerQ, addressing questions relating to the functional hierarchy between GerI and GerQ, uncertainties concerning the nature and location of the inosine binding site, and requirements for functionally crucial receptor clustering within the spore inner membrane. We also report on the observation that *Bacillus cereus*, in contrast to *Bacillus subtilis* 168, has two clusters of spoVA genes that are predicted to encode CaDPA and ion channel proteins involved downstream of activated germinant receptors, revealing insight in the functional hierarchy of these clusters, and structure-function analyses on the SpoVAF protein. The latter is notable since it is predicted to adopt a similar fold to the A-subunit of the germinant receptor complexes. Finally, we shall present a revised model depicting how the various components of the *B. cereus* 10876 germination apparatus may assemble to promote efficient spore germination.

Keywords: Spore-forming bacteria, *Bacillus cereus*, germination, biomarker CaDPA

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Differential interaction of *Bacillus* species with *Acanthamoeba castellanii* and mammalian cells

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Abstract

Pore-forming toxins (PFTs), key virulence factors in both Gram-positive and Gram-negative bacteria, have been extensively studied for their impact on mammalian cells. However, their interaction with *Acanthamoeba castellanii*, which is recognized for its diverse associations with both nonpathogenic and pathogenic bacteria, is not well understood. This study explores the interaction between *A. castellanii* and *Bacillus* species, including *B. cereus*, *B. subtilis*, and *B. thuringiensis*. These *Bacillus* species, prevalent spore-forming bacteria in soil environments, were analyzed in concert with *A. castellanii* employing high-throughput image screening, confocal microscopy, and holographic microscopy. The findings revealed intracellular localization of *B. subtilis* and *B. thuringiensis* in *A. castellanii*, whereas *B. cereus* remained extracellular. Notably, none of the bacterial species elicited significant morphological changes in A. castellanii or affected its survival. Furthermore, this study involved examining the interaction between mammalian macrophages, epithelial cells, and organoids with *B. thuringiensis* and *B. cereus*, both known for their production of PFTs. Unlike *B. subtilis*, both *B. cereus* and *B. thuringiensis* induced rapid killing of the macrophages and epithelial cells, with *B. thuringiensis* exhibiting temperature-dependent effects. Noteworthy cytotoxicity of B. cereus was observed in intestinal organoids. The study postulates a symbiotic relationship between *A. castellanii* and *Bacillus* species and proposes the use of intestinal organoids as a model system to investigate the virulence potential of different Bacillus species. Moreover, our research provides valuable insights into the complex interactions between amoebae and bacteria, with implications for comprehending microbial transmission dynamics and pathogenicity.

Keywords: *Bacillus* species, *Acanthamoeba castellanii*, interaction, mammalian cells

Citation. Nadeem, A. 2024. Differential interaction of *Bacillus* species with Acanthamoeba castellanii and mammalian cells. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 20. https://doi.org/10.51585/gtop.2024.1.0036

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Secrets of spore appendages: Biophysical marvels and aggregation mastery

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Abstract

Bacillus cereus poses a challenge to food safety as some species have pathogenic potential, and their endospores (spores)are resistant to a range of disinfection procedures. The spores are covered in long-thread-like endospore appendages (Enas), which represent a novel class of Gram-positive pili. Little is known about the biological role and the biophysical properties of these filamentous structures. Here, we use optical tweezers (OT) to investigate the biophysical characteristics of S-Enas on *B. cereus* NVH 0075/95 spores. Additionally, we examine the roles of the longer S-Enas and shorter L-Enas in spore-to-spore aggregation, both at the single-cell level and in larger population studies, using comparative analyses that involve wild-type spores and isogenic mutants with varying appendage statuses. The OT experiments unveil the robust mechanical properties of S-Ena fibers, which are both flexible and tensile stiff fibers that can withstand multiple stretches without undergoing conformational alterations or fiber breakages. By oscillating the sample chamber in the OT system, we measured that the S-Enas increased the hydrodynamic drag by 1.5, compared with S-Ena-depleted spores. Furthermore, spores featuring S-Enas exhibit reduced adhesion to glass surfaces, in contrast to spores only decorated with L-Enas. S-Enas were found to be involved in spore-to-spore connections and holding spores together in a gel-like state. Expanding our investigations to a larger scale, sedimentation assays confirm the involvement of both S-Enas and L-Enas in spore aggregation. This work reveals novel findings on the biophysical properties of the S- and L-Enas and discloses the roles they play in spore aggregation, binding to glass and their mechanical behavior upon exposure to drag forces.

Keywords: *Bacillus cereus, spore appendages,* optical tweezers, biophysical characteristics, S-Enas

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Biofilm matrix proteins in *Bacillus subtilis* and in *Clostridioides difficile*

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Abstract

Bacterial biofilms are a form of multicellular organization that confers protection against harmful conditions and creates nutrient-rich niches. Within these biofilms, distinct subpopulations of specialized cell types exist, with endospore formers like *Bacillus subtilis* and *Clostridioides difficile* inducing spore differentiation in specific cell subsets within the biofilm. The cohesiveness of cells in the biofilm is maintained by an extracellular matrix, a crucial structural element consisting of exopolysaccharides, extracellular DNA, and proteins. *B. subtilis* produces dedicated matrix proteins, for instance, TasA and BslA, which provide support for the biofilm. Strikingly, we found TasA and BslA on the coat of endospores produced within biofilms, suggesting that components of the biofilm matrix are part of mature endospores. We hypothesize that some of the structural proteins that confer integrity to the matrix biofilm, such as TasA, may function as scaffolds for the assembly of the endospore surface layers. This overlap between the assembly of the matrix and the spore surface hints at a deeply rooted connection between the two processes. In contrast, *C. difficile* utilizes abundant cytoplasmic proteins, such as Enolase, which are transported to the outside of the cell and assemble into a fibrous extracellular matrix. Enolase is a moonlighting protein that performs different functions in addition to its role in glycolysis. Enolase is encoded by the essential eno gene, the depletion of which leads to defects in growth and impaired biofilm production. Extracellular complementation with Enolase produced in *E. coli* restores biofilm formation. In the last decade, a large number of proteins have been identified that moonlight as components of the biofilm matrix in different species. The strategy of reusing cytoplasmic proteins as components of the biofilm matrix has been observed in various species, suggesting it serves as an energy-efficient approach by minimizing the need for synthesizing specialized matrix proteins

Keywords: Biofilm, *Clostridioides difficile, Bacillus subtilis,* matrix proteins

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Modeling naturally occurring mutations that potentially alter the immunogenicity and functionality of the protective antigen of *Bacillus anthracis*

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Abstract

Modelling naturally occurring mutations that potentially alter the immunogenicity and functionality of the protective antigen of *Bacillus anthracis*. Anthrax, a disease caused by the spore-forming bacteria *Bacillus anthracis*, is primarily a pathogen of animals, with humans contracting the disease following exposure to spore-contaminated animal products and environments. It is non-contagious, being released as inert spores following the death of the host, and can lay dormant in the soil for prolonged periods between infectious episodes, resulting in low genetic divergence between strains. At the molecular level, *B. anthracis* possesses two key virulence mediators, plasmids pXO1 and pXO2. The former encodes for the anthrax tripartite toxin complex, comprising the protective antigen (PA), the lethal factor (LF), and the edema factor (EF). PA binds to the cell membrane, is cleaved by a furin-like protease, and then forms a heptameric pore, enabling the translocation of LF and EF into the cell. Although naturally occurring single nucleotide polymorphisms (SNPs) have been reported within the PA gene (pagA), a comprehensive study assessing their impact on biological function and immunogenicity has yet to be conducted. To address this issue, we compared published nucleotide and amino acid sequences of PA from 140 *B. anthracis* isolates, including vaccine strains, along with 3 pXO1 homolog-positive *B. cereus* isolates. Using the PA gene sequence from the foundational paper by Welkos et al. 1988 (1) as a reference (accession number M22589.1), we found that, while extensive regions of the PA gene are highly conserved (≈99%), certain sites, termed hotspots, were prone to SNPs. The limited number of hotspots included both synonymous and non-synonymous mutations, potentially affecting the efficiency of gene expression as well as protein function and immunogenicity. We also identified subtle variations in sequences originating from the same strain. In future studies, we will explore the impact of individual mutations on the characteristics of this essential protein.

Keywords: *Bacillus anthracis,* immunogenicity, anthrax, virulence mediators

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Characterization of an open-air bio-aerosol release and its deposition and fate on urban surfaces to inform decontamination

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Abstract

A malicious attack using a biological pathogen in an urban environment is recognized by the UK in the National Risk Register. As described in the scenario, this could result in large numbers of casualties and fatalities and, in the larger-scale event, catastrophic impacts. Department of Environment, Food, and Rural Affairs partnered with Dstl to establish a National Technical Advisory Group for Recovery (NTAG-R) to enhance deployable technical options to decontaminate affected urban areas. These solutions are required to be practical, low-volume, scalable, and to be operationally ready for such an event. To understand the threat posed by aerosolized spores, we conducted open-air bio-releases using a hazard group 1 simulant, Bacillus thuringiensis HD-1 cry-. This strain is comparable to virulent strains in terms of its phenotypic properties: size, shape, and morphology. The trial characterized contamination on vehicles passing through the aerosol cloud and the potential for contamination to be spread by the vehicle. The body of the vehicle was sampled using removable coupons and swabbing. This not only showed levels of deposition but also showed areas in which spores were concentrated, making them a site of interest for further decontaminant application. Additionally, we studied the effects of plume deposition across a wide open area exposed to several environmental conditions from dry sunlit through to rain. The effect of meteorological conditions on the spore adhesion to different urban surfaces vertically orientated was investigated. Empirical methods used plate growths to quantify the concentration distribution of the downwind dispersion of the spores. We sampled and quantified the longer-term viability of spores on representative urban surfaces following their deposition. Following the simulated contamination of vehicles, we tested a gantry spray system, which was developed to decontaminate vehicles and mobile structures. The gantry applied a bespoke peracetic acid formulation to decontaminate the vehicle in a timely manner. This presentation will explore the characterization and spread of the plume to inform the logistical considerations of the decontamination strategies.

Keywords: Air bioaerosol, Decontamination

Citation. Dwarampudi, V., Jordan, J., Gazi, E., O'Sullivan, C., Ellis, C. B., Padgen, D., Hocknull, E., Stein, A., Clough, D., Whatley, P., Walker, M., Howells, J., Casey, S. and Mitchell, S. 2024. Characterization of an open-air bio-aerosol release and its deposition and fate on urban surfaces to inform decontamination. Research Workshop on *Bacillus* in Clinical and Agricultural Environments 29th Feb– 1st Mar 2024, Umeå University, Sweden. GMPC TOP. 4 (1). pp. 24. https://doi.org/10.51585/gtop.2024.1.0036

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Large-scale insights into the biosynthetic potential of the *Bacillus cereus* group

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Abstract

Members of the *Bacillus cereus* group species complex are prolific producers of secondary metabolites (SMs), which allow producer strains to interact with their respective ecological niches. Outside of their ecological roles, SMs produced by *B. cereus* group strains can have major impacts on human health (e.g., foodborne emetic toxin cereulide) or important uses in the industry (e.g., antibiotic ceresin A). Here, we used in silico approaches to mine the largest-ever set of *B. cereus* group genomes for biosynthetic gene clusters (BGCs) linked to SM production, with the goal of identifying novel BGCs of medical/industrial interest. A total of 5,976 *B. cereus* group genomes were downloaded from BTyperDB (v1), and the following methods were employed to detect BGCs in each genome: (i) GECCO (GEne Cluster prediction with Conditional random fields; v0.9.8), a machine learning-based BGC discovery tool, and (ii) antiSMASH (v6.1.1), a rule-based BGC detection tool. The resulting BGCs were clustered into gene cluster families (GCFs) using HTGCF (High-Throughput Gene Cluster Family; v1) together with experimentally validated BGCs from MIBiG (Minimum Information about a Biosynthetic Gene cluster; v3.1). Within the 5,976 *B. cereus* group genomes queried here, a total of 124,097 BGCs were detected. BGCs were clustered into 837 GCFs, of which 161 (19.2%) were singleton GCFs (i.e., with only one member). The largest GCF contained 12,834 BGCs. Interestingly, a GCF containing cereulide (MIBiG BGC0000320) and presumed cereulide-like BGCs was identified, encompassing 370 BGCs distributed across four major phylogenetic groups (i.e., panC Groups II, III, IV, and VI, detected in 7, 153, 12, and 25 genomes, respectively). Overall, the high-throughput, genome-mining approach employed here represents the most comprehensive survey of *B. cereus* group biosynthetic potential to date. Future in silico and experimental efforts will facilitate the identification of novel BGCs of interest from an infection biology and/or drug discovery perspective.

Keywords: *Bacillus cereus*, secondary metabolites, biosynthetic, genome-mining approach

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BTyperDB: A community-curated, global atlas of *Bacillus cereus* Sensu lato genomes and metadata for epidemiological surveillance

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Abstract

Public data portals linking whole-genome sequencing (WGS) data to epidemiologically relevant metadata have become a crucial resource for pathogen surveillance efforts. However, existing databases lead to dangerous pathogen misidentifications when applied to *Bacillus cereus* sensu lato (s.l). Current taxonomic assignments are inadequate for evaluating *B. cereus* s.l. pathogenic potential at the strain level, as many important virulence factors (e.g., anthrax and emetic toxins) can be gained, lost, and variably present within and across species boundaries. On top of that, incomplete metadata in general-purpose pathogen databases also makes epidemiological surveillance challenging. To combat these issues, we developed BTyperDB (www.btyper.app), an atlas of *B. cereus* s.l. genomes with standardized, community-curated metadata. Briefly, all genomes submitted to the National Center for Biotechnology Information (NCBI) Genbank database as a *B. cereus* s.l. species were downloaded (n=3,428 genomes; accessed 8 August 2022). An additional 3,473 previously unassembled genomes were assembled using Shovill v1.1.0/SKESA v2.4.0 or Unicycler v0.5.0. All 6,901 genomes underwent quality control (via QUAST v5.0.2 and CheckM v1.1.3), taxonomic assignment (via BTyper3 v3.3.3 and the Genome Taxonomy Database Toolkit v2.1.0), and in silico typing (via BTyper3). Low-quality and misidentified genomes were removed, resulting in 5,976 *B. cereus* s.l. genomes. Genomes within BTyperDB can be queried via an interactive web application to help users rapidly access and download genomic (meta)data associated with *B. cereus* s.l. strains. Users can identify strains isolated from various sources (e.g., humans, animals, foods) and geographic regions and query genomes based on the presence or absence of important virulence factors. To showcase its utility for pathogen surveillance, we used BTyperDB to identify emerging anthrax toxin- and capsule-harboring lineages. In summary, BTyperDB represents a curated genomic atlas, which can help improve *B. cereus* s.l. surveillance, source tracking, outbreak detection, and response efforts.

Keywords: *Bacillus cereus*, genomes and metadata, epidemiological surveillance

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3D printed biofilm incubator with temperature and motion control

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Abstract

Biofilm contamination threatens both our healthcare and food industry, and more effective disinfection methods are needed. However, disinfection assays require repeatability and control over the biofilm's properties, and biofilm growth is very dependent on the environmental conditions. Conventional laboratory rockers are versatile tools for mixing, staining, and washing biological samples. But when the operations require precise control of the motion and temperature, then commercial alternatives are sparse and often come with a hefty price tag, limiting their use. In this work, we show how to build a temperature-controlled rocker platform using a 3D printer, CNC milling machine, and off-the-shelf components. We evaluate and compare its performance to commercial systems, as well as in a cell cultures assay. The results show that our system performs similarly to systems costing as much as $6600 while being quick to build and costing less than $350. As a proof of concept, we show how to use this system to grow biofilms of different bacterial strains. These biofilms will aid in developing methods to treat and guard against biofilm growth in various materials and environments.

Keywords: Biofilm, cell cultures assay, 3D printer, CNC milling machine, and off-the-shelf components

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Characterization of UV-induced spectral and morphological changes in bacterial spores

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Abstract

Bacterial spores are well known for their association with food-borne illness, hospital-acquired infections, and deadly diseases e.g., anthrax. The detection and decontamination of spores are of paramount importance, with UV-radiation-mediated protocols being widely used for spore decontamination. However, unlike vegetative bacterial cells, spores are more resilient to common decontamination protocols, necessitating methods to verify spore inactivation post-decontamination. Here, we investigate the UV-radiation-mediated alterations in spectral and morphological properties of bacterial spores in a dose-dependent manner. We use a combination of spectroscopic techniques, e.g., absorption spectroscopy, fluorescence spectroscopy, Raman spectroscopy, and morphological characterizations by scanning and transmission electron microscopy for assessment of UV-mediated changes in spores. The results from these experiments indicate the dimerization of dipicolinic acid (DPA), a core component of the spore, structural degradation of protein and DNA, disintegration of the spore outer envelope, and leaking of DPA from the core of the spores. Overall, these findings have practical applications in the development of new spore decontamination and inactivation verification methods.

Keywords: Spores, anthrax, spectroscopic technique, decontamination and inactivation

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Assessing CaDPA levels, metabolic activity, and spore detection through deuterium labeling

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Abstract

Understanding the impact of environmental conditions and decontamination techniques on the metabolic activity, viability, and biomarkers of spores is crucial for combatting them. To distinguish and track spores and to understand metabolic mechanisms, spores must be labeled. Staining or genetic modification are current methods for this; however, these methods can be time-consuming and affect the viability and function of spore samples. Here, we investigate the use of heavy water for permanent isotope labeling of spores and Raman spectroscopy for tracking sporulation/germination mechanisms and evaluating decontamination. We find that steady-state deuterium levels in the spore are achieved after only 48 h of incubation with 30% D2O-infused broth and sporulation, generating Raman peaks at cell silent regions of 2200 and 2300 cm-1. These deuterium levels then decrease rapidly upon spore germination in non-deuterated media. We further find that, unlike live spores, spores inactivated using various methods do not lose these Raman peaks upon incubation in growth media, suggesting these peaks may be used to indicate the viability of a spore sample. We further observe several Raman peaks exclusive to deuterated DPA, a spore-specific chemical biomarker, at, e.g., 988 and 2300 cm-1, which can be used to track underlying changes in spore involving DPA. In conclusion, permanent spore labeling using deuterium offers a robust and non-invasive way of labeling bacterial spores for marking, viability determination, and characterizing spore activity.

Keywords: Metabolomics, CaDPA, biomarker, spores

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Lab-on-a-chip for spore disruption and detection

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Abstract

Spore-forming bacteria are responsible for a vast number of infections and costly contaminations in agriculture, the food industry, and healthcare. To minimize the spread of spores and reduce damage, rapid field detection of spores is important. However, detection is difficult since spores are resistant to many of the bacterial disruption techniques used to bring out the biomarkers necessary for detection. Because of this, quick and effective spore disruption methods are desirable. Here, we show a compact (centimetre-sized) lab-on-a-chip that can be used to disrupt bacterial spores for detection. The chip is built around a coplanar waveguide that generates a highly localized electric field with the same frequency (2.45 GHz) and intensity (10 kV/m) as a commercial microwave oven while using only 1 W of input power. We introduced a spore suspension to the chip using a microfluidic channel, and after 15 seconds of exposure, we could detect the release of biomarkers with fluorescence microscopy. Because of the low input power, no significant heating of the sample was recorded, pointing out that the disruption is likely caused by electric field-based effects. The disruption efficiency was also evaluated on an individual spore level using electron microscopy and Raman spectroscopy. Thus, we conclude that the proposed lab-on-the-chip is a reliable technique for spore disruption, which, combined with its small size of only a few centimeters, makes it suitable for handheld field detection systems.

Keywords: Spore-forming bacteria, spores, biomarkers, lab-on-a-chip

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Optical tweezers in cell, protein, and bond analysis

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Abstract

Optical tweezers are versatile tools in microscopic applications. They employ a focused laser beam, introduced through a microscope objective, to trap semi-transparent particles, including cells, spores, bacteria, and micro-spheres made of plastic, glass, or gold. These particles can be manipulated in three dimensions with nanometer precision, enabling users to handle and sort various entities. Furthermore, by functionalizing a trapped spherical plastic bead, it can be attached to proteins, fibrous protein structures, or receptors, facilitating the measurement and quantification of their biophysical properties. Understanding these properties, such as flexibility, stability, conformational changes, and interaction capabilities, is crucial for advancing our comprehension of biological mechanisms. Here, we show how optical tweezers can be used to manipulate cells and listen to molecular motors, as well as characterize the biophysical properties of nanofibers expressed by bacteria and spores.

Keywords: Optical tweezers, spores, biophysical properties

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Structure and function of a putative water channel in *Bacillus subtilis* germinant receptor protein GerAB

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Abstract

With no known water channels in the model spore-forming organism *Bacillus subtilis*, the molecular mechanism of spore water uptake during germination is unknown. Recent work showed that a subunit of the prototypical *B. subtilis* spore germinant receptor (GR) GerA, the integral spore inner membrane (IM) protein GerAB, initiates spore germination in response to L-alanine but not AGFK. Our previous work found that the GerA's GerAB subunit contains what appears to be a water channel. Using Molecular Dynamic (MD) simulations, we found water passing through the GerAB protein in silico. When analyzing the water passage in detail, we identified 13 candidate amino acid side chains that overlap with the pathway of water passage. To test the functional role of the selected residues in water permeation, two residues were chosen as examples, specifically polar residue Y97 and non-polar residue L199. Mutagenesis of these residues to alanine corroborated the assumptions with the in vivo data. The Y97A and L199A mutants showed virtually no germination with L-alanine measured as water uptake in a live-imaging experiment setting. Y97A also showed 93% germination efficiency with AGFK but only 1.5% with L-alanine. Preliminary Western-blot analyses indicate that the GR complex termed the germinosome in spores' IM is likely to present in the Y97A mutant, supporting the functional role of this residue in water passage through the *B. subtilis* spore GerAB protein. L199A showed similar germination patterns but the presence of a full germinosome was not clear in this mutant. Consequently, the Y97A mutant's function was analyzed in silico to understand the molecular picture of its function. With umbrella sampling, the free energy of water passage was calculated for both wild-type GerAB and Y97A GerAB. The result shows the free energy barrier for wt spore water passage is 9.03 kJ/mol, while that for Y97A is 12.34 kJ/mol. This agrees with the experimental data showing that the Y97A mutant encounters more difficulty in hydrating the spore. In future studies, all mutant proteins' functions will be studied in vivo and followed in silico, and the open/closed conformation of GerAB's water channel will be investigated.

Keywords: *Bacillus subtilis*, Molecular Dynamic, GerAB

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