**Spore as a Survival Strategy in Cryptogams: Emerging Applications in Biotechnology and Beyond**

**Abstract**: *Spore biology is a fascinating field with immense potential for fundamental research and its applications.* *Spores are highly resistant dormant cells with reduced metabolic activity. The spores are found naturally in soil. Spores have unique features such as haploid nature, asexual reproductive units, extremely resistant cell wall and ability to survive in extreme conditions. Due to this spores have promising future prospects in research in fields of biosensing, biocontrol, biofertilizers, biomedicine biological warfare, probiotics. and crop improvement. Because of resistance in changes in pH, temperature, and other environmental factors, they are used as efficient vaccine vehicles. Certain bacteria have been genetically manipulated to express antigens on the surface of their spores. Further bacterial spores can be used to selectively deliver drugs to tumor sites. This offers huge potential of spores in field of medicine.*

***Key words****- spore, biocontrol, biopestisides, biological warfare, biosenser*

**Introduction**- “Microorganisms colonize the external and internal surfaces of the animal body and establish mutually beneficial interactions, forming site- and individual-specific microbiota”(Hall et al. 2018; Tetz et al. 2017). “Several organisms such as bacteria, algae, fungi, yeast, actinomycetes and protozoa are well known for their ability to form spores. Formation of Spores is a distinct phase in life cycle of these small organisms making them able to resist various unfavourable environmental conditions like extreme temperature, ice, radiations, desiccation, toxic chemicals”(Hu et al. 2024). “Spore act as unit of asexual reproduction and also unit of survival in these small organisms”. (Setlow , 2023) “Spores play an essential role in microbiomes and seed banks and are a valuable tool for biotechnology” (Cottet et al 2021, Zhang et al 2020;Ting et al .2010, Oomes et al. 2004)

“Keeping in view their huge potential, the use of spores has led to several upcoming researches to develop a large number of spore-based products in the areas of biosensing, biocontrol, biofertilizers, biological warfare, medicine and probiotics”.(Woo et al. 2023) “Many of these products are now available commercially and thus offer a great potential of microbial spores. The degradation of complex polysaccharides in the animal gut, the production of useful compounds, protection against pathogenic microorganisms and contribution to the development of an efficient immune system are the main beneficial effects of a balanced microbiota”. (Gentry et al. 2004, Singh et al. 2012) “The current paper gives information about the utility microbial spores and its potential industrial applications in above mentioned fields. Furthermore, attention has also been paid to the associated challenges and future perspectives for spore-based technologies”. (Tehri et. Al 2018)

**Review of Literature:**

“Microbial spores are a special cell with thick wall, metabolically dormant structures that are produced by a process of sporulation to prevail over harsh and unfavorable climatic conditions of starvation, stress of many kinds . Spore production (sporulation) is induced by a variety of environmental conditions that impair cell growth, making the sporulation process a survival strategy”.(Setlow 2023; Hamiot et al 2023) “In addition to their metabolic dormancy and resistance, the spore has a very different structure from that of a growing cell, including several layers and many constituents that are unique to spores”. (Hu et al. 2024; Tahara YO MM 2024,2023, Pophan DL 2002)

“The outermost layer is the exosporium. Underlying the exosporium is the spore coat composed largely of proteins. There are multiple proteins in the spore coat and often multiple coat layers and this structure and its constituents are again unique to spores.”(Cho WICM 2020, Seo et al. 2021; Weaver et al. 2007; Nicholson et al. 2000) The hardest biological substance sporopollenin found in spore all, makes it resistant to harsh climatic conditions. (Stewart GC 2015)

“ The spore is metabolically dormant, and exhibits no significant metabolism of either exogenous or endogenous compounds”.(Seo et al 2021) “A striking feature of the spore’s metabolic dormancy is the virtual absence of the common high energy compounds present at high levels in growing cells such as adenosine triphosphate (ATP), reduced pyridine nucleotides and acetyl-coenzymeA13. While spores are dormant, they do have significant pools of some metabolic substrates, in particular the glycolytic intermediate, 3-phosphoglyceric acid (3PGA), as well as the enzymes needed for metabolism of this and other substrates .

While spores can remain dormant for extremely long periods, they are continually sensing their environment for the presence of nutrients using a group of receptors located in the spore’s inner membrane”. (Ramirez et al. 2021 ) “Different receptors respond to different nutrients and at least some receptors appear to act cooperatively in sensing mixtures of nutrients spores are of great significance”.(Ma et al. 2023; Fathahi et al 2022; Li et al 2022; Yu et al. 2020)

“The nutrient supplies of plants are significantly influenced by microorganisms such as bacteria and fungi. Those microorganisms that have a positive effect and stimulate plant growth are defined as plant growth-promoting (PGP) microorganisms. Spore forming microorganism can be used as biofertilizer to improve plant health and crop production” (Agake et al. 2021). Currently, some mechanisms related to plant growth promotion have been clarified, such as biological nitrogen fixation, phytohormone production, nutrient solubilization, and siderophore production It can reduce the amout of chemical fertilizers.(Mohsin et al 2021; Zhang et al. 2020) “Studies have shown that they show positive gowth ates unde limited nutrient supply. The biofetilizer made up of zeolite and contains 10 spores per gram. When applied to rice seeds,it can cause seedling to have over 30% more growth. Trichodema fungus produces growth stimulators such as IAA(Indole acetic acid) and harzianolide. It decomposes soil organic matter and inceases the supply of nutrients.The use of spores on one hand offers a number of useful applications in various fields, but at the same time, they have also become the major cause for various types of food spoilages and food-borne diseases” (Setlow 2023; Setlow and Johnson 2007; Coleman et al. 2010).

“ Spores are unique in resistance properties and environment sensing ability therefore several spore-based technologies have been developed. Spores as a biosensor have been used for the detection of various microbial and nonmicrobial contaminants in various food stuffs”. (Khan et al 2023) “Bacteria-based sensing systems have been employed in a variety of analyses as biosensors because of their selectivity and ease of use”. (Suntornsuk et al 2020, Herold et al. 2013). “Paper based biosensors have become the backbone of the diagnostic industry. The notable work on enzyme electrodes by Clark and Lyons in 1962 denoted the beginning of the field of biosensor. A biosensor is a device which combines a bio-recognition molecule with a transducer and changes the recognition event into an analytical signal”. (Dasriya et al 2021, Karanpriya et al 2020, Chamber et al 2008). “In today’s world, several biological molecules are engaged in the sensing and recognition processes. These include cells, nucleic acids, enzymes, antibodies, proteins, peptides, etc. spore-based paper strip biosensor which work on the induction principle to detect the ß-lactam group of antibiotics in milk are widely used”.(Sharma et al 2020, Thukral et al 2022). “These systems have limited applications in the field because of the inability of bacteria to survive long term under extreme environmental conditions.

The ability of spores to enable bacteria-based sensing systems with long lives, along with their ability to switch over between the vegetative spore state and the germinated living cell, contributes to their usefullness as vehicles for cell-based biosensors spores expressing certain enzymes, proteins, at their surface have been presented as a stable tool for the biospecific recognition of target molecules. A spore-based paper strip biosensor that can detect the ß-lactam group of antibiotics in milk has been developed using an enzyme induction concept”. (Ma et al 2023; Lu et al. 2022; Yu et al. 2020; Heffron JD 2009)

“Application of spores as a packaging method for whole-cell biosensors, surface display of recombinant proteins on spores for bioanalytical and biotechnological applications, and the use of spores as vehicles for vaccines and therapeutic agents”.( Ma et al 2023; Naresh et al 2021) Spores provide a packaging and storage method for whole-cell biosensing systems.(Dasriya et al 2021) “Due to the resistance of bacterial spores to changes in pH, temperature, and other environmental factors, they are used as efficient vaccine vehicles. As described previously, certain bacteria can be genetically manipulated to express antigens on the surface of their spores”.(Hu et al. 2024)

“Various studies have shown that germinating properties of bacterial spores can be used to selectively deliver drugs to tumor sites and decrease the toxic effects of chemotherapeutic drugs on patients”. (Cottet et al. 2021) “Spores have huge agricultural applications, in development of biocontrol agents and biofertilizers”.(Agake et al. 2021) “Thus the unique characteristics of spores have proved the spores as a valuable tool for fulfilling the need of present time in the area of crop protection, environment and medicine”.(Mohsin et al. 2021)

“ Due to their stability and resistance properties, they are found in different kinds of environments. They are found in soils; aquatic environment; deserts, hydrothermal sites and polar ices. The process of spore formation is known as sporulation. It involves progression through different stages such as sporulation, chromosome segregation, sporulation-specific cell division, differential gene expression and specific signal transduction mechanisms” (Cutting and Ricca 2014).

“During germination initiation of metabolism takes place resulting in ATP formation and synthesis of RNA and proteins”.(Sorg JA 2008) Eventually replication of DNA results in vegetative cell (Setlow 2023; Paidhungat and Setlow 2002) . “A new formed vegetative cell is able to grow, to duplicate and eventually to sporulate again”( Hamiot et al. 2023; Cowan et al. 2003)

“The Green Revolution significantly increased the use of synthetic pesticides, which, while causing an increase in agricultural production, also led to indiscriminate pollution in the air, soil, and water” ( Khan et al 2022; Mocali et al. 2022). “Due to this problem, alternative methods for controlling agricultural pests and pollution have been sought. Common among them are biopesticides. The biopesticides can be defined as the pesticides which have biological origin. The use of biopesticides offers several advantages over chemical pesticides”. (Amran 2004; Schisler et al 2004, ) “Biopesticides are safe to humans and the environment, are cheap and more target specific. Spores of Bacillus thuringiensis (BT) are frequently used in agriculture to target insects and pests. Spores of B. sphaericus are also known to develop a parasporal body during the process of sporulation which has toxic properties against mosquito’s larvae”(Setlow 2023). Metarhizium anisopliae and Beauveria bassiana are filamentous fungi that belong to the orders Hypocreales, family Clavicipitaceae, and Cordycipitaceae, respectively.The spores of these fungi are widely used in formulations of biopesticides.

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a beneficial effect on host health” (Hill et al., 2014). Probiotics are being developed commercially for both human use, as dietary supplements, and in animal feeds for the prevention of gastrointestinal infections,(Trotter et al. 2020) Higher resistance of microbial spores to high temperatures, low pH and [high pressures](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/hyperbarism) coupled with characteristics such as good stability and rapid germination make their use extremely desirable for the innovative formulation of several [probiotic](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/probiotic-agent) foods. Probiotics are capsules or tablets or liquid composed of lyophilized preparations of bacteria which promote a healthy gut. (Garvey et al. 2022; Brutscher et al. 2022) Spore forming probiotics have already been widely marketed as supplements (capsules) for human consumption. The effective use of spores in these areas will depend on the ability to scale up spore generation, which still needs to be optimized for batch production., (Mandarino et al 2023)Probiotic spores are resistant to several processing conditions employed in the food industry, thus presenting high feasibility of effects. Among the large number of probiotic products in use today are bacterial spore formers, mostly of the genus Bacillus, Lactobacillus (Patch et al. 2023).

“Biological warfare is the use of [biological toxins](https://en.wikipedia.org/wiki/Toxin#Biotoxins)  such as [bacteria](https://en.wikipedia.org/wiki/Bacteria), [viruses](https://en.wikipedia.org/wiki/Virus), [insects](https://en.wikipedia.org/wiki/Insects), and [fungi](https://en.wikipedia.org/wiki/Fungus) with the intent to kill or harm humans, animals or plants. [Biological weapons](https://en.wikipedia.org/wiki/Biological_agent)  are living organisms. Biological agents for [smallpox](https://en.wikipedia.org/wiki/Smallpox), [pneumonic plague](https://en.wikipedia.org/wiki/Pneumonic_plague) have the capability of person-to-person [transmission](https://en.wikipedia.org/wiki/Transmission_(medicine)) via [aerosolized](https://en.wikipedia.org/wiki/Bioaerosol) [respiratory droplets](https://en.wikipedia.org/wiki/Respiratory_droplet)”. ( Kelle et al 2009; Garfinkel et al 2007; Fraser et al. 2001) “Biological weapons, as opposed to nuclear weapons, are easier to produce and require less money investment and human resources. Infectious diseases have always played a major role in limiting military campaigns, and invading armies”.( Epstein GL 2001) “Appropriate dispersion of even a small volume of biological warfare agent may cause high morbidity and mortality, which may be multiplied by public panic and social disruption”.( Calfee et al 2011; Guillemet et al 2010,) “Biological weapons have a very high destructive potential; the consequences of biological attacks are often unpredictable and are therefore of little strategic interest in conventional war; these weapons can be cheap and relatively easy to prepare with a minimum of scientific expertise”.( Unal et al 2016; Petro et al. 2003) “Theoretically, novel approaches in biotechnology, such as synthetic biology can be used in the future to design new types of biological warfare agents, to render a vaccine ineffective; enhance the virulence of a pathogen, increase the transmissibility of a pathogen”;(Khan et al 2022; Mocoli et al 2022; Gupta et al. 2022) “Western powers, had biological research programmes for both offensive and defensive purposes, and several other nations are known or thought to have such programmes”.(Miller et al 2001)

“Anthrax is a severe disease caused by Bacillus anthracis, in which an itchy, painless sore that looks like an insect bite is produced. The sore may blister and form a black scab . Bacillus anthracis is a gram-positive capsulated bacterium that can survive in the presence or absence of oxygen and can develop spores when exposed to the environment through various body fluids of a deceased animal . This disease impacts both domestic animals and wild animals., It can also affect humans, leading to a global spread of the outbreak. Anthrax can be transmitted through inter-animal or zoonotic transmission Anthrax endospores exhibit resistance to desiccation, high temperatures”. ( Cote et al 2015; Oliva et al. 2008; Hart 2001)

“The World Health Organization estimated that 50 kg of *B anthracis* released upwind of a population center of 500000 would result in up to 95000 fatalities, with an additional 125000 persons incapacitated”(Huxsoll et al 1989)

Smallpox (variola) was a devastating disease with a high case-fatality rate. Although the disease was eradicated in 1977, the remaining stocks of smallpox virus constitute one of the most dangerous threats to humanity. The smallpox virus is highly specific for humans and non-pathogenic in animals. There is no antiviral treatment and a vaccine is active only if administered in the first four days post-exposure. Smallpox virus is a a potential biological weapon that could be used by terrorists, Variola is highly contagious and is transmitted from patients by droplets and sometimes by aerosols. Smallpox has proved potential as a biological warfare agent even before any possible genetic enhancement in weapons programmes. Smallpox is a potential biological weapon because it can be used to intentionally spread the variola virus, which causes the disease. Although smallpox has been eradicated, public health authorities prepare for the possibility of a bioterrorist attack. Smallpox can be grown in large quantities, It can spread through the respiratory route It has a 30% mortality rate There is no specific therapy for smallpox

**Conclusion:**

In addition, Botulism is a rare but severe illness caused by the neurotoxin produced by the vegetative form of C. botulinum after spore germination. (Wilcox et al. 2011)In a case of a bioterrorist attack using aerosolized C. botulinum spores it might be predicted that C. botulinum, after spore germination, could produce an infection with release of the neurotoxin in gastrointestinal tract of injured persons.(Gaur et al. 2014;Paredes-Sabja 2011; Wilcox et al. 2003) Although there are no reports on the use of C. botulinum spores in bioterrorism events, the spore dormancy makes spores harmless to anyone (a bioterrorist) handling them. Furthermore, the ease of handling spores in comparison with manipulation of toxins makes spores as the ideal weapons to disseminate C. botulinum . Each of the described pathogens (B. anthracis, C. botulinum, C. difficile, C. perfringens and C. burnetii) is relatively easy to grow in specific culture media to allow the production of high titres of the resistant forms: spores.

**Emerging themes and future directions**

Future trends in spore biology include the use of artificial cells and the development of new therapies for spore-forming infections.  Artificial cells  cells are like lipid vesicle protocells, can help study molecular biology. These cells can help study how molecular concentration variations and macromolecular crowding affect cells. Further by using metabolomics and other omics tools to gain biochemical insights and link molecular insights with spore behaviors. Combining omics with single-spore techniques and available literature can lead to new possibilities in the field of spore biology. This can help in developing new agents for the biocontrol of pathogens in agriculture.

Further, as a substrate for delivery of biomolecules and second as a source for understanding self assembling molecules spore research offers huge potential.. As a delivery vehicle the spore coat has been shown to provide a suitable surface for display of heterologous antigens. As vaccine vehicles spores have a number of advantages. It is probable that other spore coat proteins can be used for surface expression and delivery and indeed spore forming species other than B. subtilis could be developed. The spore coat carries scores of different protomers of which some self-assemble although little is currently known about how this occurs. Accordingly, the assembly processes that form the spore coat as well as the nature of individual proteins could provide a rich source of hitherto unknown self-assembling molecules. In addition, microfluidic technologies have the potential to visualise biological events at the single-cell level, leading to important scientific discoveries, revealing phenomena and behaviors that had remained masked by macro-scale, population-level approaches. This can lead to phenomenal exploration in the field of spore research.

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**References**

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Cowan A.E., Koppel D.E., Setlow B., Setlow P. (2003) A soluble protein is immobile in dormant spores of Bacillus subtilis but is mobile in germinated spores. Proc. Natl. Acad. Sci. USA 100. 4209–4214.

Nicholson W.L., Munakata N., Horneck G., et al. (2000) Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. 64. 548–572.

Schisler, D. A., Slininger, P. J., Behle, R. W., and Jackson, M. A. (2004). Formulation of Bacillus spp. for biological control of plant diseases. Phytopathology 94:1267-1271.

Ting, A.S.Y.; Fang, M.T.; Tee, C.S. (2010) . An in vitro assessment on the efficacy of clay-based formulated cells of Pseudomonas isolate UTAR EPA2 for petrol degradation. American Journal of Applied Science

Oomes S. J. C. M. and S. Brul (2004) .The effect of metal ions commonly present in food on gene expression of sporulating Bacillus subtilis cells in relation to spore wet heat resistance Innovative Food Science & Emerging Technologies . 5( 3): 307-316 .

Amran, M. (2006). Biomass production and formulation of Bacillus subtilis for biological control. Indonesian journal of Agricultural 7(2):51- 65

Gentry, T.J., C. Rensing and I.L. Pepper (2004). New approaches for bio augmentation as a remediation technology. Crit. Rev. Environ. Sci. Technol., 34: 447-494.

Herold, K. E., Rasooly, A. & Sandwall, P. Biosensors and molecular technologies for cancer diagnostics. Med. Phys. 40(6), 067301 (2013).

Tetyana, P., Shumbula, P. M. & Njengele-Tetyana, Z. Biosensors: Design, Development and Applications (IntechOpen, 2021).

Suntornsuk, W. & Suntornsuk, L. Recent applications of paper-based point-of-care devices for biomarker detection. Electrophoresis 41(5–6), 287–305 (2020).

Chambers, J. P., Arulanandam, B. P., Matta, L. L., Weis, A. & Valdes, J. J. Biosensor recognition elements. Curr. Issues Mol. Biol. 10(1–2), 1–12 (2008)

Karanpriya. Paper Strip Based Assay for Detection of Heavy Metals in Milk (Doctoral dissertation, NDRI, Karnal) (2020)

Dasriya, V. et al. Rapid detection of pesticide in milk, cereal and cereal based food and fruit juices using paper strip-based sensor. Sci. Rep. 11(1), 1–9 (2021).

Singh, B., Kaur, J. & Singh, K. Biodegradation of malathion by Brevibacillus sp. strain KB2 and Bacillus cereus strain PU. World J. Microbiol. Biotechnol. 28(3), 1133–1141 (2012).

Thukral, H., Dhaka, P., Bedi, J. S. & Aulakh, R. S. Occurrence of aflatoxin M1 in bovine milk and associated risk factors among dairy farms of Punjab, India. World Mycotoxin J. 15(2), 201–210 (2022).

Sharma, H., Jadhav, V. J. & Garg, S. R. Aflatoxin M1 in milk in Hisar city, Haryana, India and risk assessment. Food Addit. Contam. Part B 13(1), 59–63 (2020)

Miller J, Engleberg S, Broad W. Germs: America’s secret war against biological weapons. New York: Simon and Shuster, 2001.

Hart CA, Beeching NJ. Prophylactic treatment of anthrax with antibiotics. BMJ 2001;323:1017-8.

Kelle A (2009). "Security issues related to synthetic biology. Chapter 7.". In Schmidt M, Kelle A, Ganguli-Mitra A, de Vriend H (eds.). Synthetic biology. The technoscience and its societal consequences. Berlin: Springer.

Garfinkel MS, Endy D, Epstein GL, Friedman RM (December 2007). ["Synthetic genomics: options for governance"](https://dspace.mit.edu/bitstream/1721.1/39141/1/Synthetic%20Genomics%20Options%20for%20Governance.pdf) (PDF). Industrial Biotechnology. 3 (4): 333–65. [doi](https://en.wikipedia.org/wiki/Doi_(identifier)):[10.1089/ind.2007.3.333](https://doi.org/10.1089%2Find.2007.3.333). [hdl](https://en.wikipedia.org/wiki/Hdl_(identifier)):[1721.1/39141](https://hdl.handle.net/1721.1%2F39141). [PMID](https://en.wikipedia.org/wiki/PMID_(identifier)) [18081496](https://pubmed.ncbi.nlm.nih.gov/18081496)

Gaur K, Iyer K, Pola S, Gupta R, Gadipelli AK, et al. (2014) The clostridium perfringens epsilon toxin as a bioterrorism weapon. J Microb Biochem TechnolS8: 009.

Wilcox MH (2003) Clostridium difficile infection and pseudomembranous colitis.Best Pract & Res Clin Gastroenterology 17: 475–493.

Fraser CM, Dando MR (2001) Genomics and future biological weapons: The need for preventive action by the biomedical community. Nat Genet 29: 253-256.

Epstein GL (2001) Controlling biological warfare threats: resolving potential tensions among the research community industry and the national security community. Crit Rev Microbiol 27: 321-354.

Petro JB, Plasse TR, McNulty JA (2003) Biotechnology: Impact on biological warfare and biodefense. biosecurity and bioterrorism: biodefense strategy practice and science.

Epstein GL (2012) Preventing biological weapon development through the governance of life science research. biosecurity and bioterrorism: biodefense strategy practice and science..

Setlow P (2007) I will survive: DNA protection in bacterial spores. Trends Micro biol 15: 172-180.

Weaver J (2007) Protective role of bacillus anthracis exosporium in macrophage-mediated killing by nitric oxide. Infect Immun 75: 3894-3901.

Stewart GC (2015) The exosporium layer of bacterial spores: a connection tothe environment and the infected host.

Calfee MW, Choi Y, Rogers J, Kelly T, Willenberg Z, et al. (2011) Lab scale Citation: Cogliati S, Costa JG, Ayala FR, Donato V, Grau R (2016) Bacterial Spores and its Relatives as Agents of Mass Destruction. J Bioterror Biodef 7: 141. doi: 10.4172/2157-2526.1000141 Page 11 of 12 Volume 7 • Issue 1 • 1000141J Bioterror BiodefISSN:2157-2526 JBTBD an open access journal assessment to support remediation of outdoor surfaces contaminated with bacillus anthracis spores J Bioterr Biodef 2: 1-8.

Cote CK, Welkos SL (2015) Anthrax toxins in context of bacillus anthracis spores and spore germination. Toxins 7: 3167-3178.

Oliva CR, Swiecki MK, Griguer CE, Lisanby MW, Bullard DC, et al. (2008) The integrin Mac-1 (CR3) mediates internalization and directs Bacillus anthracis spores into professional phagocytes. PNAS 105: 1261-1266.

Guillemet E, Cadot C, Seav-Ly T, Guinebretiére MH, Lereclus D, et al. (2010) The InhA metallo proteases of bacillus cereus contribute concomitantly to virulence. J Bacteriol 192: 286-294.

Popham DL (2002) Specialised peptidoglycan of the bacterial endospore: the inner wall of the lockbox. Cell Mol Life Sci 59: 426-433.

Henriques AO (1998) Involvement of superoxide dismutase in spore coat assembly in bacillus subtilis. J Bacteriol 180: 2285-2291.

Wilcox MH, Fraise AP, Bradley CR, Walker J, Finch RG (2011) Sporicides for clostridium difficile: the devil is in the detail. J Hosp Infect 77: 187-188.

Paredes-Sabja D (2014) Clostridium difficile spore biology: sporulation germination and spore structural proteins. Trends in Microbiology.

Paredes-Sabja D (2011) Germination of spores of bacillales and clostridiales species: mechanisms and proteins involved. Trends in Microbiology 19: 85-94.

Setlow P (2003) Spore germination. Curr Opin Microbiol 6: 550-556.

Sorg JA (2008) Bile salts and glycine as co-germinants for clostridium difficile spores. J Bacteriol 190: 2505-2512.

Paredes-Sabja D (2009) SleC is essential for cortex peptidoglycan hydrolysis during germination of spores of the pathogenic bacterium clostridium perfringens. J Bacteriol 191: 2711-2720.

Heffron JD (2009) Roles of germination-specific lytic enzymes CwlJ and SleB in bacillus anthracis. J Bacteriol 191: 2237-2247.

Carr KA (2010) The role of bacillus anthracis germinant receptors in germination and virulence. Mol Microbiol 75: 365-375.

van der Voort M, García D, Moezelaar R, Abee T (2010) Germinant receptor diversity and germination responses of four strains of the bacillus cereus group. Int J Food Microbiol 139: 108-115.

Abee T, Groot MN, Tempelaars M, Zwietering M, Moezelaar R, et al. (2011) Germination and outgrowth of spores of bacillus cereus group members: diversity and role of germinant receptors. Food Microbiol 28: 199-208.

Setlow P (2013) Summer meeting 2013 when the sleepers wake: the germination of spores of Bacillus species. J Appl Microbiol 115:1251-1268.

Setlow P (2014) Germination of spores of bacillus species: what we know and do not know. J Bacteriol 196: 1297-1305.

Inglesby TV, O’Toole T, Henderson DA (2002) Anthrax as a biological weapon. JAMA 287: 2236-2252.

Fowler RA, Shafazand S (2011) Anthrax Bioterrorism: prevention diagnosis and management strategies. J Bioterr Biodef 2: 1-5.

Center for Civilian Biodefense Studies, Johns Hopkins University Schools of Medicine, Baltimore, MD 21202, USA.

Wein LM, Craft DL, Kaplan EH (2003) Emergency response to an anthrax attack. Proc Natl Acad Sci USA 100: 4346-4351.

Dudley JP (2005) Review and analysis of reported anthrax related military mail security incidents in washington DC metropolitan area during march. J Bioterr Biodef pp: 1-6.

Hugh-Jones ME, Rosenberg BH, Jacobsen S (2012) Evidence for the source of the 2001 attack anthrax. J Bioterr Biodef 3: 1-8.

Unal B, Aglani S (2016) Use of chemical biological radiological and nuclear weapons by non-state actors. Lloyd’s emerging risk report Chatham House The Royal Institute of International Affairs.

Hamiot A, Lemy C, Krzewinski F, Faille C, Dubois T. (2023) Sporulation 423 conditions influence the surface and adhesion properties of Bacillus subtilis 424 spores. Front Microbiol 14:1219581.

Setlow PC, G. (2023) New thoughts on an old topic: secrets of bacterial spore resistance slowly being revealed. . Microbiology and molecular biology reviews: MMBR 87(2):0008022

Tahara Y O MM. (2023) Visualization of peptidoglycan structures of Escherichia coli by quick-freeze deep-etch electron microscopy. Methods Mol Biol 2646:299-307.

Li L, Jin J, Hu H, Deveau IF, Foley SL, Chen H. (2022) Optimization of sporulation and purification methods for sporicidal efficacy assessment on Bacillus spores. J Ind Microbiol Biotechnol 49.

Tahara YO, Miyata M, Nakamura T. (2021) Correction: Tahara et al. quick freeze, deep-etch electron microscopy reveals the characteristic architecture of the fission yeast spore. . J Fungi (Basel) 7:930.

Hu S, Tahara YO, Tezuka T, Miyata M, Ohnishi Y. (2024) Architecture of Actinoplanes missouriensis sporangia and zoospores visualized using quick freeze deep-etch electron microscopy. Biosci Biotechnol Biochem 88:225-229.

Cho WI CM. (2020) Bacillus spores: a review of their properties and inactivation processing technologies. Food Sci Biotechnol 29:1447-1461.

J.-W. Seo, J. Y. Kim, J.-J. Oh, Y. J. Kim and G.-H. Kim, Selection and characterization of toxic Aspergillus spore-specific DNA aptamer using spore-SELEX, *RSC Adv.*, 2021, **11**(5), 2608–2615

H. Lu, J. Zhu, T. Zhang, X. Zhang, X. Chen and W. Zhao, *et al.*, A rapid multiplex nucleic acid detection system of airborne fungi by an integrated DNA release device and microfluidic chip, *Talanta*, 2022, **246**, 123467

Z. Fattahi and M. Hasanzadeh, Nanotechnology-assisted microfluidic systems for chemical sensing, biosensing, and bioanalysis, *TrAC, Trends Anal. Chem.*, 2022, **152**, 116637

M. Asal, Ö. Özen, M. Şahinler, H. T. Baysal and İ. Polatoğlu, An overview of biomolecules, immobilization methods and support materials of biosensors, *Sens. Rev.*, 2018, **39**(3), 377–386

V. Naresh and N. Lee, A review on biosensors and recent development of nanostructured materials-enabled biosensors, *Sensors*, 2021, **21**(4), 1109

L.-S. Yu, J. Rodriguez-Manzano, N. Moser, A. Moniri, K. Malpartida-Cardenas and N. Miscourides, *et al.*, Rapid detection of azole-resistant Aspergillus fumigatus in clinical and environmental isolates by use of a lab-on-a-chip diagnostic system, *J. Clin. Microbiol.*, 2020, **58**(11), e00843–20

X. Ma, Y. Zhang, X. Qiao, Y. Yuan, Q. Sheng and T. Yue, Target-Induced AIE Effect Coupled with CRISPR/Cas12a System Dual-Signal Biosensing for the Ultrasensitive Detection of Gliotoxin, *Anal. Chem.*, 2023, **95**(31), 11723–11731

G. Woo, D. H. Lee, B. Y. Lee and T. Kim, *Real-time bioaerosol detecting via combination of cyclone based collecting system and SiNW biosensor*, ed. IEEE, 2023, IEEE International Symposium on Medical Measurements and Applications (MeMeA), 2023

S. Khan, M. Naushad, M. Govarthanan, J. Iqbal and S. M. Alfadul, Emerging contaminants of high concern for the environment: Current trends and future research, *Environ. Res.*, 2022, **207**, 112609

J. Ma, G. Jiang, Q. Ma, H. Wang, M. Du and C. Wang, *et al.*, Rapid detection of airborne protein from Mycobacterium tuberculosis using a biosensor detection system, *Analyst*, 2022, **147**(4), 614–624

Agake, S.; Ramirez, M.D.A.; Kojima, K.; Ookawa, T.; Ohkama-Ohtsu, N.; Yokoyama, T. Seed coating by biofertilizer containing spores of Bacillus pumilus TUAT1 strain enhanced initial growth of Oryza sativa L. Agron. J. 2021, 113, 3708–3717.

Mocali, S.; Gelsomino, A.; Nannipieri, P.; Pastorelli, R.; Giagnoni, L.; Petrovicova, B.; Renella, G. Short-Term Resilience of Soil Microbial Communities and Functions Following Severe Environmental Changes. Agriculture 2022, 12, 268. [

Gupta, A.; Mishra, R.; Rai, S.; Bano, A.; Pathak, N.; Fujita, M.; Kumar, M.; Hasanuzzaman, M. Mechanistic Insights of Plant Growth Promoting Bacteria Mediated Drought and Salt Stress Tolerance in Plants for Sustainable Agriculture. Int. J. Mol. Sci. 2022, 23, 3741.

Ramírez, M.D.A.; España, M.; Sekimoto, H.; Okazaki, S.; Yokoyama, T.; Ohkama-Ohtsu, N. Genetic Diversity and Characterization of Symbiotic Bacteria Isolated from Endemic Phaseolus Cultivars Located in Contrasting Agroecosystems in Venezuela. Microbes Environ. 2021, 36, ME20157.

7. Mohsin, M.Z. et al. (2021) Advances in engineered Bacillus subtilis biofilms and spores, and their applications in bioremediation, biocatalysis, and biomaterials. Synth. Syst. Biotechnol. 6, 180–191

Cottet, J. and Renaud, P. (2021) Introduction to microfluidics. In Drug Delivery Devices and Therapeutic Systems (Chappel, E., ed.), pp. 3–17, Academic Press

Zhang, X. et al. (2020) Applications of Bacillus subtilis spores in biotechnology and advanced materials. Appl. Environ. Microbiol. 86, e01096-20

Hall, E.K. et al. (2018) Understanding how microbiomes influence the systems they inhabit. Nat. Microbiol. 3, 977–982

Galiana, E. et al. (2019) Guidance of zoospores by potassium gradient sensing mediates aggregation. J. R. Soc. Interface 16, 20190367

Tetz, G. and Tetz, V. (2017) Introducing the sporobiota and sporobiome. Gut Pathog. 9, 1–6 4.

Jones, S.E. and Lennon, J.T. (2010) Dormancy contributes to the maintenance of microbial diversity. Proc. Natl. Acad. Sci. U. S. A. 107, 5881–5886 5.

Zhang, X. et al. (2020) Applications of Bacillus subtilis spores in biotechnology and advanced materials. Appl. Environ. Microbiol. 86, e01096-20

Trotter, R.E.; Vazquez, A.R.; Grubb, D.S.; Freedman, K.E.; Grabos, L.E.; Jones, S.; Gentile, C.L.; Melby, C.L.; Johnson, S.A.; Weir, T.L. Bacillus subtilis DE111 intake may improve blood lipids and endothelial function in healthy adults. Benef. Microbes 2020, 11, 621–630.

Brutscher, L.M.; Borgmeier, C.; Garvey, S.M.; Spears, J.L. Preclinical Safety Assessment of Bacillus subtilis BS50 for Probiotic and Food Applications. Microorganisms 2022, 10, 1038. [

Garvey, S.M.; Mah, E.; Blonquist, T.M.; Kaden, V.N.; Spears, J.L. The probiotic Bacillus subtilis BS50 decreases gastrointestinal symptoms in healthy adults: A randomized, double-blind, placebo-controlled trial. Gut Microbes 2022, 14, 2122668.

Penet, C.; Kramer, R.; Little, R.; Spears, J.L.; Parker, J.; Iyer, J.K.; Guthrie, N.; Evans, M. A Randomized, Double-blind, Placebocontrolled, Parallel Study Evaluating the Efficacy of Bacillus subtilis MB40 to Reduce Abdominal Discomfort, Gas, and Bloating. Altern. Ther. Health Med.

Mandarino, F.V.; Sinagra, E.; Barchi, A.; Verga, M.C.; Brinch, D.; Raimondo, D.; Danese, S. Gastroparesis: The Complex Interplay with Microbiota and the Role of Exogenous Infections in the Pathogenesis of the Disease. Microorganisms 2023, 11, 1122.

Patch, C.; Pearce, A.J.; Cheng, M.; Boyapati, R.; Brenna, J.T. Bacillus subtilis (BG01-4(TM)) Improves Self-Reported Symptoms for Constipation, Indigestion, and Dyspepsia: A Phase 1/2A Randomized Controlled Trial. Nutrients 2023, 15, 4490.

Huxsoll  DLParrott  CDPatrick  WC Medicine in defense against biological warfare. *JAMA.* 1989;262677- 679

Tehri, N., Kumar, N., Raghu, H. V., Shukla, R., & Vashishth, A. (2018). Microbial spores: concepts and industrial applications. *Microbial bioprospecting for sustainable development*, 279-289.