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Microbial Spores: Concepts and Industrial Applications

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Abstract

Several microorganisms like bacteria, fungi, yeast, algae, actinomycetes and protozoa are well known for their ability to form spores. Spores have inherently distinct life cycle as compared to vegetative cells making them able to resist various unfavourable environmental conditions like extreme temperature, radiations, desiccation, toxic chemicals, etc. Keeping in view their vast potential, the use of spores has led to several breakthrough researches in order to develop a large number of spore-based products in the fields of biosensing, biocontrol, biofertilizers, biocatalysis, biosorption, biopolymers, biological warfare, medicine, probiotics and surface display. Most of these products are now available commercially and thus indicate the indispensable potential of microbial spores. The current chapter gives information about the concept of microbial spores and its potential industrial applications in aforementioned fields. Furthermore, attention has also been paid to the current status, associated challenges and future perspectives for spore-based technologies.

Keywords

Spores · Biosensing · Biocontrol · Probiotics · Applications

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15.1 Introduction

Microbial spores are robust and metabolically dormant structures that are produced by a process of sporulation to prevail over harsh and unfavourable climatic conditions of starvation and stress. They are formed by microorganisms belonging to different groups such as bacteria, fungi, actinomycetes, algae, etc. Spores are of great significance. The use of spores on one hand opens the door to 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 2006; Setlow and Johnson 2007; Coleman et al. 2010).

Physiological behaviour of spores that occurs as a part of their life cycle holds great promise for immense industrial applications. It is well known that the life of spores revolves around two phases, the dormant and the metabolically active vegetative state, and perhaps this exclusive biphasic phenomenon of spores could have great potential to form the basis of development of various techniques ranging from biosensing and biocontrol to probiotics and medicine. Development of these techniques based on the use of spores takes the advantage of the process of germination by examining the physiological changes occurring upon addition of spore-specific dormancy-breaking signals (Yung 2008). Induction of germination in spores by exposure to specific germinant converts them into vegetative cells which are metabolically very active and fragile. Conversion from dormant to vegetative forms takes place when spores sense favourable environmental conditions.

Taking advantage of the spore's unique resistance properties and environment sensing ability, several spore-based technologies have been developed (Table 15.1). Spores as a biosensor have been used for the detection of various microbial and nonmicrobial contaminants in different food stuffs. Because of the metal-binding properties of their surface protein, they have been exploited in the field of biosorption. Being resistant to harsh environmental conditions, spores have been used to develop probiotics that can easily pass through the acidic conditions of the stomach in order to reach the intestine. Spores have also been used for agricultural applications, in development of biocontrol and biofertilizers. There other useful applications including development of biopolymers, enhancing durability of building materials and biocatalysis, etc. Thus the unique inherent characteristics of spores have proved the spores as a valuable tool for fulfilling the need of current time in the area of food, environment and medicine. The presented information highlights about such breakthrough spore-based technologies. Moreover, the chapter also unfolds unlimited scope and hidden potential spores that could lead to many more demanding applications as per the need of times to come.

15.2 Microbial Spores

Microbial spores are ubiquitous in nature. Due to their inherent stability and resistance properties, they are distributed in different kinds of environments. They are found in soils; aquatic environment; extreme environments such as deserts,

Table 15.1 Applications of microbial spores

Field	Spores	Principle	Application	References
Biosorption	*Bacillus subtilis*	Genetically engineered surface proteins (Cot B) for enhanced metal-binding affinity	Bioremediation of heavy metals contaminated sites	Hinc et al. (2010)
Biosensors	*Bacillus megaterium*	Spore germination and enzyme inhibition	Pesticide detection in milk	Kumar et al. (2015)
	Bacillus stearothermophilus	Spore germination and DPA release	Antibiotic detection in milk	Kumar et al. (2006)
	Bacillus megaterium	Enzyme release and spore germination	*Listeria monocytogenes* detection in milk	Balhara et al. (2013)
	Cladosporium cladosporioides	Spore inhibition	Fungicides detection	Kanatiwela and Adikaram (2009)
Biocontrol	*Bacillus thuringiensis*	Endotoxins	Lepidopteran larvae	Whalon and McGaughey (1998)
	Pythium oligandrum	Applied as seed treatment	Damping-off disease caused by *P. ultimum* in sugar beet	Lewis et al. (1989) and Khetan (2001)
	Beauveria bassiana	–	*Anopheles gambiae*, mosquito control	Farenhorst et al. (2011)
Biopolymer	*Saccharomyces cerevisiae*	Presence of chitosan in spore coat layer	Bioadsorption and enzyme immobilization	Zhang et al. (2014)
Biofertilizer	*Frankia*	Nitrogen fixation	*Casuarina cunninghamiana*	Lalonde and Calvert (1979) and Burleigh and Torrey (1990)
Medicine	*Bacillus subtilis* LTB antigen (spore-based delivery system)	Chromosomal-encoded C-terminal fusion with spore coat CotC protein	*Escherichia coli* (ETEC); induction of serum and faecal antibody responses	Mauriello et al. (2004)
Microalgal industry	Fungal strains, isolated from compost, straws and soil	Algal flocculation	Waste water treatment and biofuel production	Muradov et al. (2015)

hydrothermal sites and arctic ices; food system; etc. Spores from different microbial groups are also known to form associations with insects, animals, plants and other organisms. Most of the times, such associations also help them to carry out their entire life cycle comprising of germination-growth-sporulation, within the animal host (Cutting and Ricca 2014).

Being a survival strategy, spores are well resistant to varying ranges of temperature and pressure, UV radiation and many noxious chemical substances. A number of theories were proposed in earlier times during 1970–1975, to understand the resistance of microbial spores. These theories included "contractile cortex theory", the "expanding osmoregulatory cortex theory", the "anisotropic swollen cortex theory", the "high-polymer matrix theory" and the "calcium-dipicolinate complex theory". These theories were established based on the heat resistance mechanism as determined by observations from analysis of spore components and electron microscopy (Murata 1993). Recent advancements in biochemical, genetic and molecular techniques have further contributed to the better understanding of structural and functional properties of spores from different microbial groups.

The process of spore formation is known as sporulation. It involves progression through different stages including commitment to sporulation, chromosome segregation, sporulation-specific cell division, differential gene expression and specific signal transduction mechanisms (Cutting and Ricca 2014). Spores can remain in dormant form for long time periods; however they persistently scrutinize their environment for favourable conditions, the presence of germinants that trigger germination. Furthermore, spores possess the unique properties to recognize stereoisomerically distinct forms of germinants (Tehri et al. 2017). During germination initiation of metabolism takes place resulting in ATP formation and synthesis of RNA and proteins. Eventually replication of DNA results in vegetative cell (Setlow 1983; Paidhungat and Setlow 2002). A new vegetative cell is able to grow, to duplicate and eventually to sporulate again. This exclusive biphasic phenomenon of spores holds great potential to form the basis of development of several spore-based techniques, mentioned below under section of industrial applications.

15.3 Industrial Applications of Microbial Spores

Spores of various types of microorganisms, especially bacterial spores, have been evaluated for their potential for industrial applications in several fields (Fig. 15.1). A number of such spore-based technologies developed till date have been discussed below.

15.3.1 Biosensors

Spores from microorganisms such as bacteria and fungi have been used to develop sensing systems that can easily detect the presence of various microbial and nonmicrobial contaminants in food, clinical and environmental samples. Spore-based

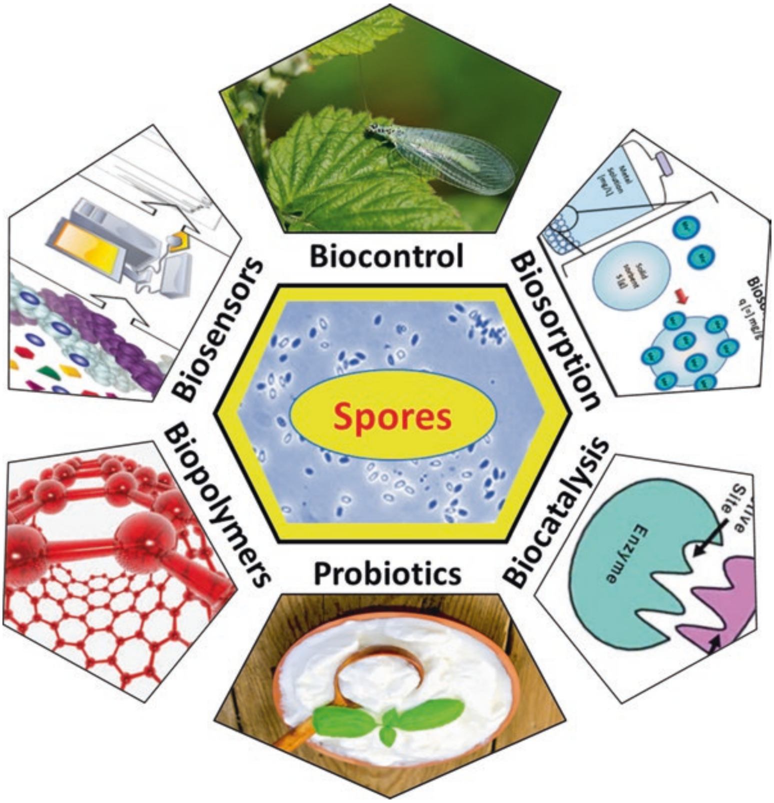

Fig. 15.1 Applications of microbial spores

sensors are cost-effective, have long shelf-life and give a real-time response. Keeping in view, several spore-based biosensors have been developed to target different analytes. Spore-based sensors have been developed to detect β-lactam and several other groups of antibiotics in milk (Das et al. 2011; Kumar et al. 2014). The developed assays are chromogenic and work on the principle of inhibition of spore germination in the presence of antibiotic residues. Furthermore, spores have also been used to target the presence of pathogens that are of public health significance. One such sensor has been developed for detection of *Listeria monocytogenes* in milk. This is a two-stage assay: the first stage is pre-enrichment and the second stage includes the detection of pathogen with the help of *B. megaterium* spores. The assay works on the principle of conversion of complex sugar into simpler form with the help of a marker enzyme present in *L. monocytogenes.* The resulting simpler sugar acts as a nutrient germinant for spores of *B. megaterium.* The germination in spores, indicating the presence of pathogen, is assayed by means of a fluorogenic enzymatic assay (Balhara et al. 2013). In addition to bacterial spores, spores of fungi have also been used to develop spore-based sensing systems. A convenient, cost-effective and sensitive fungal spore-based thin layer chromatography bioassay has been developed for detection of fungicidal residues. The assay has successfully been used for detection of fungicides on tomato. The assay works on the principle of *Cladosporium* spore inhibition in the presence of fungicides. Thus the presence of fungicides is indicated by the lack of aerial mycelium (Kanatiwela and Adikaram 2009).

15.3.2 Biopesticides

The biopesticides can be defined as the types of pesticides which have biological origin. They are usually derived from animals, plants, bacteria and certain minerals. The most common type of biopesticides is based on microorganisms that are pathogenic to the pest to be killed. They can be classified as biopesticides, biofungicides and bioherbicides. The use of biopesticides offers several advantages over chemical pesticides. Biopesticides are safe to humans and the environment, are cheap and have more target specificity. Spores from bacteria and fungi are promising candidates for their use as biopesticides. Spores of *Bacillus thuringiensis* (BT) are widely used in agriculture to target insects and pests. BT is known to produce crystal protein inclusions, with toxic insecticidal properties, during the process of sporulation. Apart from this, various subspecies of BT have been identified with varying levels of toxicity against 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 (Arora et al. 2016). The commercial products based on BT are available in powdered form containing chiefly dried spores and toxin crystals. Spores of *B. sphaericus* are also known to develop a parasporal body during the process of sporulation. It has toxic properties against mosquito's larvae.

15.3.3 Biosorption

The use of biological agents to bind and accumulate pollutants such as heavy metals is known as biosorption (Fourest and Roux 1992). It offers a low-cost, high efficiency, cost-effective treatment for contaminated sites and regeneration of adsorbed materials like recovery of metals (Kratochvil and Volesky 1998a, b). In this context, bacterial spores have also been evaluated to act as biosorbents. Spores of a marine *Bacillus* sp. strain (SG- 1) have been found to bind and oxidize Mn (II) and Co (II) (Lee and Tebo 1994). The Cot B surface protein in spore coat of *B. subtilis* has been engineered at molecular level for expression of 18 residues of histidine fused to promoter and its N-terminal part. The resulting recombinant spores were reported to exhibit greater binding affinity of spores to Ni ions than that of wild type (Hinc et al. 2010). Thus the metal-binding properties reveal the unique nature of bacterial spores to carry out bioremediation in heavy metal-contaminated sites. Furthermore, the peculiar properties such as resistance and stability against harsh environmental conditions make the spores potentially more interesting as a bioremediation tool.

15.3.4 Biopolymers

Chitosan is a polymer made up of β-1, 4-linked D-glucosamine. It has unique adsorptive properties. It acts as a chelator for transition metal ions and has positively charged amino groups that can bind molecules bearing negative charge. Because of these characteristics, chitosan finds several industrial applications in food, chemicals, environment and medicine. Production of chitosan by chemical means faces many challenges including generation of hazardous waste. In order to solve this issue, various biological approaches have been studied for the production of chitosan (Shahidi et al. 1999; Lee et al. 2009; Kardas et al. 2012). Spores of budding yeast *Saccharomyces cerevisiae* have successfully been used for production of chitosan beads. The spore wall of yeast has outer layer of dityrosine and second layer of chitosan. The gene coding for dityrosine, i.e. DIT1 gene, was removed leaving chitosan layer at the spore surface. Thus resulting spores resemble chitosan beads. The practical utility of such spore-based chitosan beads for removal of heavy metals was also examined.

Spores with dityrosine layer were found capable of adsorbing heavy metals such as Cu(II), Cr(III) and Cd(II). However increased rate of absorption was reported upon removal of dityrosine layer. Mutants with removed chitosan layer demonstrated reduced adsorption properties. This suggests the potential of chitosan from spores to act as an adsorbent. Chitosan can easily undergo chemical modifications, and therefore spores acting as chitosan beads can also be used as a carrier for enzyme immobilization (Zhang et al. 2014). Thus wild-type and mutant yeast spores both can be used as chitosan beads that can further find a number of applications in different fields.

15.3.5 Biocatalysis

Spores are considered as dormant and metabolically inactive, but at the same time they have been reported to contain all enzymes similar to those found in vegetative cells (Shigematsu et al. 1993). This suggests that microbial spores are unique bags of enzymes and have the potential to act as biocatalysts. In reference to these conclusions, endospores of *B. subtilis* and ascospores of *S. cerevisiae* have been evaluated for the biocatalytic activities. Two enzymes, namely, adenosine 5′-triphosphatase and alkaline phosphatase, from the spores of bacteria and yeast, respectively, have been used as model enzymes to assess their activity for industrial application. Spores were found to express both enzymes at a significant rate when subjected to physical (sonication or electric field pulse) and chemical (organic solvents or detergents) treatments. Furthermore the spore-based catalysis was used to produce chemicals in bioreactor systems. The use of spores as a source of enzymes was found to be advantageous over vegetative cells. This is because when vegetative cells are lysed to release enzymes for accomplishment of biocatalysis, enzymes come in contact with several biophysical and biochemical factors making them inactive. On the other hand, when spores are used, enzymes can remain stable in spores in bioreactor system. Moreover, spores have also been successfully immobilized for continuous utilization to produce useful chemicals (Murata 1993). Hence, this finding indicates the superiority of spore (bacterial and fungal groups) usage over vegetative cells for their application to bioreactor systems in order to work as a biocatalyst.

15.3.6 Building Material

One of the most commonly used building materials is concrete. However cracks in concrete are a major problem. Cracks in concrete generally occur due to freeze thawing, shrinkage and mechanical forces. Ingression rate of corrosive chemicals like water and chloride ions in the structure of concrete gets increased (Meldrum 2003). In order to improve the strength of cement concrete, an attempt based on the use of bacterial spores has been made. Dormant spores of *B. sphaericus* have been incorporated in concrete matrix. Entry of water in the concrete begins the process of activation of bacterial spores. This results in the precipitation of calcium carbonate by the process of germinated spore-mediated metabolic activities (Gavimath et al. 2012). Thus this work clearly represents the potential application of microbial spores in improving the strength and durability of concrete, a building material.

15.3.7 Probiotics

The term "probiotics" means "for life". It has been derived from a Greek word (Reid et al. 2003). These are defined as, "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2005). Bacterial spores mainly those belonging to genus *Bacillus* have widely been used as probiotics for humans, animals and feed supplements. Probiotics aid to an improved digestion in host (le Duc et al. 2004; Hong et al. 2005). Administration of probiotics in the form of spores is advantageous. As being resistant to acidic conditions, spores upon ingestion can easily pass through the acidic conditions of stomach. They reach the intestine, and the conditions prevailing over therein are less acidic pH, nutrient support germination and subsequent cell growth (Cutting 2011). Several clinical trials on the human and animal models have been done to study the effect of spore-based probiotics. As a result oral administration of *Bacillus* spores was found to stimulate the immune system by triggering both specific humoral and cell-mediated immune responses (Suva et al. 2016). Furthermore, a study carried out with rabbits administered orally with spores of *B. subtilis* has shown the significant contribution of spores in development of the gut-associated lymphoid tissue (GALT) than other commensal bacteria (Rhee et al. 2004).

The spores of *Bacillus subtilis* have inherent properties of acting as probiotics, but addition of certain proteins, such as feed enzymes, on the surface of spores can further improve them for feed applications. Feed enzymes are generally added to feed in order to enhance the digestion of animals. But to retain their activity while passing through stomach acids to reach the gut is very challenging. To solve this issue, inner coat proteins OxdD of spores have been engineered genetically to display the feed enzyme phytase on the surface of *B. subtilis* spores (Potot et al. 2010). Phytase is an enzyme that acts on the phytate-bound phosphorus, releasing free phosphorus and thus enhancing the nutritional value of feed.

Thus the spore-based probiotics offer several advantages such as prolonged persistence in the GI tract, the formation of robust biofilms, rapid sporulation and the stimulation of innate immune responses, room temperature stability without the need of refrigeration, etc. (Chen et al. 2014). These distinctive properties have led to their faster commercialization.

15.4 Current Status and Future Perspectives

The presented information uncovers the vast potential of microbial spores for their industrial applications. Spore-based technologies offer several advantages: (1) amenable to incorporation into portable devices, thus facilitating their use in field applications; (2) versatile performance during multiple germination and sporulation cycles, which further enhances their potential for reusability; (3) cost-effectiveness; (4) a wide range of aforementioned applications ranging from food to medicine; and (5) inherent stability of resisting harsh unfavourable environmental conditions. However, to get advantages of such spore-based applications, preference is usually given to those microbial spores (a) with the availability of detailed genetic and structural information, (b) that can be easily undergo genetic manipulation, (c) that have the safety record for their use on humans and animals (probiotic, medicine) and food systems and (d) that have potential robustness and heat stability. Till date, many such spore-based technologies mainly in the area of biosensing, biocontrol, biofertilizers and probiotics are available commercially, and others are in the queue of technology transfer process.

The use of spores to a wide range of applications in various fields has a bright future ahead. The research work to explore the unseen potential of microbial spores is still in continuation process. One such research work is going on at Harvard University which is attempting on the use of *B. subtilis* spores for generating electricity. Recently the forensic and archaeological value of fungal spores has also been recognized (Hawksworth and Wiltshire 2011), and related work is in progress at the University of Southampton. Apart from this, spores of bacteria have also been explored to provide possible mechanism for moving life among worlds and creating a new research area (Dehel 2006).

Thus unique features of spores make them an attractive tool for broad range of utility in various fields of science and technology. Furthermore, modern molecular-based studies are providing new paradigm on characteristics (biochemical and biophysical) of microbial spores which may further aid to more promising new applications.

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