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Spore: Potential of Invaluable Bacterial Wrap

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ABSTRACT- Bacterial Spores are robust and dormant life forms. The enthralling controlling system can maintain the spore dormancy for years yet allow the reappearance into active state within minutes thus provide resistance to the bacterium to heat, freezing, chemicals, radiations and other adverse environments. In spite of being considered as a spoilage and disease cause, Bacterial spores have been emerging as a miracle package. The survivability of bacterial spores under harsh conditions provides various solutions to human needs. Thus bacterial spores are drawing increased interest of the researchers as a solution to get work done under tough conditions. Bacterial spores have been exploited successfully to develop Biological Detection Systems as they can sense environmental changes and respond rapidly. Recently several spore based biosensors have been developed for the detection of different contaminants from different sources. More valued Probiotic Products based on bacterial spores have also been developed as spores can travel through GIT safely due to their resistant to digestive enzymes.Taking advantage of spore survivability, Pest Control Products based on spores are being used for making innovations in pest control. Different strains of *Bacillus thuringiensis* have been used to protect crops. More recent Bt genes have been expressed in transgenic plants to provide inherent resistance. *Bacillus* spores also have been exploited for vaccine delivery as a non- invasive and thermostable vaccine delivery system. Bioremediation and Electricity generation is also another applied corner of bacterial spores. This reevaluation highlights the potential of this simpler microbial structure and recent growth in the applied bacterial spore biology.

Key-words- Bacterial Spore, Biological detection system, Probiotics, Vaccine delivery, Bioremediation

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INTRODUCTION

Under normal conditions, the bacterial cell acquires the nutrients from surroundings that results in an orderly increase of all the chemical constituents of the bacterial cell and the multiplication of the cell is the consequent. During inability of bacteria to acquire nutrients, the bacterial cell loses its ability to reproduce and resultant is the death. However, some bacteria particularly members of *Firmicutes*, exist in two configurations depending upon the nutrient swing. Under cordial conditions, these bacteria exist as metabolically committed and dividing vegetative cells.

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However under deprived growth environment, they mold themselves into metabolically dormant survival forms as spores. The first published data on spores was found with Ferdinand Cohn, a german botanist, stating the survival of some bacteria even after boiling [1]. Cohn believed that these bodies represented a stage in the life cycle of the bacilli and suggested that they were real spores, from which new Bacilli may develop [2]. Later, a number of spore forming species were discovered from various extreme environments. In contrast to fungi, Bacterial spores are extremely resilient to critical environmental stress, for instance heat, desiccation, UV and ϒ- radiation, mechanical disruption, enzymatic digestion and toxic chemicals. In addition to spore's resistance to stress, they can survive for highly extended periods under milder conditions. In 1983, archaeologists found viable spores in sediment lining Minnesota's Elk Lake. The sediment was over 7,000 years old [3]. Even 25 million years old spores have also been isolated [4]. Moreover, spores of some species can survive for extremely millions of years. As a result of the persistence of spores, they are common contaminants of food stuffs and major contributors to food spoilage and food poisoning. In addition, spores are responsible for a large number of other human illnesses, including gas gangrene, tetanus and anthrax. Spores of *B. anthracis* are major bioterrorism threat. Spores of *Clostridium difficile* are emerging as new food threat. A step forwarded to the resistance studies of spores, some recent studies reveal that spores of common soil inhabitant bacteria are highly resistant to digestion by bacteriophages and soil macroflora [5]. Protective structures of spore like spore coat hinder access of digestive enzymes such as lysozyme to sensitive structure as the cell wall and thus enable the bacteria to pass through the worm's digestive tract. Nevertheless of the dormant nature of spore, it maintains its ability to sense the environment and give the immediate response to any favorable alteration as reconversion into the metabolically active state [6]. The ability to sense the environmental changes at micro level and subsequent germination make the spores used in various positive aspects. This review will enlighten the positive aspects of spore resistance and sensing properties. Brief description of important inventions made in relevant subject will be addressed particularly describing the applied research on spores. Some very recent studies and future possibilities will also be discussed.

Spore: The phenomenon

The process of conversion of vegetative bacterial cell into spore is known as Sporulation and is introduced by nutritional or environmental stress [7]. Sporulation is a well-studied phenomenon. During early exposure of active cells to the limited nutrient, the cells undergo into a stationary phase. The stationary phase progressions allow the cells to get adapted to adverse environment with minimal damages. In the early stages of nutrient harsh conditions, DNA replicates and the cell divides asymmetrically, generating a larger mother cell and a smaller fore spore. As a series of developmental events, the spores mature within the sporulating cell and are released free as the mother cell lyses. These spores are well capable to survive under most environmental stress. The mechanism of spore resistance lies in its well-structured multi-layered morphology [8,9]. For a long time, the structural components of bacterial spore have been widely studied. Beginning from the outside, the spore layers include the exosporium, coat, outer membrane, cortex, germ cell wall, inner membrane and central core. Each spore compartment shows a great resistance potential. However, the role of the exosporium is still in dark. The fundamental to spore resistance is thought to be the spore coat, a tough protein layer that provides mechanical strength and also rejects large toxic molecules, while allowing small nutrient molecules to access germination receptors lying beneath the coat. The coat also appears to contain enzymes that have important roles in germination. The spore coat contributes protection against various chemicals and lytic enzymes. The cortex i.e. made of peptidoglycan, osmotically removes water from the interior of the endospore. The resulting dehydration is thought to be very important in the endospore's resistance to heat and radiation. Likewise, the extremely low permeability of the spore inner membrane prevents both hydrophobic and hydrophilic molecules from entering into the core and is thought to be responsible for the endospore's resistance to chemicals such as formaldehyde, nitrous acid and others which target their action on spore core [10]. Changes in the inner membrane permeability illustrate altered resistance to chemicals that act in spore core in contrast to those, act outside the core.The spore core, the genetic material province contains a lesser amount of water that is mainly responsible for resistance of spore to wet heat. Calcium di-picolinate, abundant within the endospore, protects by giving more heat resistance. In addition, specialized DNA-binding proteins (Small acid soluble proteins) saturate the endospore's DNA and protect it against many types of damages. The SASP are synthesized during sporulation only and are degraded as spore germinates into vegetative form. The DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination. Furthermore the bacterial spore is able to survive under the scarce nutrient conditions as the metabolic rate slows down. Thus the dormancy or the inertness of the spore is important for its survival.

Despite of the dormant form, the bacterial spore sustain the ability to sense the environment and give the immediate response to any favorable alteration as reconversion into the metabolically active state. The reversion of favorable growth conditions triggers a signal for bacterial spore germination. The spore germination events lead to the sequential loss of spore specific properties. The basic apparatus required for germination is already present in the mature dormant spore. Germination starts in response to specific chemical nutrients that acts upon the specific receptor proteins, located at the inner membrane of the spore. As a consequence of this binding, the mono-valent cations move out from the spore core, followed by Ca^{2+} and dipicolinic acid (DPA). This results in a break in the spore wall and outgrowth of a new vegetative cell. This newly formed cell behaves similarly under nutrient favorable conditions, as a normal bacterial cell and capable to develop into the spore again, on the reversion of any environmental stress.

Bacterial spore resistance: Upshots

Although, the resistance feature of bacterial spore triggers the first mark in the mind as spoilage of food stuff. However, with this resistance phenomenon, several potential valuable inventions have been made.

Spores: The Detectives

Taking advantage of the environment sensing ability of the spores, several spore based sensing systems have been developed for the detection of various microbial and non-microbial contaminants in different food stuffs. The spore based sensing systems are much more superior over the other existing systems. These systems have a longer shelf life due to the dormant nature of the spore. The production cost of is also very economic thus the cheaper product systems are better able to complete with the other technologies in the market. Spore based systems are extremely rapid and produce a real time response as the environment sensing and subsequent germination of spores takes only minutes [11]. One emerging technology developed by BCR Corp., (Jameston, RI) uses microbial spores as nano-detectors, which emit fluorescence light signals in response to specific bacteria in the surroundings. LEXSAS, the biological operating system is based on the hydrolysis of alanine-alanine by analyte bacterium to produce L-alanine, which acts as specific germinant for spores.

This chain reaction is monitored at time intervals using fluorescent output due to DAF hydrolysis by acetyl esterase i.e. produced during spore germination [12]. Furthermore, a spore based detection system for monitoring β-lactam antibiotics specifically in milk matrix has been developed. The test is based on the novel β-lactamase induction principle that specifically detects β-lactam antibiotic residues in dairy foods [13]. Another method targeting spores as bio-sensing element is designed for monitoring broad spectrum antibiotics in milk [14,15]. The assay involves the transformation of resistant spores into active vegetative cells that is inhibited in the presence of antibiotic residues. The test system comprises of a small tube containing the test medium and spores i.e. embedded within. The change in the color of the test system indicates the presence and absence of antibiotic residues in the milk sample. The field of spore based biosensor widened its area for the detection of various pathogenic microorganisms. A spore based system for the detection of *Listeria monocytogenes* in milk has been invented. This comprises a two stage assay, in which a pre-enrichment is given as a first step after that spore based detection is performed. The assay is based on the specific marker enzyme of *L. monocytogenes* that converts the complex sugar into simpler sugar. This simpler sugar provides the nutrient for germination of spores in the system. The detection assay has been specialized for milk based products with the detection time of 20 hrs that can detect up to 1 log cells in milk [16]. Furthermore, Research on immobilized of spores on the solid surface of biochip to miniaturize the tests is in trend. A typical working of biochip based assay using spore germination principle is presented in Fig. 1.

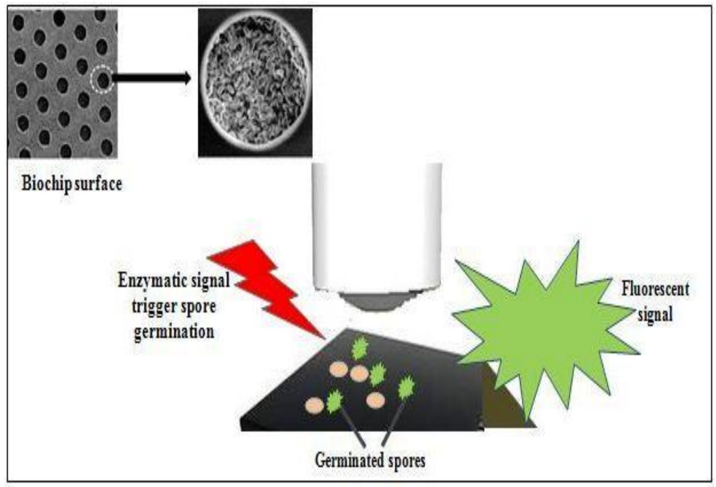

Fig. 1: Working Phenomenon of a spore based Biochip

Biochip provides the miniaturized test sites (microarrays) arranged on a solid substrate that permits many tests to be performed at the same time in order to achieve higher throughput and speed. Typically, a biochip's surface area is very small. Industries currently desire the product with ability to simultaneously screen a wide range of contaminants, with purposes like testing public water systems and foods for disease agents. Thus, such spore based systems provide a novel strategy to ensure the food safety. Numerous advancements continue to be made in spore sensing research that enables new platforms to be developed for new applications.

Spores: The Pest killers

Pest management is very crucial so as to prevent the damages to crops and humans. Synthetic chemical pesticides provide a great solution, but so pose some hazards. However, an alternative is the use of microbial insecticides or pesticides that contain microorganisms or their by-products. Microbial insecticides are especially valuable because their toxicity to non-target animals and humans is extremely low. Compared with other commonly used insecticides, they are safe for both the pesticide user and consumers of treated crops. Bacterial pathogens, used for insect control are spore forming, rod-shaped bacteria in the genus *Bacillus*.Strains of BT along with *Bacillus sphaericus* have been successfully used to control the mosquito vectors of diseases, such as dengue and malaria. The spores of *Bacillus thuringenesis*, commonly referred to as BT are widely used as biopesticides in agriculture [17]. This microorganism produces chemicals toxic to insects. BT was registered in the United states for use as a pesticide in 1961 [18]. BT toxicity is insect specific. Scientists have identified BT subspecies that differ in toxicity to different insects e.g. the subspecies *aizawai*is effective against moths, *kurstaki* is for moths, *israelensis*is for mosquitoes and flies and *tenebrionis*is for beetles [19]. Commercial BT products contain the crystalline protein toxin containing spores. When a susceptible insect ingests BT, the protein toxin is activated by alkaline conditions and enzyme activity in the insect's gut. When activated toxin attaches to receptor sites, it paralyzes and destroys the cells of the insect's gut wall, allowing the gut contents to enter the insect's body cavity and bloodstream. Poisoned insects die readily by the activity of the toxin or may die within 2 or 3 days because of septicemia. Although a few days may elapse before the insect dies, it stops feeding or in another words stops damaging plants, soon after ingesting BT [20]. The most common trade names for these spores containing commercial products include Dipel®, Javelin®, Thuricide®, Worm Attack®, Caterpillar Killer®, Bactospeine®, and SOK-Bt®. These products are widely available as liquid concentrates, wettable powders, and ready-to-use dusts and granules.

Spores: The Probiotics

Probiotics has arisen as a new solution alternative to antibiotics. Among the large number of probiotic products in use today are bacterial spore formers, Mostly of the genus Bacillus. Although the most common types of probiotics available are the lactic acid bacteria, but the bacterial spores are the most stable probiotic strains as spores are extremely resistant and more stable [21]. Spores of *Bacillus* spp. were the first commercial probiotics, the prescription probiotics starting in 1958 as Enterogermina® (Sanofi-Aventis, Italy) and Bacti-Subtil® (Aventis Pharma, France). Most widely used and well-studied strains in humans are *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans*, *Bacillus clausii* and *Bacillus indicus.* The spores of these probiotic strains have been found to colonize very effectively in the GIT of several different animals. Spores of this *Bacillus* spp. have also been found to produce a significant number of potent antibiotics that control bacterial over-growth in the GIT e.g. Coagulin, Subtilisin, Amicoumacin, Surfactin, Iturins A and Bacilysin [22]. *Bacillus* probiotics have been known to produce key digestive enzymes that relieve bloating, cramping and discomfort. Enzymes like alpha-amylase, lactase, protease and lipase are improved in GIT with the use of *Bacillus* spp. [23]. *Bacillus* probiotics produce important vitamins like menaquinones and biotin in the large intestines that can be absorbed easily as they are produced at the site of absorption. *Bacillus* spp. also digest starch and non-starch polysaccharides to short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. SCFA stimulate colonic blood flow and electrolyte uptake. In addition, *Bacillus* spp. have been proven to detoxify the genotoxic compounds encountered in the GIT e.g. Vomitoxin i.e. found in wheat, corn and other grains is neutralized by *Bacillus* spp.in the GIT and Zearalenone, naturally produced by the fungus found on cattle and in milk is also neutralized by *Bacillus* spp. Primarily seen with the consumption of *Bacillus subtilis* from natto, *Bacillus sp.* has also been shown to digest cholesterol in the GIT. The commercial *Bacillus* spp. products carry a number of attributes that support its use as a good probiotic which include prolonged persistence in the GI tract, the formation of robust biofilms, rapid sporulation and the stimulation of innate immune responses [24]. In addition, spores of *Bacillus* spp. provide applications as probiotic additives into a number of food products like chocolate, cakes etc. that could not be considered for the more common *Lactobacilli* and *Bifidobacteria* probiotics [25]. And the *Bacillus* probiotic products are more stable and do not require refrigeration and able to be stored on the shelf at room temperature.

Spores: The Vaccine car

In recent years, the developments in molecular biology and cell biology have led to the discovery of enormous bacterial and viral antigens. However, transformation of these antigens into an effective vaccine is governed by a delivery system i.e. able to avoid the loss of biological activity that often impairs antigen efficacy. Recently, spores of *Bacillus* spp. have shown their potential to be used for designing next generation vaccines [26]. The bacterial spore is a quiescent cell form and offers a potentially powerful tool to deliver antigens to mucosal surfaces. Spore contains unique resistance properties and can survive extremes of temperature, desiccation, and exposure to solvents and other noxious chemicals. These unique attributes make the spore an attractive vehicle for delivery of heterologous antigens or, indeed, any bioactive molecule to extreme environments such as: the gastrointestinal tract. A typical transfer mechanism of vaccine through spores is depicted in Fig. 2.

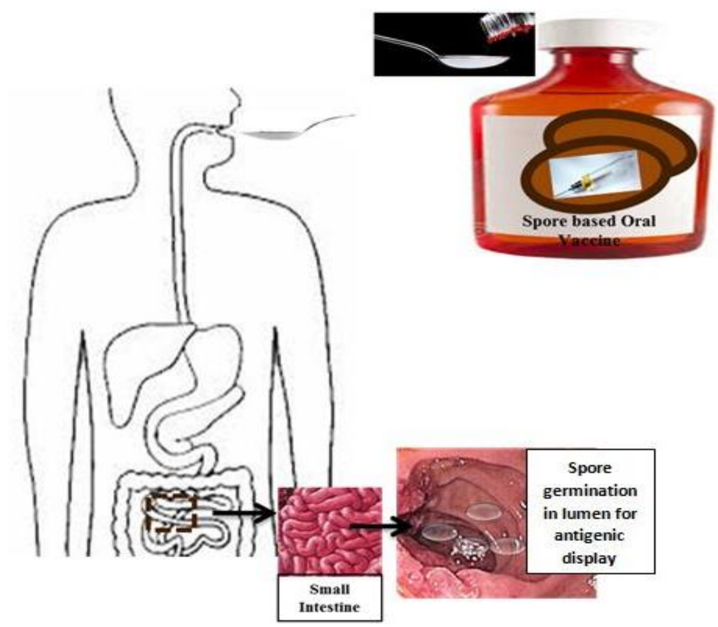

Fig. 2: Spore based vaccine delivery system for Intestinal mucosa

Spores of *Bacillus* spp. also give a significant adjuvant effect that leads to evoked immune response [27-28]. The use of spores as vaccines is credible, providing a robust and heat-stable bio-particle that can be engineered to display more than one protein antigen on the spore surface as a fusion with spore coat proteins [29,30]. Spores of *B. subtilis* are able to germinate to some degree in the small intestine [31]. The foreign antigen coding regions are incorporated into *B. subtilis* nuclear material and expressed by using a strong promotor i.e. expressed only during vegetative cell growth. Use of spores as vaccine delivery systems for gastrointestinal mucosa allows the passage of entire inoculum across the gastric region and enables the germination in the small intestine. Rather than requiring needle delivery, vaccines based on spores can be delivered via a nasal spray, or as on oral liquid or capsule [32]. Alternatively, they can be administered via a small soluble film placed under the tongue, in a similar way to modern breath fresheners. As spores are exceptionally stable, vaccines based on spores do not require cold-chain storage alleviating a further issue with current vaccine approaches.

Spores: The Bioremediation Gadgets

Recently, the recombinant spores of *B. subtilis* have been reported as a potential bioremediation tool for adsorption of nickel ions [33-34]. The spore surface protein CotB has been engineered to express eighteen histidine residues within the spore coat. Wild type and recombinant spores when analyzed to assess their efficiency in adsorbing nickel ions, the latter proved to be significantly more efficient than wild type spores in metal-binding. The efficiency of nickel binding of *B. subtilis* spores together with the simple purification procedure, the high robustness and safety makes the recombinant spore a new and potentially powerful tool for the treatment of contaminated ecosystems.

Spores: The Electric Generators

A very recent research on application of spores for generating electricity has been made at Harvard University. The special electricity generators employ the power of evaporating water. *B. subtilis* wrinkles as it dries out like a grape becoming a raisin, forming a tough, dormant spore. Spores can take on water and almost immediately restore themselves to their original shape. Since changing moisture levels deform these spores, it followed that devices containing these materials become able to move in response to changing humidity levels. The prototype generators work by harnessing the movement of a sheet of rubber coated on one side with spores. The sheet bends when it dries out, much as a pine cone opens as it dries or a freshly fallen leaf curls, and then straightens when humidity rises. Such bending back and forth means that spore-coated sheets or tiny planks can act as actuators that drive movement, and that movement can be harvested to generate electricity. In fact, moistening a pound of dry spores generate enough force to lift a car one meter off the ground.

CONCLUSIONS

Bacteria develop into spores as a way to defend themselves. Man as of human nature exploits this resistant form in adverse environments, where ever biological activity is needed. A spore is a hard casing. This physically and chemically resilient package protects the genetic material during periods. With a large range of commercial applications of bacterial spore, it also provides a good research model for the existence of life on other planets. As per an assumption, there is a possibility that the forces of uplift ‘magnetosphericplasmoids’ on a charged bacteria particle are sufficient to bring at least some lighter types of bacteria higher into the ionosphere, and subsequently move the charged spore onto magnetic field lines. These plasmoids are capable of reaching Mars or other planets, and even others systems, eventually depositing the bacterial spores either via comets or direct interaction with the receiving planet. Thus the bacterial spores are providing another possible mechanism for moving life among worlds and creating a new research area. But the threat posed by the use of spores in the mail is difficult to counter. Researchers are working to develop sensors that detect the spores without even opening the letter.

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