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AGING IN LIGNINOLYTIC FUNGI IN SPACE CONDITIONS

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ABSTRACT: *Aging is considered in the context of various abiotic stresses, such as water deficit, high temperature, salinity, cold, heavy metals, mechanical wounding, UV radiation, etc. It is well known that the extreme level of these factors can be reflected in sharply increased production of reactive oxygen species. Reactive oxygen species interact with cellular biomolecules, such as lipids, proteins and DNA and can play an important role in cell injury. At the same time, fungi are an appropriate model system in different research areas, including aging. Testing of ligninolytic fungi in space conditions is forthcoming. However, it is unknown how the conditions of microgravity would affect fungal cell aging and enzyme production. Determination of the long-term physiological responses and adaptation to the space environment is of great importance for fungal enzyme production as well.*

KEYWORDS: *AGING, SPACE, ROS, LIGNINOLYTIC FUNGI.*

1. Aging and oxidative stress

Among all existing theories, the free radical/oxidative stress theory is the most plausible and currently acceptable mechanism to explain the ageing process. This theory is based on highly reactive oxygen species (ROS) and their interactions with biomolecules [1]. Aging is considered in the context of various abiotic stresses, such as water deficit, high temperature, salinity, cold, heavy metals, mechanical wounding, UV radiation, etc. It is well known that the extreme level of these factors can be reflected in sharply increased production of reactive oxygen species (ROS), e.g. hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O_2^{\cdot -}$) [2,3]. ROS interact with cellular biomolecules (lipids, proteins and DNA), and can play an important role in cell injury [4]. Aging is a process involving progressive loss of tissue and organ function over time [5]. Various diseases such as rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, cancers, etc. are also initiated by reactive oxygen species [6].

2. Fungi are an appropriate model system for studying aging

Many authors postulated that aging is ubiquitous among animals and even fungi. They assumed that mechanisms of aging are well-conserved among living organisms and those conclusions drawn from studies carried out on the *Saccharomyces cerevisiae* may be extrapolated to human beings [7, 8].

At the same time, fungi are an appropriate model system in different research areas [9,10], including aging [11,12,3]. They are easy to cultivate under laboratory conditions, possess a complexity of organization that is much lower than that of higher organisms, and different species are well suited for genetic analysis of various phenomena and pathways [13].

3. Lignocellulolytic fungi in space

Lignocellulolytic fungi produce a variety of lignocellulolytic enzymes which are responsible for the biodegradation of lignocellulosic agro-wastes in nature. Fungi producing lignocellulolytic enzymes include species of ascomycetes, basidiomycetes, including white rot fungi, brown rot fungi and several anaerobic species that break down cellulose in the gastrointestinal tract of ruminants. Most of these lignocellulolytic fungi have been found to express enzyme activities like filter paper activity (FPase), endoglucanase, exocellulase, β-glucosidase, xylanase, glucoamylase, manganese peroxidase (MnP) and protease. Extracellular oxidative enzymes (oxidoreductases) secreted by lignin degrading fungi, play an important role in degradation of plant cell wall containing the lignocellulose components during the fungal growth [14].

The fungal species *Daedalea quercina* [15] and *Trametes trogii* [16] have been an object of research in various studies. The brown-rot fungus *Daedalea quercina* (Fig.1) produces the ligninolytic enzymes laccase and Mn-dependent peroxidase. This species has been examined for its capability of producing antioxidant and anti-inflammatory compounds as well [17]. *Trametes trogii* (Fig.1) is a member of the white-rot fungi family. Its representatives can produce unique extra-cellular oxidative enzymes, including lignin peroxidase (LiP), Mn-peroxidase (MnP) and laccase, and the latter is the dominant one [18].

Daedalea quercina *Trametes trogii*

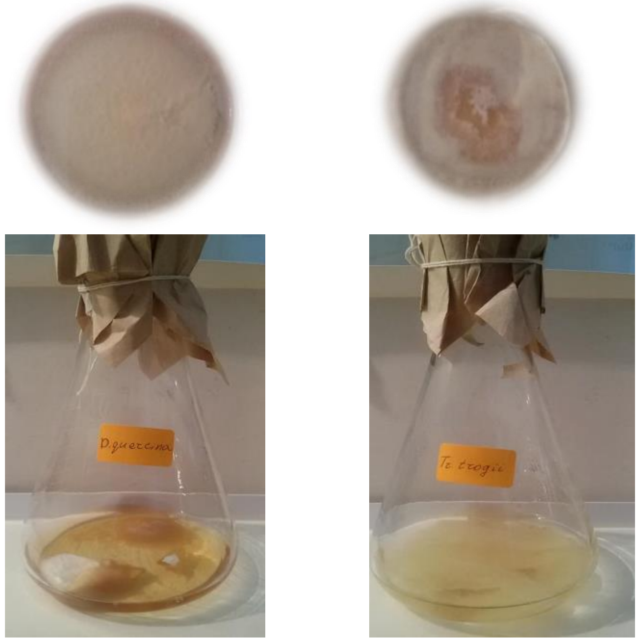

Fig. 1. Both strains belong to the Mycological collection of the Institute of Microbiology, Sofia, and they are maintained at 4°C on beer agar, pH 6.3.

Testing of fungi that degrade lignocellulosic wastes in space is forthcoming. It has been reported that many laccase isozymes show exceptionally high thermal stability [19]. However, it is unknown how the conditions of microgravity would affect fungal cell aging and enzyme production. Determination of the long-term physiological responses and adaptation to the space environment is of great importance for fungal enzyme production as well. The effect of space and space flights on the survival of microorganisms was first recorded in 1935 [20]. Although cellular response mechanism in response to microgravity condition have been studied in *S. cerevisiae* [21] analogous research in ligninolytic fungal species has yet to be undertaken. There are no data about changes in ROS level under space conditions in fungal strains that may prove useful in future biomass conversion strategies involving lignocellulosic materials.

4. The challenges of spaceflights

Spaceflights expose all organisms to several major stress conditions, such as space radiation, microgravity, vibration, pressure and other low shear environment. These factors affect their survival rate and physiology. To evaluate the risks of causing infections or allergies by fungi on board spacecraft, it is necessary to understand how they grow in the new environment and how their characteristics change in space.

Safety is an important issue with fungi space experiments. They may 'escape' from the closed experimental environment into International Space Station (ISS). Because of this, tests should be done for the viability of fungi and their spores in the environment of ISS. The atmosphere of ISS has a pressure and gas composition substantially similar to earth atmosphere at sea level. Pressure is kept stable because if the pressure were to drop too far, it could cause problems with the Station equipment. The main difference between Earth and ISS atmosphere is the high concentration of carbon dioxide in ISS air, typically about 3000 ppm. The temperature on board is 20-25 ^{o}C, typically about 22 ^{o}C. Low relative humidity is maintained, 40-70 %, in order to avoid microorganism proliferation and possible infections. A set of experiments is designed to test the behavior of the fungus in a simulated ISS environment. The ISS atmosphere will be created on earth and the development of the fungus on different substrates will be tested. To investigate the effects of these stresses, model strains have to be cultured in conditions that mimic the conditions seen in space flight [22,23]. Design, modeling and fabrication of a camera maintaining optimal air temperature, relative humidity and pressure for the cultivation of both model strains is necessary.

5. Conclusion

Despite the wide interest to explain ageing process, there are a number of questions waiting for answers, e.g. how is the ROS level changing in cells exposed to the extreme space conditions, because most of abiotic stresses evoke an increased production of ROS. Remarkably little is known about the effects of microgravity, space radiation, or other elements of spaceflight on fungal physiology, developmental processes in eukaryotic cells and their ability to synthesize important enzymes. It should be noted that ligninolytic fungi have not been used in aging research in space.

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