How the space environment influences organisms: an astrobiological perspective and review

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Abstract

The unique environment of space is characterized by several stress factors, including intense radiation, microgravity, high vacuum and extreme temperatures, among others. These stress conditions individually or in-combination influence genetics and gene regulation and bring potential evolutionary changes in organisms that would not occur under the Earth's gravity regime ($1 \times g$). Thus, space can be explored to support the emergence of new varieties of microbes and plants, that when selected for, can exhibit increased growth and yield, improved resistance to pathogens, enhanced tolerance to drought, low nutrient and disease, produce new metabolites and others. These properties may be more difficult to achieve using other approaches under $1 \times g$. This review provides an overview of the space microgravity and ionizing radiation conditions that significantly influence organisms. Changes in the genomics, physiology, phenotype, growth and metabolites of organisms in real and simulated microgravity and radiation conditions are illustrated. Results of space biological experiments show that the space environment has significant scientific, technological and commercial potential. Combined these potentials can help address the future of life on Earth, part of goal e of astrobiology.

Introduction

The field of astrobiology is broad and interdisciplinary encompassing not just life's evolution and its occurrence throughout the universe, but also the future of life on Earth and beyond. Life and the environment in which it lives are inextricably intertwined. Here we address the future of life on Earth with respect to its changing environment especially in light of global climate change. Astrobiology has as one of its core goals to understand the evolving relationship between Earth and its biota and how life responds to environmental change on a planetary scale. In this paper, we review how the space environment can help us lessen the negative impact of environmental change, especially global climate change, on terrestrial life.

Earth's changing environment

The human population is growing exponentially and is estimated to reach between 9.4 and 10.2 billion people by 2050 (Roser *et al.*, 2020). With the rapid spread of industrialization and the human population's growth to unprecedented levels, environmental pollution has become a global problem. Human activities, such as the overuse of fossil fuels and land, unrestrained deforestation and intensive animal farming, to meet the growing population's demands have led to climate change (Masson-Delmotte *et al.*, 2018; Grossi *et al.*, 2019). This is adversely affecting and transforming life on Earth (Edenhofer, 2015; Xu *et al.*, 2015; Zhang *et al.*, 2018; Grossi *et al.*, 2019). Changes in weather and climate are associated with the occurrence, attack and spread of opportunistic invasive species (Anderson *et al.*, 2004; Backlund *et al.*, 2008; Kernan, 2015; Bellard *et al.*, 2016; Gallardo *et al.*, 2017), thus increasing the stress on affected ecosystems. Climate warming is affecting plant growth and development, as well as causing the extinction of many species of plants and animals (Moritz and Agudo, 2013). It is predicted that nearly 37% of species would become extinct by 2050 because of climate change (Thomas *et al.*, 2004) and up to 50% by 2080 (Warren *et al.*, 2013). This clearly illustrates that global climate change impacts the future of life on Earth negatively and directly addresses goal 6 of the astrobiology roadmap to understand the principles that will shape the

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future of life, both on Earth and beyond. To address the Earth's problems listed above, several paths must be taken. One potential path is to use the space environment to quickly obtain mutant stains that could help alleviate these problems and simultaneously be advantageous for space exploration.

Food security is a serious issue facing humanity. It is predicted that food demand will increase by 60% by 2050 (Breene, 2016). Intensive farming and poor soil management practices have destroyed the soil structure and composition in many areas, making it unsuitable for plant growth (Vitousek *et al.*, 2009; Borrelli *et al.*, 2017). It is essential to increase the efficiency of crops and food systems to reduce pressure on soil and the environment. With climate change, extreme climate events and the emergence of new pathogens, agriculture's long-term sustainability is questionable. Agricultural losses due to attacks by pests, pathogens and invasive species are a major threat to agricultural production (Oerke and Dehne, 2004; Vurro *et al.*, 2010; Savary *et al.*, 2019). The use of agrochemicals (pesticides and fertilizers) has significantly increased crop production globally (Gomiero, 2013; Popp *et al.*, 2013), and has also caused severe adverse effects to beneficial soil organisms (e.g. bacteria, fungi, worms, etc.), animals, humans and plant growth and nutritional values (Udeigwe *et al.*, 2015). Also, the extensive use of pesticides has led to the development of pesticide-resistant pests and pathogens (Gomiero, 2013). Furthermore, modern agriculture practices have significantly reduced the diversity of crops making agriculture more vulnerable to pests, pathogens and environmental stresses (e.g. water scarcity, high and low temperatures, increased salinity, etc.) (Vurro *et al.*, 2010; Dean *et al.*, 2012; Huang *et al.*, 2016; Savary *et al.*, 2019). For high and stable yield, crops require protection from biotic and abiotic stresses. Creating genetic diversity within crops can help restrain disease development, enhance yield, increase adaptability to abiotic factors and improve biodiversity (Schöb *et al.*, 2015; Newton, 2016; Savary *et al.*, 2019). For the sustainability of agriculture, it is important to consider the system's flexibility as a whole (holistic approach) and not adopt the reductionist strategy to improve tolerance by selecting traits (Newton, 2016). For example, the focus should be on developing strains that are productive as well as disease tolerant and resistant to pests, pathogens and other hostile environmental conditions, such as drought, extreme temperatures, soil salinity, etc.

The most relevant results for developing sustainable, robust organisms and biomaterials for agricultural and health sectors will come from new biological research methods. The conventional strategies have been, to date, to search for well-adapted and well-performing organisms (Pickens *et al.*, 2011; King *et al.*, 2016; Wohlleben *et al.*, 2016; Zhang *et al.*, 2018). We hypothesize that the simple and most redefining strategy would be to subject organisms to stress conditions that modulate evolutionary processes (recombination, mutation, epigenetic modification and gene transfer) and support the emergence of genetically diverse or new varieties of strains (Boto, 2010; Lynch, 2010; Nei, 2013; Lind and Spagopoulou, 2018; Prasad *et al.*, 2020). Environmental stressors, including both biotic (e.g. parasites, pathogens, pests, etc.) and abiotic factors (e.g. chemicals, temperature, light, other climatic factors, etc.), are viewed as selection elements and play a significant role in shaping the acclimation, adaptation and evolution of species (Bijlsma and Loeschcke, 1997; Hoffmann and Hercus, 2000; Badyaev, 2005; Parsons, 2005; Nevo, 2011). For example, the development of antibiotics, pesticide and heavy metal resistance in different organisms in response to the selection pressure exerted by these agents

(Davies and Davies, 2010; Lerminiaux and Cameron, 2019). The space environment consists of many unique stress factors that cause both mutagenic and/or non-mutagenic effects and can be exploited to induce evolutionary changes in cells and organisms (Horneck *et al.*, 2010; Prasad *et al.*, 2020).

This review focuses on the distinctive environment of space and discusses the potential to explore it to develop space-engineered products that can find application in the agri-food and health-pharma sectors on Earth. But these same space environmental stressors can also be used to explore their potential to develop products and processes applicable to space exploration now and in the future. The development of biological processes producing more efficient pharmaceutical and health products crucial for astronauts' health needs to be addressed. Drugs required aboard the International Space Station (ISS) are either brought along with the mission or resupplied regularly. While this may be possible on the ISS, it is impractical for a mission to Mars. The development of biological processes that produce drugs on demand is of tremendous value for realizing a human mission to Mars. We will not just go to Mars but eventually set up a permanent base on Mars, and to do that, we require to establish life-support systems that are robust with long lifetimes and needs few repairs – a technology that we currently do not have. However, we can build systems that are biological-based reactors, potentially using organisms engineered in the space environment to purify water by adapting forward osmotic systems (Flynn *et al.*, 2016) and air (Nelson and Bohn, 2011). In fact, microbial processes that will enhance food productivity that has been developed in space are uniquely adapted to improve food productivity on Earth. Last but certainly not least, all of what we have learned and are learning about the space environment on organisms is directly applicable to planetary protection (Craven *et al.*, 2021). The influence of space environment stress factors, mainly microgravity and radiation, that bring notable changes in cells or organismal systems are discussed in the review. Adopting the natural evolution process as a systemic, non-presumed approach for the production of sustained, robust organisms to overcome environmental obstacles is very tempting.

Space environmental factors inducing evolutionary changes

The hostile environment of space is characterized by factors that stress organisms in ways that cannot be mimicked on Earth, such as microgravity (weightlessness), intense ionizing radiation, high vacuum, pressure, space magnetic fields and extreme temperatures (Horneck *et al.*, 2010; Kennedy, 2014; Prasad *et al.*, 2020). These factors would not occur on Earth. Studies show that the space environment exerts distinct effects on genetics, mutation rate, gene expression, epigenetics, metabolite production and metabolic pathways and alters the shape, structure, function, physiology and growth behaviour of organisms. Thus, space can be explored as a 'casting tray' for the 'guided evolution' of species leading to the emergence of strains with novel properties (e.g. high yield, rapid growth, improved pathogen resistance, enhanced drought, disease tolerance, etc.) (Fig. 1) (Horneck *et al.*, 2010; Milojevic and Weckwerth, 2020; Prasad *et al.*, 2020). The two major space environmental features that are widely studied include microgravity and ionizing radiation. These space stressors, which cannot be duplicated on Earth, can be used for the evolution of strains of organisms that will be more robust and more productive than their respective wild types on Earth.
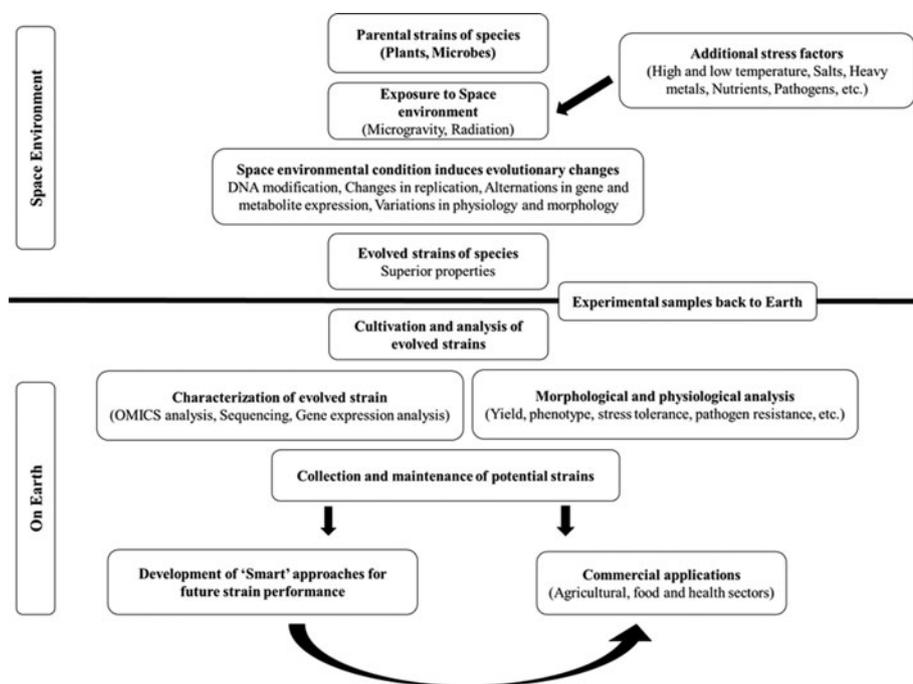
Fig. 1. Scheme of the utilization of space environmental conditions to support the emergence of species with novel characters. The unique space environment (microgravity and radiation) can be combined with abiotic and biotic stress factors to modulate novel evolutionary changes in species that cannot be achieved on Earth under the influence of gravity. These strains can find potential applications in food, agricultural and health sectors in a constantly changing and challenging environment. A systematic characterization of the naturally evolved strains on Earth will enlighten the underlying mechanisms of changes. In addition, 'Smart' strategies, including the engineering of gene regulatory networks by conventional breeding, mutagenesis and genetic engineering approaches and alteration of biological systems' function by external or internal means such as environmental stimuli, can be implemented to enhance strain performance further. The knowledge can be applied to develop other novel strains as well.

Microgravity

Microgravity is, in essence, the absence of gravity, which is one of the four basic forces in physics that leads to the acceleration of the mass. The physical environment is entirely different in microgravity than under $1 \times g$ (Todd, 1989; 1992; Horneck et al., 2010). Various physical phenomena are based on gravity's acceleration force, such as sedimentation, convection, hydrostatic pressure, etc. Under space microgravity conditions, sedimentation/stratification and convection are negligibly small since no acceleration force acts on solids, liquids and gases to separate them based on their density differences. This means that no buoyancy-driven convection occurs in liquids or gases under microgravity, whereas the constant exchange of volume occurs under $1 \times g$. Due to the lack of convection in microgravity, metabolites' transport is reduced to the range (and rate) of diffusion. There is also no hydrostatic pressure in a water column in microgravity because hydrostatic pressure occurs due to acceleration forces. Also, shear forces are strongly reduced in microgravity because moving or rotating objects no longer work against acceleration vectors.

Microgravity can produce drastic effects on cell systems. For example, the fluidity of cell membranes is increased in microgravity (Sieber et al., 2014) that affects membrane proteins' functionality, such as membrane channel conductance (Sieber et al., 2016; Kohn et al., 2017). Due to the increase in membrane fluidity, drugs' pharmacokinetic (incorporation of amphipathic drugs into membranes) are changed in microgravity (Kohn and Hauslage, 2019). It is also observed that the bacterial virulence, pathogenicity and resistance to antibiotics increase in space microgravity conditions (Wilson et al., 2008). Thus, space's unique microgravity environment can be used to understand microbes' physiology and pathogenesis and develop novel treatment strategies (Amselem, 2019).

Gravity cannot be eliminated on Earth to perform experiments without its influence. There are several methods of achieving simulated microgravity conditions for scientific experiments that differ in duration, frequency and level of gravity, as well as on the type of research (Sabbatini, 2014; Amselem, 2019; Prasad et al., 2020). Several devices providing simulated microgravity conditions have been developed with an advantage in cost, easy accessibility and reproducibility. Examples include 2D- or 3D-clinostat, random positioning machines, rotating wall vessels, rotating-wall bioreactor, rotary cell culture system, etc. (Horneck et al., 2010; Prasad et al., 2020). However, none of these methods provide real microgravity conditions similar to space since gravity cannot be shielded or reduced. Real microgravity can only be achieved under free-fall conditions. Platforms, such as drop towers, sounding rockets, aircraft flying parabolic manoeuvres, spacecraft (research satellites) and the ISS are utilized for microgravity research (Pletser, 2004; Ruyters and Friedrich, 2006; Baglioni et al., 2007; Horneck et al., 2010; Böhmer and Schleiff, 2019). The duration of sample preparation, exposure and recovery significantly differs among the various platforms used to achieve real microgravity conditions. Depending upon the type of platform used, microgravity values between 10^{-3} and $10^{-6}g$ can be achieved (Horneck et al., 2010; Böhmer and Schleiff, 2019). Also, depending upon the method, the degree of automatization varies. During spaceflights, test samples encounter a complex environment consisting of acceleration, microgravity, vibration, radiation and electromagnetism, among other environmental stresses, which are impossible to simulate completely on the ground (Horneck et al., 2010; Taylor, 2015).

Space ionizing radiation

Among space stress factors, ionizing radiation is probably the primary constraint for organisms' growth and human-crewed space missions (Kennedy, 2014; Furukawa et al., 2020). Space radiation is composed of galactic cosmic radiation, solar cosmic radiation and radiation trapped by Earth's magnetosphere (van Allen belts) (Ohnishi and Ohnishi, 2004). Galactic cosmic radiation is produced by the Sun and supernova remnants. It consists of 87% high-energy protons, 12% α-particles and 1% heavier nuclei

(Horneck *et al.*, 2010; Kennedy, 2014; Li *et al.*, 2018). The heavier nuclei comprise high-charge and high-energy particles called HZE particles (*H*igh charge *Z* and high *E*nergy). The solar cosmic radiation consists of low solar wind particles to highly energetic charged particles (up to several GeV) originating from solar energy (Horneck *et al.*, 2010; Kennedy, 2014; Li *et al.*, 2018). The solar energy particle events arise from the sun's magnetically disturbed regions and generally last for a few hours. About 92% protons, 6% helium ions and 6% HZE ions make up these highly energetic particles. The Earth's magnetic field provides latitude-dependent protection against solar particle events. The galactic and solar cosmic radiation trapped within the Earth's magnetic field and atmosphere form the van Allen belt, consisting of electrons, protons and some heavier particles (Horneck *et al.*, 2010). The inner belt particles emerge from the decay of neutrons produced in cosmic particle interactions with the atmosphere, while the outer belt mainly consists of trapped solar particles. The average dose of radiation aboard the ISS is about 180 μGy day^{-1} (Goossens *et al.*, 2006; Vanhavere *et al.*, 2008), which is up to 80-fold higher than the natural radiation levels on Earth.

The impact of space radiation on organisms is an essential aspect of study for space missions. The biological effects of radiation depend upon the quality and quantity of radiation, exposure duration, the quality of the inflicted damage and characteristics and developmental stage of the cell/tissue/organism (Horneck *et al.*, 2010; de Micco *et al.*, 2011; Matallana-Surget and Wattiez, 2013). The significant effect of space ionizing radiation (γ-ray, X-ray, β-ray, α-radiation and neutron radiation) is DNA damage (single- or double-strand breaks), which activates cellular responses leading to cell cycle arrest and appropriate DNA repair mechanisms (base-excision repair, homologous recombination or non-homologous end-joining repair) depending on the nature of the damage (Zhou and Elledge, 2000). The HZE particles have high linear energy transfer (LET) values (> 10 keV μm^{-1}) and are considered as the main component of radiation-induced cellular damages (complex, difficult-to repair DNA damages) and health risks to astronauts (Cucinotta *et al.*, 2001; Nikjoo *et al.*, 2001; Cucinotta and Durante, 2006; Friedland *et al.*, 2011; Arena *et al.*, 2014). The LET values of electromagnetic ionizing radiations (X-rays and γ-rays) are relatively lower than high LET radiations (α particles, neutrons and protons) but have a high penetration power into matter (Reisz *et al.*, 2014). The biological damages produced by the X-rays and γ-rays are lower than those induced by the same dose of high-LET radiations. The significant cellular effects produced by X- and γ-rays is due to the generation of reactive oxygen species (ROS) (H_2O_2, $O_2\bullet^-$ and $\bullet$OH) and other reductants ($H\bullet$ and e_{aq-}) (Reisz *et al.*, 2014; Desiderio *et al.*, 2019). These highly reactive agents cause DNA breaks, protein fragmentation, protein structure deterioration and cell membrane function and permeability changes.

Several experiments have been performed in space aboard space stations and different satellites as well as on Earth to study the effects of cosmic radiation on cells/tissue/organisms from the perspective of manned space missions (Horneck *et al.*, 2010; Arena *et al.*, 2014; Furukawa *et al.*, 2020). Different facilities simulating space-like radiation are available to evaluate ionizing radiation's biological effects (Furukawa *et al.*, 2020). However, space ionizing radiation cannot be simulated to its full complexity on Earth (Moissl-Eichinger *et al.*, 2016). In space, radiation may act independently or synergistically with other stress factors, such as microgravity, space vacuum and low shear forces,

among others, to produce significant effects on cells/tissue/organisms.

Experiments in space and ground-based space simulation facilities

The first space experiments, mostly done by Russian scientists in the 1960s, revealed that microgravity does not produce any considerable effects on living organisms (Zhukov-Verezhnikov *et al.*, 1962; Montgomery *et al.*, 1978; Tairbekov *et al.*, 1983). However, further experiments in the space environment showed that space-specific conditions change the biological properties of cells. Changes in genetics, gene expression, metabolite production and function, and morphology, growth and behaviour of cells/organisms have been observed in response to the space environment and space analogue conditions. It is proposed that microgravity primarily alters cell growth kinetics and behaviour, while radiation contributes to increased mutation rates (Taylor, 2015; Senatore *et al.*, 2018).

Effect of microgravity on microorganisms

Many microorganisms can survive and thrive in extreme environments, including space hostile environment (Bronikowski *et al.*, 2001; Benoit *et al.*, 2006; Horneck *et al.*, 2010; Vaishampayan *et al.*, 2012; Aunins *et al.*, 2018; Huang *et al.*, 2018). A diverse range of microorganisms has been sent into space to study the effects of the space environment (Taylor, 1974; Juergensmeyer *et al.*, 1999; Kacena *et al.*, 1999a; Nickerson *et al.*, 2004; Clément and Slenzka, 2006; Wilson *et al.*, 2007; 2008; Horneck *et al.*, 2010; Crabbé *et al.*, 2011; 2013; Rosenzweig and Chopra, 2012; Kim *et al.*, 2013a; 2013b; Huang *et al.*, 2015). Also, several experiments have been performed in simulated microgravity conditions on Earth to study the response of different microorganisms (Nickerson *et al.*, 2000; Baker *et al.*, 2004; Chopra *et al.*, 2006; Tucker *et al.*, 2007; Altenburg *et al.*, 2008; Gao *et al.*, 2011; Searles *et al.*, 2011; Pacello *et al.*, 2012; Arunasri *et al.*, 2013; Lawal *et al.*, 2013; Rosenzweig *et al.*, 2014; Soni *et al.*, 2014; Huang *et al.*, 2015; Xu *et al.*, 2015; Kim *et al.*, 2016). Both real and simulated microgravity conditions have been shown to influence microbial growth, physiology, virulence, pathogenesis, antibiotic resistance, stress tolerance and secondary metabolite production (Lapchine *et al.*, 1986; Nickerson *et al.*, 2000; Ilyin, 2005; Horneck *et al.*, 2010; Mermel, 2012; Kalpana *et al.*, 2016; Senatore *et al.*, 2018; Tirumalai *et al.*, 2019).

A significant number of studies have investigated the growth response of different microorganisms, including bacteria, fungi, archaea and microalgae, to real and simulated microgravity conditions. Studies with *Escherichia coli* (Bouloc and D'Ari, 1991; Thévenet *et al.*, 1996; Kacena *et al.*, 1997; 1999b; Brown *et al.*, 2002; Benoit and Klaus, 2005; Tucker *et al.*, 2007; Arunasri *et al.*, 2013; Tirumalai *et al.*, 2017), *Bacillus subtilis* (Kacena *et al.*, 1997; 1999b), *Salmonella typhimurium* (Wilson *et al.*, 2002a), *Pseudomonas aeruginosa* (England *et al.*, 2003; Kim *et al.*, 2013a; 2013b), *Staphylococcus* (Rosado *et al.*, 2010), *Streptococcus* (Allen *et al.*, 2007; Orsini *et al.*, 2017), *Streptomyces* (Fang *et al.*, 1997c; 2000), *Saccharomyces cerevisiae* (Purevdorj-Gage *et al.*, 2006; van Mulders *et al.*, 2011), *Candida albicans* (Altenburg *et al.*, 2008; Crabbé *et al.*, 2013; Jiang *et al.*, 2014), *Deinococcus radiodurans* (Ott *et al.*, 2019), *Haloarchaea* (Dornmayr-Pfaffenhuemer *et al.*, 2011; Thombre *et al.*, 2017), *Euglena* (Häder *et al.*, 2005; Nasir *et al.*, 2014; Krüger *et al.*,

2019), *Chlamydomonas* (Yoshimura *et al.*, 2003; Häder *et al.*, 2005), etc., have been conducted. Most studies have reported reduced lag phase and increased cell population across several bacterial species in the space environment (Benoit and Klaus, 2007; Horneck *et al.*, 2010; Huang *et al.*, 2018). The differences in growth rate are attributed to the type of cell and species used, the property of cell motility, culture conditions (suspension and solid-state agar culture), concentrations of medium nutrient (high and low), the extracellular microenvironment, the type of bioreactors used inside the spacecraft and microgravity conditions (Klaus *et al.*, 2004; Horneck *et al.*, 2010; Taylor, 2015; Huang *et al.*, 2018). Also, it is proposed that the indirect physical effects of microgravity (viz. fluid dynamics, extracellular transport) have a significant influence on bacterial cell growth rather than the direct impact during spaceflight (Horneck *et al.*, 2010; Huang *et al.*, 2018). In microgravity, decreased mass diffusion, low shear forces and changes in fluid dynamics alter a cell's microenvironment influencing growth and physiology. The direct effect of microgravity on cell growth is mainly observed in higher organisms that possess unique molecules or organelles to sense gravity (Limbach *et al.*, 2005; Horneck *et al.*, 2010; Huang *et al.*, 2018; Nasir *et al.*, 2018; Prasad *et al.*, 2020).

A significant knowledge gap remains regarding the specific mechanisms involved in altering virulence, pathogenesis and resistance to antibiotics of pathogens in the microgravity environment. Based on studies conducted to date, it is speculated that global regulatory signals may control these change (Wilson *et al.*, 2002b, 2007; Nickerson *et al.*, 2003; Crabbé *et al.*, 2011; Aunins *et al.*, 2018; Ott *et al.*, 2020). Studies have shown the involvement of the global transcriptional regulator Hfq, a RNA-binding protein, in the induction of virulence-related genes in pathogenic bacteria (e.g. *P. aeruginosa, S. typhimurium*) in spaceflight or spaceflight analogue conditions (Wilson *et al.*, 2007; Crabbé *et al.*, 2011; Ott *et al.*, 2020). In a low-shear modelled microgravity (LSMMG) environment experiment, Wilson *et al.* (2002b) observed the differential expression of 163 genes representing functionally diverse groups (transcriptional regulators, virulence factors, lipopolysaccharide biosynthetic enzymes, iron-utilization enzymes and proteins of unknown function) and discovered the role of ferric uptake regulator (Fur) in the regulation of virulence, stress resistance and protein expression in *Salmonella enterica* in microgravity. Nickerson *et al.* (2000) found *S. enterica* serovar Typhimurium cells grown in simulated microgravity more virulent and resistant to acid stress and macrophage killing than normal-gravity-grown cells. Higher cell counts in murine spleen and liver and significant differences in protein synthesis were also found in simulated microgravity (Nickerson *et al.*, 2000). They indicated that the microgravity environment regulates the expression of genes and proteins, contributing to these effects in *S. enterica*. Alteration of gene and protein expression was also found in bacteria that showed higher virulence and better adherence to human cells under simulated microgravity conditions than in normal $1 \times g$ (Poudrier, 2003). Morphological alterations can contribute to microbial pathogenicity. Induction of cell aggregation, clumping and extracellular matrix formation have been observed in virulent strains of *S. typhimurium* cultivated in spaceflight (Wilson *et al.*, 2007). Also, the increased frequency of filamentous forms contributing to virulence has been noted in *C. albicans* under microgravity conditions (Altenburg *et al.*, 2008).

Very recently, Gilbert *et al.* (2020) found that *Serratia marcescens* was more lethal to *Drosophila melanogaster* on the ISS than in ground-based controls and suggested that spaceflight induces a physiological change leading to increased virulence in the pathogen. In contrast, the virulence of clinical pathogens (*Listeria monocytogenes*, methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecalis* and *C. albicans*) against larvae and adult *Caenorhabditis elegans* reduced in the space environment (Hammond *et al.*, 2013). No effect on the virulence of MRSA and *Listeria* against both adult and larvae was noted in simulated microgravity conditions (clinorotation), while *Candida* and *Enterococcus* were less virulent for larvae, but not adult worms of *C. elegans*. These data suggest that real and simulated microgravity environments alter the interactions between host and virulent bacteria.

An increase in resistance to antibiotics and various stresses, such as acid, thermal and osmotic, is also observed in a real and simulated space environment (Leys *et al.*, 2004; Klaus and Howard, 2006; Horneck *et al.*, 2010; Taylor, 2015; Thombre *et al.*, 2017; Aunins *et al.*, 2018). Higher concentrations of antibiotics are required to inhibit bacterial growth in microgravity. This is probably due to the lower transport rate (mass transport is limited to diffusion) and changes in cell proliferation, membrane permeability, rate and morphology (Klaus *et al.*, 1997, 2004; Kacena and Todd, 1999; Sieber *et al.*, 2014; Zea *et al.*, 2016, 2017; Kohn *et al.*, 2017; Aunins *et al.*, 2018; Kohn and Hauslage, 2019). In a study with the halophilic archaeon, *Haloarcula argentinensis* under simulated microgravity condition (clinostat), an increase in the activity of antibiotic efflux pumps resulting in the development of multi-drug resistance to antibiotics, such as augmentin, norfloxacin, tobramycin and cefoperazone was observed (Thombre *et al.*, 2017). Studies have also indicated the involvement of global regulators (*hfq* and *rpoS*) in cross-resistance to antibiotics in *Salmonella* and *E. coli* under microgravity conditions (Wilson *et al.*, 2002a; Lynch *et al.*, 2004; Horneck *et al.*, 2010).

Increases in antibiotic resistance have also been observed in some microorganisms and have been associated with mutations of genes in the space environment (Fajardo-Cavazos and Nicholson, 2016; Fajardo-Cavazos *et al.*, 2018; Tirumalai *et al.*, 2019). Dramatic differences in the spectrum of mutations in the regulator gene *rpoB* leading to rifampicin-resistance (Rif[R]) have been observed in *B. subtilis* and *Staphylococcus epidermidis* cultivated in the ISS compared to ground controls, indicating the mutagenic potential of spaceflight environment (Fajardo-Cavazos and Nicholson, 2016; Fajardo-Cavazos *et al.*, 2018). The frequency of mutation to Rif[R] in *S. epidermidis* cells flown aboard the ISS was observed to be significantly higher (24-fold) than ground controls (Fajardo-Cavazos and Nicholson, 2016). In a recent study, Tirumalai *et al.* (2019) noted mutations in multiple genes associated with antibiotic resistance (*ompF*, *acrB*, *marR* and *mdfA*) in *E. coli* cells grown under LSMMG for over 1000 generation leading to resistance against chloramphenicol, cefalotin, cefuroxime, cefuroxime axetil, cefoxitin and tetracycline. The resistance was stable in successive generations grown under normal gravity conditions (Tirumalai *et al.*, 2019). Authors have linked the spread and increase of virulence and antibiotic resistance in spaceflight conditions to gene transfer events as well (Timmery *et al.*, 2011; Senatore *et al.*, 2018; Tirumalai *et al.*, 2019). Efficient gene transfer events have been noticed in the Gram-positive bacteria *Bacillus* sp. in spaceflight compared to ground controls, whereas no differences have been observed in the Gram-negative species *E. coli* and *Cupriavidus metallidurans* (de Boever *et al.*, 2007). Changes in conjugal transfer rates and phage induction have also been noted under space microgravity conditions (Mattoni, 1968; Ciferri *et al.*, 1986;

Alpatov *et al.*, 1989). Recently, Aunins *et al.* (2018) suggested that microgravity-induced stress response genes also contribute to the increased resistance of bacteria to antibiotics in space conditions.

Overexpression of stress response genes and proteins have been observed in bacteria and fungi under simulated and space microgravity conditions (Leys *et al.*, 2009; Mastroleo *et al.*, 2009, 2013; van Mulders *et al.*, 2011; Nicholson *et al.*, 2012; Vukanti and Leff, 2012). In a study, Vukanti and Leff (2012) observed the over-expression of stress response genes (viz. *csiD*, *cstA*, *katE*, *otsA* and *treA*) in *E.coli* cells grown in simulated microgravity (clinorotation) compared to $1 \times g$ controls. Proteins involved in response to carbon limitation and oxidative stress were differentially expressed in *C. metallidurans* grown aboard the ISS (Leys *et al.*, 2009). Orsini *et al.* (2017) reported up- and down-regulation (both $\geq$2-fold) of 153 and 94 genes, respectively, in *Streptococcus mutans* grown under simulated microgravity conditions. Among others, genes of extrachromosomal elements, carbohydrate metabolism, translation and stress responses were affected (Orsini *et al.*, 2017). Investigations have shown the induction of various ROS scavengers (e.g. superoxide dismutase (SOD)) and redox-active proteins (e.g. thioredoxin, catalase, peroxiredoxin, thiol peroxidase, sulfoxide reductase MsrA) in space-exposed *Bacillus* spores, *E. coli*, *Rhodospirillum rubrum* and *S. typhimurium*, indicating the up-regulation of antioxidant defence mechanisms to cope with the space-induced oxidative damage occurring during long-term spaceflight (Wilson *et al.*, 2008; Mastroleo *et al.*, 2009; Nicholson *et al.*, 2012; Vaishampayan *et al.*, 2012). Increased synthesis of protective carotenoids has been reported in simulated-microgravity-exposed *H. argentinensis* against the oxidative damage caused by the harmful effects of stress (Thombre *et al.*, 2017). Studies also show that cells activate pathways similar to those induced during osmotic stress and produce osmoprotective compounds (e.g. compatible solutes) to increase osmotolerance and survive in the space environment (Li *et al.*, 2015; Zhang *et al.*, 2015; Milojevic and Weckwerth, 2020).

Noticeable effects on the growth kinetics, spores, mycelia formation and morphogenic switches have been noted in the fungal pathogen *C. albicans* under modelled microgravity conditions (rotary cell culture system) as well (Jiang *et al.*, 2014). Differential regulation of genes involved in transcriptional regulation, ABC transport, cell aggregation and resistance against antifungals and oxidative stress, among others, have been observed in spaceflight-subjected *C. albicans* compared to ground controls (Crabbé *et al.*, 2013). Genes were found to be differentially expressed. Also, increased random budding was noted in spaceflight-cultured cells compared to bipolar budding in $1 \times g$ samples (Crabbé *et al.*, 2013). Random budding and induction of stress-responsive proteins involved in stress/protein folding and oxidative stress have also been detected in *S. cerevisiae* in a space mission (Soyuz TMA-9) (van Mulders *et al.*, 2011). In contrast to bacteria, the SOD and peroxiredoxin were found to be down-regulated in *S. cerevisiae*, indicating a shift of the culture towards anaerobiosis in microgravity (van Mulders *et al.*, 2011). The up-regulation of genes involved in anaerobic metabolism has also been observed in *P. aeruginosa* during spaceflight (Crabbé *et al.*, 2011).

Microbial biofilm formation is also reported to be altered in microgravity. Increased number of viable cells, thick and higher biomass biofilms, differences in biofilm-forming extracellular polymeric substances, cell aggregation and cell clumping, and differential expression of genes involved in cell aggregation and biofilm formation are observed in different bacterial biofilms in microgravity (Wilson *et al.*, 2007; Crabbé *et al.*, 2011, 2013; Kim *et al.*, 2013b). Transcriptome analysis showed that spaceflight conditions caused up-regulation of biofilm-related genes in *B. subtilis* (Morrison *et al.*, 2019). Space-exposed cells of the human pathogen *Klebsiella pneumoniae* exhibited increased biofilm formation, which is an essential characteristic of virulence (Li *et al.*, 2014). Increased tolerance to salt and ethanol treatments and antibiotic resistance has also been reported in microbes of biofilms under microgravity conditions (Lynch *et al.*, 2006; Tucker *et al.*, 2007).

The extracellular environmental signals and stress factors, like heat, nutrients, chemicals, osmotic stress and shear stress, greatly influence the production of secondary metabolites in microbes (Gao *et al.*, 2001, 2011; Viollier *et al.*, 2003; Bibb, 2005). Considering the immense impact of space environmental conditions on microbial growth and physiology, several research groups investigated the production of secondary metabolites of commercial interest under stressful microgravity conditions (Table 1) (Horneck *et al.*, 2010; Huang *et al.*, 2018; Prasad *et al.*, 2020). Very interesting and diverse results of secondary metabolites yields (e.g. antibiotics, bioactive compounds, anti-tumour agents, vaccines, immunosuppressant, etc.) have been reported (Lam *et al.*, 1998, 2002; Demain and Fang, 2001; Horneck *et al.*, 2010; Gao *et al.*, 2011; Huang *et al.*, 2018). An increase in the antibiotic monorden's specific productivity by *Humicola fuscoatra* WC5157 has been found in solid-state fermentation carried on T8 and PG agar media aboard the Space Shuttle mission STS-77 (Lam *et al.*, 1998). The production of monorden in the T8 and PG agar medium increased up to 30 and 190%, respectively, in spaceflight conditions compared to ground control (Lam *et al.*, 1998). The group has also reported an increased production of the anticancer agent actinomycin D by *Streptomyces plicatus* WC56452 grown in defined and complex media under dark, anaerobic and thermally controlled (20 °C) conditions onboard the Space Shuttle mission STS-80 (Lam *et al.*, 2002). In the study by Benoit *et al.* (2006), increased production of actinomycin D by *S. plicatus* was noted only on days 8 and 12 during the space mission, while decreased production was noted in all subsequent sample days (4 days interval for 72 days) compared to the ground controls. In a study, Huang *et al.* (2015) investigated the growth, morphological and secondary metabolic responses of *S. coelicolor* exposed to simulated (2D-clinostat) and real (Shenzhou-8 space mission) microgravity conditions. They observed morphological and secondary metabolite production changes and increased bacteriostatic activity of the culture against *B. subtilis* in microgravity environments, indicating that *S. coelicolor* A3(2) produces more bioactive substances under microgravity conditions. In the study of Luo *et al.* (1998), the antifungal agent nikkomycin production by space-exposed strains of *Streptomyces ansochromogenus* (15 days of spaceflight aboard a satellite) was found to be increased by 13–18%. Enhanced production of the toxin microcystin by the cyanobacteria *Microcystis aeruginosa* under simulated microgravity conditions has also been demonstrated (Xiao *et al.*, 2010).

Investigations under LSMMG conditions have shown decreased production of metabolites, such as the β-lactam antibiotic (cephalosporin C) and polyketide macrolide (rapamycin) by *Streptomyces* sp. (Fang *et al.*, 1997c, 2000). In another study, Fang *et al.* (1997a) demonstrated a decreased production of the peptide antibiotic microcin B17 by *E. coli* ZK650 under LSMMG condition (high aspect rotating vessel (HARV)). Also, cultivation in the HARV led to extracellular production of

Table 1. Examples of observed changes or products of commercial interest derived by exposing microbes and plants to the real space environment

Organism(s)	Observed changes/products in the real space environment	Reference
Microorganisms		
Streptomyces coelicolor A3	Changes in secondary metabolite production and morphology; production of bioactive (antibacterial) substances	Luo *et al.* (1998)
Streptomyces fradiae	Increased production of the antibiotic tylosin	Fang *et al.* (2005)
Streptomyces plicatus	Increased actinomycin D production after 17 days in space	Lam *et al.* (2002)
Streptomyces avermitilis	Increase of avermection production after 40 h in space (Shenzhou VII-mission)	Gao *et al.* (2011)
Streptomyces plicatus	Increased actinomycin D production onboard the ISS	Benoit *et al.* (2006)
Humicola fuscoatra WC5157	Increased production of monorden onboard the Space Shuttle mission STS-77	Lam *et al.* (1998)
Nocardia mediterranei var. kanglensis 1747-64	Increased production of the immunosuppressant Kanglemycin C after 6 days of space exposure (Shenzhou III)	Zhou *et al.* (2006a, 2006b)
Salmonella	Development of new vaccines against diarrhoea-causing *Salmonella* strains aboard the ISS	Horneck *et al.* (2010)
Plants		
Rice	The space environment induces heritable epigenetic changes and activation of transposable elements	Ou *et al.* (2010)
Seeds of more than 140 plant species, including field crops, flowers, vegetables, fruits, medicinal plants and forest trees	Chinese 'Space-mutation breeding'-program from 1987 to 2005. According to the reports, >200 promising varieties were determined with superior quality, yield and disease resistance. More than 50 strains are successfully planted on large scales bringing socio-economic benefit	Xianfang *et al.* (2004), He *et al.* (2006), Liu *et al.* (2008)
Tomato	Mutants of strain CK produced by space mutation breeding induced more pronounced growth inhibition as well as an increase in apoptosis of colon cancer cells compared to the parent strain	Shi *et al.* (2010)
Seeds of licorice (*Glycyrrhiza uralensis*)	Increase in glycyrrhizic acid and liquiritin in root extracts after exposure to the space environment (18 days, Shenzhou)	Zhang *et al.* (2011)
Glycyrrhiza uralensis	Changes in secondary metabolites quantity in rhizomes grown from seeds after exposure to space	Dong *et al.* (2012)
Robinia pseudoacazia	Variation in morphological traits and a significant increase in the mutation rate in plants grown from 15-day space-exposed seeds	Yuan *et al.* (2012)
Acer Mono Maxim	Genetic diversities and variations in 15-day space-exposed seedlings	Sun *et al.* (2015)

microcin compared to the intracellular production in control shake flasks. de Gelder *et al.* (2009) demonstrated increased production of poly-β-hydroxybutyrate by *C. metallidurans* LMG 1195 after 24 h of cultivation under LSSMG conditions but reduction after 48 h of culture compared to $1 \times g$ controls, indicating fluctuation of metabolite production in microgravity. No simulated-microgravity-related changes in the production of secondary metabolites (e.g. gramicidin S) have also been reported (Fang *et al.*, 1997b).

The variation in microbial secondary metabolite production (increased, decreased, fluctuate over time or uninfluenced) under microgravity conditions is mainly attributed to differences in cell strains, medium, fermentation processes and conditions and extracellular microenvironment, as well as microgravity-related physical effects on fluid dynamics, secretion and transport of metabolites (Benoit *et al.*, 2006; Horneck *et al.*, 2010; Huang *et al.*, 2018).

Collectively, studies have provided substantial evidence on the effect of microgravity on microbial growth, behaviour, virulence, pathogenicity, resistance and metabolite production. Future experiments in space and space analogue conditions employing comparative omics techniques (genomics, transcriptomics, proteomics and metabolomics) and bioinformatics tools will further

enhance our understanding of the molecular mechanisms of microgravity-induced alterations in microorganisms. In-depth knowledge of space-induced changes can be utilized to engineer microbes with new properties and develop new approaches to produce antibiotics and other metabolites of commercial interest in terrestrial facilities more efficiently, as well as establish novel treatment strategies for preventing and controlling infectious diseases on Earth (Benoit *et al.*, 2006; Horneck *et al.*, 2010; Aunins *et al.*, 2018; Milojevic and Weckwerth, 2020).

Effect of microgravity on plants

Whole plants and their parts (organs, tissues, cells and protoplast cultures) have been studied in several space experiments onboard satellites, space shuttles and orbital stations, as well as under simulated microgravity conditions on Earth to elucidate gravity-related changes. These experiments demonstrate that changes in gravity (microgravity and hypergravity) affects the growth, development and phenotype of plants and enhance our understanding of cell-division processes, gravity perception, signal transduction and gravity-induced distribution of organelles in plants (Kiss, 2000; Ferl *et al.*, 2002; Hemmersbach and Braun, 2006; Stutte

et al., 2006; Paul *et al.*, 2012a, 2012b; Correll *et al.*, 2013; Kordyum and Chapman, 2017).

Morphological, anatomical and histological changes have been observed in plants grown in the space environment. *Arabidopsis* grown on the ISS was found to be smaller with abnormal root growth than $1 \times g$ controls (Millar *et al.*, 2011; Paul *et al.*, 2012a). *Arabidopsis* seedlings germinated and grown under real microgravity in space were reported to show a significant decrease in ribosome production (Matía *et al.*, 2010). Compared to $1 \times g$-samples, cell proliferation rate increased, but cell growth decreased. The authors suggest that microgravity affects cell cycle regulation and leads to a shortened G2 period (Matía *et al.*, 2010). Leaf ultrastructure and chloroplast shape were also found to be different in dwarf wheat grown under microgravity (Stutte *et al.*, 2006). Changes in the actin cytoskeleton organization involved in transporting cell wall components to the cell periphery have also been noted under microgravity conditions (Correll *et al.*, 2013). In a study by Kamal *et al.* (2018), alteration in the cell cycle (lower fraction of cells in G1-phase), levels of proteins involved in cell-cycle regulation and nucleolus and fibrillar structures were observed under simulated microgravity or Martian gravity.

Significant changes in the transcriptome and proteome of *Arabidopsis* under microgravity conditions are recorded (Martzivanou and Hampp, 2003; Martzivanou *et al.*, 2006; Paul *et al.*, 2012b, 2013; Correll *et al.*, 2013; Hausmann *et al.*, 2014; Fengler *et al.*, 2015, 2016; Schüler *et al.*, 2015). Among others, genes involved in reactive oxygen pathways, stress response, cell wall remodelling, diverse metabolic pathways and calcium signalling were found to be differentially expressed. Studies with *Arabidopsis* callus cells also show that acceleration or microgravity induces changes in gene expression, protein translation and modification and metabolites production (Martzivanou and Hampp, 2003; Babbick *et al.*, 2007; Barjaktarović *et al.*, 2009a, 2009b; Hausmann *et al.*, 2014; Neef *et al.*, 2015). Mainly, changes in proteins involved in primary metabolism, carbohydrate metabolism (e.g. glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate-isomerase), signalling, radical scavenging, detoxification of ROS and chaperone functions (HSP70) were found (Barjaktarović *et al.*, 2009b). Both short- and long-term microgravity exposure influences the expression of genes of ROS-related enzymes, such as ascorbate and glutathione peroxidases, calcium-dependent signalling, stress response and detoxification (Salmi and Roux, 2008; Barjaktarović *et al.*, 2009a, 2009b; Salmi *et al.*, 2011; Paul *et al.*, 2012b, 2013; Hausmann *et al.*, 2014).

Alterations in expression of genes involved in detoxification, biotic-induced stress, water stress, oxidative stress, salt stress and other stress responses have also been observed in *Arabidopsis thaliana* seedlings (Paul *et al.*, 2012b, 2013; Correll *et al.*, 2013). Changes in genes coding for proteins involved in pathogen response, touch-wounding response, stress response (cold and drought stress), cell wall remodelling, growth, auxin-mediated root development, signal transduction, etc., were found in seedlings exposed to the space environment. Several experiments have shown the effects of acceleration changes on protein synthesis and gene expression in other plants as well (*Ceratopteris richardii*, *Solanum lycopersicum*, dwarf wheat and *Oryza sativa*) (Stutte *et al.*, 2006; Salmi and Roux, 2008; Bushart *et al.*, 2013; Jin *et al.*, 2015). These studies observed alteration in genes involved in the cell wall structure, primary metabolism, transcription, calcium regulation and physiological and metabolic processes (protein kinases, oxidoreductase, transferases, hydrolases, etc.), among others.

Studies have shown that altered gravity produces significant epigenetic changes in plants (Kamal *et al.*, 2018; Xu *et al.*, 2018; Zhou *et al.*, 2019). In a recent study, Zhou *et al.* (2019) found that 20% of the protein-coding genes to be differentially methylated in leaves of *Arabidopsis* subjected to real microgravity conditions aboard the ISS. Determination of DNA methylation in *Arabidopsis'* cell culture showed that artificial microgravity and Martian gravity increased DNA methylation levels (Kamal *et al.*, 2018). In the study by Ou *et al.* (2010), both genetic changes and hypo- and hyper-methylation (CG and CNG) were observed in rice plants grown from space-flown seeds. The frequency of genetic changes ranged from 0.7 to 6.7% across investigated plants, while hypomethylation frequency was found to be 0.76% (CG) and 0.80% (CNG). The hypermethylation was 1.95% (CG) and 1.44% (CNG) (Ou *et al.*, 2010). Analysis of *Arabidopsis* seedlings flown onboard of an SJ-10 recoverable satellite for 60 h revealed that among others, genes involved in metabolic pathways, biosynthesis of secondary metabolites and starch and sucrose metabolism were methylated in microgravity (Xu *et al.*, 2018), suggesting epigenetic modifications in plants under space environment.

Available literature data strongly indicate that the space environment can also induce heritable and stable DNA changes in plants (both positive and negative mutations), resulting in genetically diverse strains (Table 1) (Cowles *et al.*, 1994; Dutcher *et al.*, 1994; Nechitailo *et al.*, 2005; He *et al.*, 2006; Liu *et al.*, 2008; Gao *et al.*, 2009; Wu *et al.*, 2010; Chengzhi, 2011; Dong *et al.*, 2012; Yuan *et al.*, 2012). In the course of a 'Space-mutation breeding' program, Chinese scientists sent seeds of crops, floriculture, vegetable, fruits, medicinal plants and forest trees to space (Xianfang *et al.*, 2004; He *et al.*, 2006; Liu *et al.*, 2008). According to He *et al.* (2006) report, more than 200 varieties of plants exhibiting promising properties, such as high yield, resistance to pathogens and good quality, were obtained from the space-exposed seeds. A remarkable increase in potency and germination rate was found in space-mutated seeds of wheat, maize, barley, triticale, soybean, sunflower, cucumber, tomato and cotton, while no significant difference was noted in rice, pea, millet, lettuce, sweet pepper and tobacco. The seed germination rate was also found to be reduced in seeds of sorghum, watermelon, eggplant, radish and towel gourd (Dutcher *et al.*, 1994; Liu *et al.*, 2008). These indicate that the effect of spaceflight differs between different species or strains of plants. Flowering plants (purple shamrock, scarlet sage, garden petunia, orchids) with brighter flowers, unpredictable flower colour pattern and more extended flowering periods and more tolerant to insects and pathogens as well as vegetables (chili pepper, cucumber, eggplant, tomato and lotus) with higher yields and contents of vitamins and elements have been developed through the space-induced mutation breeding strategy (Li *et al.*, 1999; Yan *et al.*, 2002; Huai *et al.*, 2005; He *et al.*, 2006; Liu *et al.*, 2008). China has officially approved several new varieties of crops, including rice, wheat, pepper, tomato, alfalfa, sesame and cotton, developed by space breeding techniques and successfully planted on large scales (He *et al.*, 2006; Liu *et al.*, 2008).

Yuan *et al.* (2012) observed higher levels of genetic diversity as determined by simple sequence repeat and sequence-related amplified polymorphism molecular marker analyses and also apparent variation in morphological traits (height, basal diameter, number of branches, leaflet vertex angle and tippy leaf vertex angle, among others) were observed in the space-mutagenized black locust plants (*Robinia pseudoacacia*, a forest legume)

from seeds that endured 15-day spaceflight compared to plants from parallel ground-based control seeds (Yuan *et al.*, 2012). Genetic diversities and variations have also been observed in 15-day space-exposed *Acer Mono Maxim* seedlings than ground control seedlings (Sun *et al.*, 2015). Inheritable changes in proteins and phenotypes have been observed in rice mutants grown from seeds subjected to short-term (15 days) spaceflight conditions (Lu *et al.*, 2008). In a study on the effects of long-term exposure to spaceflight physical factors on tomato plants grown from dormant seeds flown for 6 years aboard the space station MIR, Nechitailo *et al.* (2005) found that upon return to the Earth, the seeds germinated and grew to maturity. Significant differences in growth, development, yield and molecular changes were observed between plants (first and second generation) from space-exposed seeds and ground-based controls (Nechitailo *et al.*, 2005). Of the 12 cauliflowers plants grown from space-exposed seeds, two first-generation plants showed significant phenotypical changes in plant size and flower head weight (Wu *et al.*, 2010). Also, the space-flown cauliflowers plants were found to be resistant to the black rot, suggesting that space-induced mutagenesis has the potential to result in disease-resistant strains as well. Similar mutations were observed in plants grown from seeds obtained from first-generation plants (Wu *et al.*, 2010). However, in the case of sprout broccoli, the rate of emergence from the flown seeds was 30% lower than the control (Wu *et al.*, 2010). Spaceflight-induced heritable mutations have been observed in different rice varieties grown from 6-day spaceflight-subjected seeds (Shenzhou-3) (Yu *et al.*, 2007). Changes in the height of plants, colour, shape and angle of leaves, panicle length and type and rice shape and colour, among others, were observed in the first and successive generations of plants (Yu *et al.*, 2007). These studies collectively show that DNA changes have occurred in the space-flown seeds, and some of the changes are inherited from the first to successive generations.

Alterations in the flower colour (golden, yellow, light yellow or yellowish-green), ligulate petals of the unisexual floret (broadened or thin), short tubular petals of bisexual floret (elongated or turned into semi-ligulate or ligulate petals) and shape and thickness of leaves were observed in the plants grown from space-subjected seeds(Yang *et al.*, 2012). Also, sequence analysis showed changes in a DNA sequence by deletion, insertion and replacement, indicating the high mutagenic efficacy of space flight (Yang *et al.*, 2012). Significant changes in the rate of DNA variation (6.34%) as well as various morphological (increase in the plant height, number of leaves and fresh weight) and anatomical modification were observed in space-flown rose seedlings compared to ground controls (Huai *et al.*, 2005). Ultrastructural alterations included twist, contraction, deformation of the cell wall, curvature and loose arrangement of lamellae of some chloroplasts and an increase in the number of starch grains per chloroplast (Huai *et al.*, 2005).

Taken together, a growing body of evidence shows the significant impact of the space environment on the genetic integrity, epigenetics, gene expression, physiology, metabolite production and morphology of plants. Studies demonstrate that the microgravity environment acts as a stressor and induce heritable mutagenic changes in plants. The space-induced mutation technique can be used as a novel method to support the emergence of new varieties of plants and develop and enhance characters of crops, such as yield, quality, short-growing period, resistance to pathogens, etc.

Effects of ionizing radiation on microorganisms

The effect of space ionizing radiation on microorganisms' response is not as widely studied as the effects of microgravity. An in-depth understanding of the impact of space ionizing radiation on different microbes is essential from the perspective of life-support systems for space exploration as well as to create novel microbial strains for agricultural and biotechnological applications. Studies show that ionizing radiation produces negative or positive effects that mainly depend on radiation (type, quality, dose and duration) and the microbe itself (species, shape, structure, growth stage, metabolism and genome). On exposure to ionizing radiation, alterations in genetics, metabolite expression, physiology and phenotype have been observed in microbes (Fukuda *et al.*, 2000; Yatagai *et al.*, 2000; Kimura *et al.*, 2006; Novikova *et al.*, 2006; Mastroleo *et al.*, 2009; Senatore *et al.*, 2018).

The whole-genome oligonucleotide microarray analysis and high throughput gel-free proteomics of the α-proteobacterium *Rhodospirillum rubrum* S1H sent twice to the ISS revealed that a low dose (2 mGy) of simulated ISS-ionizing radiation (a combination of single beams of low-LET γ rays and high-LET neutron rays) caused significant changes at the transcriptomic level but only a few considerable variations in the expression of proteins, and no alteration in cell viability (Mastroleo *et al.*, 2009).

Spores are highly resistant to a wide range of stressors, including extreme temperatures, radiation, pressure, vacuum, oxidizing agents and chemicals. Nicholson *et al.* (2002) used *B. subtilis* strains defective in various DNA repair systems and spore structural components to investigate the types of DNA damage induced by sunlight UV-B and UV-A components. The strains were also used to determine the role of spore DNA repair systems and spore structural components in resisting the mutagenic and lethal effects of solar UV radiation. They concluded that *B. subtilis* spores employ a wide range of protective and repair mechanisms that enable them to survive the stresses of solar UV radiation exposure. Long-term exposure (nearly 6 years) of *B. subtilis* spores in monolayers or multilayers to the space environment showed that solar UV radiation reduced spores' survival, but up to 10^4 viable spores were still recovered (Horneck *et al.*, 1994). The study also reported the survival of 70% of the multilayered spores exposed to space vacuum and protected against solar UV radiation. *B. pumilus* spores were found to be highly resistant to the open space environment as well as simulated Martian atmospheric conditions aboard the ISS (Vaishampayan *et al.*, 2012). The survival rate of spores was found to be between 10 and 40% in open space and between 85 and 100% under simulated Martian conditions. The proteomic analysis also showed an increase of proteins inducing stress tolerance (e.g. SOD) in space-exposed samples compared to ground controls (Vaishampayan *et al.*, 2012). Also, the first-generation cells and spores derived from space-exposed samples exhibited high resistance to UV-C than ground control samples (Vaishampayan *et al.*, 2012).

Many fungal species also exhibit high resistance to radiation. In a study, Kimura *et al.* (2006) analysed the effect of ionizing radiation (X- and γ-rays) on the transcriptional response of *S. cerevisiae* strain S288C by cDNA microarray analysis and revealed a time-course transcriptional profile of changed gene expressions. They noted the up-regulation of genes mainly related to cell cycle and DNA processing, cell rescue defence and virulence, protein and cell fate and metabolism in irradiated cells (50 Gy dose of ionizing radiation). The down-regulated genes primarily included transcription and protein synthesis, cell cycle and DNA processing, control

of the cellular organization, cell fate and C-compound and carbo-hydrate metabolism categories (Kimura *et al.*, 2006). Space-related conditions of UV-C irradiation and vacuum induced significant stearic acid production, a biofilm-associated compound, in the radioresistant bacterium *D. radiodurans*, which may have led to cell survivability and maintenance of cell membrane integrity under stress conditions (Ott *et al.*, 2017). This compound is gener-ally produced in lower quantities under non-stressed conditions (Melin *et al.*, 1998). The integrated proteomic and metabolomic analysis also revealed molecular alterations in several metabolic and stress response pathways, including the tricarboxylic acid cycle, the DNA damage response systems, ROS scavenging systems and transcriptional regulators to cope with the space-related stres-sors (Ott *et al.*, 2017).

A survey of environmental biocontamination aboard the ISS showed the presence of several fungal and bacterial species (opportunistic) pathogens, and strains degrading structural mate-rials (Novikova *et al.*, 2006). The study suggested that the surviv-ability of the microorganisms in the extreme environment of space is due to the observed physiological and morphological changes (Novikova *et al.*, 2006). A long-term spaceflight experi-ment performed aboard the Russian space station Mir exposed to space radiation containing high-LET of 10–21 mGy (0.25–0.51 mGy day^{-1}) revealed that space-subjected samples had higher mutation frequency than ground control samples of *B. Subtilis* and *S. cerevisiae* (Fukuda *et al.*, 2000; Yatagai *et al.*, 2000). No significant difference in the frequency of mutations in the ribosomal protein L (*rpsL*) gene leading to spectinomycin resistance (Spc^R), but distinct differences in the spectrum of mutations leading to Spc^R in *B. subtilis* spores were noted between spaceflight and ground control samples (Yatagai *et al.*, 2000). In another study by Fukuda *et al.* (2000), no significant difference in point mutation rates but total or large deletion in the bacterial ribosomal protein L (rpsL) gene were observed between space and ground samples, proposing that space radiation comprising high-LET caused deletion-type mutations. However, in the study by Takahashi *et al.* (2002), the mutation frequencies of space samples were not different from the ground control samples in plasmid DNA containing the *rpsL* gene as well as in both the repair-deficient and wild-type cells of *E. coli* strains, suggesting no effect of space radiation on the mutation frequency.

In the study of Zhou *et al.* (2006a), the production of the immunosuppressant Kanglemycin C (K-C) by one of the mutant strain (F-16) derived from *Nocardia mediterranei* var. *kanglensis* strain U-7 spores exposed to space radiation during a spaceflight (Shenzhou III) was found to be increased up to 200% compared to the ground control strain U-7. In another mutant strain, F-210 derived from spaceflight-subjected spores of strain M-13, morphological and physiological changes and reduced K-C prod-uctivity were observed (Zhou *et al.*, 2006a).

Studies as a whole indicate that space ionizing radiation exerts mutagenic effects (e.g. increase in mutation rates, variations in the spectrum of mutation) on microbial cells bringing alteration in gen-ome organization, physiology and morphology. The observed effects or changes depend on the type of investigated species, the quality and quantity of ionizing radiation and the presence of other stress factors, e.g. microgravity, in the case of real space experiments.

Effect of ionizing radiation on plants

Several experiments have been performed in space and on Earth to analyse the effect of ionizing radiation on plants and elucidate the physiological mechanisms of tolerance to radiation. Ionizing radiation can alter genetics, growth, development and physiology of whole plants, seeds, meristems and germ cells (de Micco *et al.*, 2011; Arena *et al.*, 2014). The severity of the effect is dependent on several factors, including radiation type and dose and plant species, cultivar, age, physiology, morphology and genome organ-ization (Holst and Nagel, 1997; Kim *et al.*, 2011; de Micco *et al.*, 2011; Arena *et al.*, 2014). Low doses of ionizing radiation can cause stimulatory effects, while intermediate to high doses can be harmful or detrimental (de Micco *et al.*, 2011; Arena *et al.*, 2014). The visible changes in plants upon exposure to ionizing radiation include alterations in the morphology (abnormal devel-opment, dwarf or tall phenotypes, increased pubescence, etc.), the anatomy of organs and reproductive capabilities (Wi *et al.*, 2007; de Micco *et al.*, 2011; 2014b; Arena *et al.*, 2014). The physiological and cytological modification include changes in photosynthetic machinery and efficiency of photosynthetic apparatus oxygen evo-lution, dilation of thylakoid membranes, accumulation of antho-cyanin, phenolics and antioxidant compounds and variation of the antioxidative system (Kovács and Keresztes, 2002; Kim *et al.*, 2005; 2011; Arena *et al.*, 2013). The photosynthetic machin-ery components that are mainly influenced by ionizing radiation include electron transport carriers, light-harvesting complexes and carbon reduction cycle enzymes (de Micco *et al.*, 2011; Fan *et al.*, 2014). The detrimental effects of deleterious mutations due to ionizing radiation include the death of embryonic cells, loss of reproduction, abnormal phenotypes, shortened lifespan, lower yield and increased vulnerability to diseases (de Micco *et al.*, 2011; Arena *et al.*, 2014).

Plants are more resistant to space ionizing radiation than ani-mals and humans (Kumagai *et al.*, 2000; Real *et al.*, 2004; Arena *et al.*, 2014). This is most likely due to the structural properties (e.g. thick cell wall), presence of absorbing materials in the cuticle (e.g. phenolic substances and aliphatic organic molecules), genetic organization (e.g. polyploidy), higher efficiency in repairing DNA damages and/or higher rates of DNA methylation (Kranz *et al.*, 1994; Shikazono *et al.*, 2002; Yokota *et al.*, 2005; Esnault *et al.*, 2010; Pecinka and Mittelsten Scheid, 2012; Arena *et al.*, 2014; de Micco *et al.*, 2014a; Beysens and van Loon, 2015). Indeed plant seeds exhibit stronger resistance against radiation compared to organs at different stages of life because of their metabolism and intrinsic characters, such as water content and structure, that influence the rate of ion penetration and production of free radicals (Kumagai *et al.*, 2000; Wu and Yu, 2001; Qin *et al.*, 2007; Esnault *et al.*, 2010; de Micco *et al.*, 2011).

Plant cells with a specialized thick secondary wall coated with phenolic compounds (lignins and suberin) provide high resist-ance to ionizing radiation. The cell wall's role in radioprotection is not yet entirely proved since its structural components, like cel-lulose and pectin, show increased sensitivity to ionizing radiation (Kovács and Keresztes, 2002). The phenolic compounds occur-ring in the cuticle, trichomes and cells of sub-epidermal layers act as screens and provide a photoprotective function. Some spe-cific phenolic compounds also act as antioxidant agents (Agati *et al.*, 2009). Due to an effective anti-oxidative system triggered by radiation-induced oxidative stress, plants protect themselves against higher radiation (Zaka *et al.*, 2002; Arena *et al.*, 2013). Also, polyploid species show a higher resistance to radiation than species with low chromosome numbers within the same genus. Due to the increased regeneration capability, plants are more tolerant against ionizing radiation or other environmental stresses (de Micco and Aronne, 2012). Enhanced tolerance of

plants to high doses of ionizing radiation is attributed to the activation of the scavenging enzymatic machinery and the enzymes regulating stress response pathways (like the nuclear enzymes poly-ADP-ribose polymerases) that recognizes and repairs damaged DNA (Arena *et al.*, 2014). It has also been reported that higher doses of X- and γ-rays (50 and 100 Gy) increase scavenger enzymes activity and overproduce pigments (anthocyanins, carotenoids and flavonoids) as well as ascorbic acid, glutathione and phenolic compounds that potentially remove free radicals and counteract cell oxidative damage (Arena *et al.*, 2013; Fan *et al.*, 2014; de Micco *et al.*, 2014a). The scavenger enzymes include SOD, catalase, ascorbate peroxidase, glutathione-S-transferase and glutathione reductase (Gill and Tuteja, 2010; Foyer and Shigeoka, 2011; Hasanuzzaman *et al.*, 2019). These enzymes work in harmony to protect plant cells from radiation-induced oxidative damage by scavenging ROS, which affects the expression of several genes and numerous processes, like growth, development, cell cycle, cell death, signalling, abiotic stress responses and pathogen defence.

A study by Kim *et al.* (2004) demonstrated that low doses of γ radiation altered photosynthesis and antioxidant capacity of red pepper (*Capsicum annuum* L.) grown from γ-irradiated seeds. Enzyme activity assays and pigment analyses of two cultivars showed that irradiation modified the activities of antioxidant enzymes (SOD and glutathione reductase) and the composition of photosynthetic pigments (chlorophylls and carotenoids) (Kim *et al.*, 2004). In a study, Marcu *et al.* (2013) showed that an irradiation dose of ionizing radiation between 2 and 30 Gy enhanced the growth factors (final germination percentage, germination index and root and hypocotyl length) and the photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) content of lettuce plants compared to untreated ones. A higher dose (70 Gy) significantly decreased plant growth as well as assimilatory pigments. Similarly, Arena *et al.* (2014) showed that 50 and 100 Gy of X-rays reduced the ratios of Chl *a/b* and Chl $(a + b)$/Car$(x + c)$ in dwarf bean (*Phaseolus vulgaris*) leaflets, indicating that chlorophylls and carotenoids are sensitive to higher doses of irradiation.

Nevertheless, the effects of ionizing radiation on photosynthetic pigments vary among plant species and cultivars (Kim *et al.*, 2004). Changes in the plant height, leaf area and mass, stem, above-ground biomass, photosynthesis and respiration rates and chlorophyll fluorescence (f_v/f_m ratio and yield) have been observed in irradiated flowering plants like *A. thaliana* (Kurimoto *et al.*, 2010). In a study, Arena *et al.* (2019) determined the effect of ionizing radiation (25 Gy calcium ions) on the morphology, photosynthetic efficiency, leaf anatomical functional traits and antioxidant production in leaves and fruits of a tomato plant (*Solanum lycopersicum* L. 'Microtom'). Plants germinated from irradiated seeds showed various physiological and structural changes. Better photochemical efficiency, higher amounts of D1 protein and photosynthetic pigment content, leaves with smaller cells and a lower number of chloroplasts were observed in plants germinated from irradiated seeds than controls. Also, plants grown from irradiated seeds produced larger berries, richer in antioxidants (carotenoids, anthocyanins and ascorbic acid) than controls (Arena *et al.*, 2019). In an experiment, Desiderio *et al.* (2019) investigated the effects of X- and γ-rays on tomato hairy root cultures and found variation in the proteome of cultures exposed to 5 or 10 Gy of X- or γ-rays but no morphological changes compared to the unexposed control. These studies confirm that plants trigger various molecular, physiological, and morphological changes for the acclimation or adaptation to ionizing radiation.

Space ionizing radiation (real and simulated) has been widely used in plant breeding program to create plants with desirable traits, such as high yield, shorter growth period, flower colour, temperature tolerance, pathogen-resistance, etc. (Mei *et al.*, 1998; Cyranoski, 2001; Okamura *et al.*, 2003; Yamaguchi *et al.*, 2003, 2010; Wei *et al.*, 2006; Zhou *et al.*, 2006b; Li *et al.*, 2007; Chengzhi, 2011; Wong *et al.*, 2016; Yamaguchi, 2018). New cultivars of several ornamental plant species (e.g. chrysanthemum, carnation, rose, *Lilium*, *Limonium*, *Gypsophila*, *Gentiana*, *Tulipa*, *Delphinium*, *Dahlia*, *Dianthus*, *Tricyrtis hirta*, *Rhododendron*, *Asclepias*, *Hydrangea*, *Petunia*, *Cyclamen*, *Dendrobium*, *Cymbidium*, *Osteospermum*, *Salvia*, *Prunus*, *Torenia*, etc.) with new flower colours and shapes, fewer lateral buds and malformed flowers and the ability to flower early at a low temperature have been produced using ion beams and sold as potted plants and cut flowers (Ueno *et al.*, 2013; Yamaguchi, 2018). Considerable DNA alternations, point-like mutations and null mutations have been observed in mutants of chrysanthemum, carnation and *Arabidopsis* induced by ion beams (Tanaka *et al.*, 2010). Appropriate doses of ion beam radiation have also been utilized to generate novel mutants of crops, such as faba bean, rye, rice and linseed (Yamaguchi *et al.*, 2009; Khazaei *et al.*, 2018).

In the study of Mei *et al.* (1998), mutations and morphological features of maize plants germinated from seeds exposed to space radiation (Chinese satellite for 15 days) were investigated. Morphological changes, such as yellow stripes on leaves, dwarf, yellow-green seedlings, etc., were observed in first-generation plants grown from irradiated seeds. The results showed that radiation-induced mutagenic changes are inherited in successive generations (Mei *et al.*, 1998). Alteration of cell growth, various chromosome aberrations (micronuclei, chromosomal bridges, fragments and laggards) were found in spaceflight-subjected as well as artificially-irradiated (heavy energy ion of) rice seeds (Wei *et al.*, 2006). The frequency of mitotic index and chromosome aberrations was higher in space-exposed seeds than ground-irradiated ones (Wei *et al.*, 2006).

Space ionizing radiation-induced changes in secondary metabolites production have been appreciated in rhizomes of a Chinese traditional medical plant Licorice (*Glycyrrhiza uralensis*) grown from seeds exposed in space to a radiation dose of 0.102 mGy day^{-1} for 18 days (Gao *et al.*, 2009; Dong *et al.*, 2012). In another study, an increase in glycyrrhizic acid and liquiritin was measured along with significant changes in other secondary metabolites production (e.g. flavonoids, triterpene saponins, coumarin) in the root extracts of Licorice after space exposure (Zhang *et al.*, 2011). Spaceflight-induced inheritable genetic mutations in the glycyrrhizic-acid-related gene have also been noted in *G. uralensis* plants grown from seeds receiving a radiation dose of 0.102 mGy day^{-1} and suggested that space conditions can be used for accelerating the progress in plant breeding (Yan *et al.*, 2009).

Taken together, the experiments conducted with ionizing radiation on Earth and in the space environment show that the ionizing radiation produces useful structural modifications as well as stable genetic alterations (wide spectrum and high rate of mutations), suggesting the application of space ionizing radiation for the development of new varieties of plant species that can thrive and produce under stress conditions.

Conclusion and future perspectives in space research

Space environmental conditions, in particular microgravity and radiation, exert significant pressure on organisms resulting in

changes in genes, physiology, metabolites production and morphology that are difficult to achieve on Earth. Recent studies using advanced molecular tools and analytics expand our understanding of these changes in cells and organisms that lead to adaptation and evolution in the space environment. Due to the unique environmental factors offered by space, scientists emphasize the commercial exploration of space factors for the production of novel strains, drugs and products for the health and agricultural sectors. Several infrastructure and space operation capacities are currently available or will be available in the near future as standard carriers (e.g. Shepard spacecraft by Blue Origin, Cygnus spacecraft by Orbital ATK or Dragon spacecraft by SpaceX) to execute various biological experiments in space. Satellite missions allow long-term experiments (days to decades) in microgravity at far lower costs than experiments on the ISS (fewer safety regulations). However, all experiments in a satellite need to be controlled fully automatically, as no operator is available to perform necessary tasks. Sounding rockets, parabolic flights and drop-tower campaigns offer short microgravity periods. Nevertheless, these facilities play an integral role as testbeds for hardware development and initial experiments with organisms because of the lower costs than a satellite or an ISS mission and relatively easy accessibility.

National space agencies are currently encouraging private entrepreneurs to participate actively and conduct various space research in different fields of life and material sciences. Recently, private stakeholders and companies are supporting space research programs. For example, the biotech company Space Biology Unlimited (SBU), France (https://space-cu.com/space-biology-unlimited/), a subsidiary of Space Cargo Unlimited, Luxembourg, aims to bring life science research in space with innovative technologies. In collaboration with academia, research institutes and industries, SBU is performing six experiments of an ambitious project 'WISE' (Vitus Vinum in Spatium Experientia) on the ISS to obtain fruitful outcomes for the future of food, agriculture and health on Earth. Very recently, SBU sent 12 wine bottles to the ISS for 12 months of storage. The objective of this mission is to investigate how space radiation and real microgravity conditions affect the wine components during the ageing process in space since wine is a multi-component system consisting of key elements, such as yeast, bacteria, but also crystals, colloids or polyphenols (Zanchi et al., 2008; Markoski et al., 2016).

As space experiments are expensive, one must narrow down the research to a limited number of model organisms. For example, grapevines (Vitis vinifera) can be studied as a representative of agricultural plants because of their substantial scientific promise and relevance in food quality, food production, nutrition and health (e.g. resveratrol and oligomeric procyanidins). Very recently, in an experiment named 'CANES', which is a part of the WISE project, SBU sent grapevine canes to the ISS (https://space-cu.com/space-cargo-unlimited-debuts-the-third-space-experiment-of-mission-wise-in-partnership-with-cnes-and-esa/). The aim is to investigate the genetic, epigenetic and metabolic changes in grapevine canes due to the long-term storage in real microgravity conditions and create diverse novel varieties of grapevine cuttings. Microbes, such as yeast (e.g. Saccharomyces) and bacteria (e.g. Lactobacillus spp. and Oenococcus oeni), are interesting model organisms. S. cerevisiae has been used in winemaking, baking and fermented food production since ancient times. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology. The Gram-positive

bacterium O. oeni does not contain a DNA repair system and plays an important role in the fermentation process and winemaking.

Collectively, the space environment can be used as a unique stress selection factor to develop novel and rare variants of microbes and plants that will have the immense potential to occupy a niche in the modern biotechnological market and agriculture on Earth and beyond.

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