REVIEW

BY: Assoc. Prof. Maria Gerginova Gerginova, Institute of Microbiology - BAS. Member of the Scientific Jury according to Order N I-113 of 04.08.2025 of the Director of the Institute of Microbiology – BAS

REGARDING: Competition for the academic position of "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, in the professional direction 4.3. Biological Sciences for the needs of the laboratory "Biodiversity and Molecular Biology of Micromycetes", Department of Mycology, Institute of Microbiology "Stefan Angelov" - BAS, announced in the State Gazette No. 55 of 08.07.2025.

The sole candidate in the competition is Senior Assistant Professor Dr. Jeny Georgieva Miteva-Staleva from the Laboratory "Biodiversity and Molecular Biology of Micromycetes", Department of Mycology, Institute of Microbiology - Bulgarian Academy of Sciences. The competition documents comply with the requirements of the ADAS RB and the regulations for its implementation.

1. *Professional biography of the candidate*

Jeny Miteva-Staleva obtained her Master's degree in 2003 from Sofia University "St. Kliment Ohridski" in the field of Ecology and Environmental Protection. She joined the Institute of Microbiology of the Bulgarian Academy of Sciences as a microbiologist in the "Mycology" laboratory in 2005 and has more than 23 years of work experience. In 2017, she received her PhD on the topic "Low-temperature stress and cell aging in Antarctic fungi of the genus Penicillium" under the supervision of Prof. Maria Angelova and, after a competition, took up the scientific position of Assistant Professor in the Department of Mycology of the Institute of Microbiology.

In 2013, Dr Jeny Miteva-Staleva completed a two-month specialisation at the University of Pavia, Italy Department of Earth Science and Environment, in the field of classical and molecular biological methods for the identification of mycetes.

Dr Jeny Miteva-Staleva works mainly in the field of the impact of low temperature and other extreme conditions on the oxidant - antioxidant status and aging processes in filamentous fungi. She continues to develop issues related to the topic of her dissertation, which is an indicator of the consistency of her scientific interests.

2. *Analysis of the candidate's document and assessment of compliance with national and institutional requirements.*

The documents presented by Dr. Miteva-Staleva are in accordance with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Regulations for its implementation, as well as the Regulations for the Development of the Academic Staff of the Bulgarian Academy of Sciences and the Institute of Microbiology, BAS, for holding the academic position of "Associate Professor".

Reference for the minimum requirements under ADSRB

- Group A; indicator 1. This indicator includes the PhD thesis - 50 points;
- Group B indicator 1. Six scientific articles in journals referenced in Scopus: 3 in the journals quartile Q1, 1 - Q2 and 2 - Q3 (total 125 points).
- Group D. Total 241 points. Indicator 7 - The submitted documents include 14 scientific articles referenced in Scopus, outside of those in group B. The distribution by quartiles is as follows: 2 with Q1, 4 with Q2, 5 with Q3 and 3 with Q4.
- Group D Indicator 1. 198 citations from the Scopus database are indicated, which form a total of 396 points.

In total, according to the listed indicators, Dr Jeny Miteva-Staleva accumulated 812 points, which covers and exceeds the required 430 points according to the PP ADASRB.

The review of the competition documents showed that the candidate also meets the additional criteria for academic development according to the Regulations for the Development of the Academic Staff of IMicB-BAS.

Attached are 20 scientific publications (excluding those for PhD), published in journals with an Impact Factor (IF) or Scimago Journal Rank (SJR), of which she is the first author in 5. All publications with the participation of Jeny Miteva-Staleva have a total IF of 96.835, and the h-index of the candidate is 9, with a requirement of - 5. The candidate demonstrates significant activity in scientific research, having participated in 4 international scientific projects and 23 national research projects, 11 of which are funded by the National Science Foundation. Her scientific activity is represented by 3 participations with reports and 14 posters in international scientific forums, 2 reports and 5 posters in national forums with foreign participation and 2 reports and 6 posters in national scientific forums.

Dr Jeny Miteva-Staleva was the supervisor of a graduate student from NBU with a bachelor's thesis on the topic: "Filamentous fungi from Alaska: isolation, identification and characterisation".

3. **Contributions from scientific research activities.**

Dr. Miteva-Staleva has categorised her principal scientific contributions into three primary groups, as elaborated in the comprehensive habilitation report.

The first section is entitled "*Biodiversity and characterisation of the biotechnological potential of filamentous fungi isolated from habitats with extreme living conditions*". Specific results on the topic are presented in a large part of her publications (13 scientific publications) under numbers 2, 3, 4, 8, 9, 11,12, 13, 14, 16, 20, 21, 23.

The first part of this section highlights research in the field of biodiversity of filamentous fungi from habitats with extreme conditions. The objects of analyses are isolated from Antarctica and Alaska but also from other sources such as saline and heavy metal-contaminated soils. The most significant are the studies of psychrotolerant and mesophilic representatives of the genus Penicillium and the genus Aspergillus, isolated from habitats characterized by extreme living conditions. The second part of the section presents results related to the purification and characterization of cold-active enzymes from filamentous fungi isolated from extreme habitats.

Basic research has demonstrated that low-temperature cultivation clearly induces the generation of reactive oxygen species (ROS) in the cells and mitochondrial fractions of Antarctic fungi. The response of cells to cold stress depends on the developmental stage of the model strains (13).

An improved laboratory technology for the production of superoxide dismutase (SOD) from *Aspergillus niger* 26 has been developed, based on a new fermentation strategy. A short-term heat shock for 6 hours leads to a significant increase in the efficiency of superoxide dismutase (SOD) synthesis. The enzyme obtained by this method retains its temperature optimum, which allows its medical and pharmaceutical application (20).

Related to the role of ROS is the study of the effect of copper stress in *Humicola lutea*. It has been proven that high concentrations of Cu^{2+} induce an increase in the production of SOD, thereby improving the ability of the cell to detoxify superoxide radicals and survive stress (9). Studies on the relationship between the influence of heavy metals and oxidative stress processes have been expanded and show the different responses of two strains of *Aspergillus fumigatus*, isolated from habitats with varying degrees of heavy metal contamination, when treated with a combination of heavy metal ions (Cd, Cu, Ni, and Zn). The strains differ in their antioxidant enzyme activity, with one strain exhibiting higher tolerance due to more active antioxidant defense. One strain stimulates SOD activity, while the other shows reduced catalase (CAT) activity under multiple metal stresses (12).

Regarding the growing interest in the study of halophilic and halotolerant microorganisms, the participation of antioxidant enzyme protection in the adaptive strategy of halotolerant fungi against elevated NaCl concentrations was investigated. The fungal strain *Penicillium chrysogenum* P13, isolated from saline soils around Pomorie Lake, Bulgaria, was studied. Changes in cellular biomarkers of oxidative stress, such as oxidatively damaged proteins, lipid peroxidation, and levels of reserve carbohydrates of the studied strain, were evaluated (3).

An important part of the research is the study of fungal biodiversity and proteolytic activity of filamentous fungal strains isolated in Antarctica and Alaska. New data are presented on the predominant species belonging to the genera Aspergillus and Mucor in soils from Denali National Park and Preserve, Alaska. Most of the identified fungi are classified as mesophiles. The proteolytic activity of all isolated strains was studied, with significant enzymatic activity being found in 7 of them. As inhabitants of extremely cold environments, these fungal strains could be used to produce industrially important cold-active enzymes (23). A study comparing Antarctic and temperate strains of the genus Penicillium showed that cold stress leads to increased protease activity, with a more pronounced and rapid response in Antarctic strains. The action of protease is associated with protection against oxidative damage to proteins under low-temperature stress conditions. Protease activity was generally lower in stationary phase cells compared to younger exponential phase cells, regardless of the temperature class of the strain (21).

The results obtained in the study of enzymes with catalase activity are also original and significant. Catalase is an enzyme actively involved in cellular antioxidant defense. It plays a key role in protecting a wide range of Antarctic organisms against cold stress. Cold-active catalase was produced from the *Penicillium griseofulvum* P29 strain isolated from Antarctic soil. The enzyme was purified and characterised. By optimising the nutrient medium and cultivation conditions, improved enzyme production was achieved. An effective laboratory technology has been developed based on changing the level of oxidative stress by increasing dissolved oxygen in the bioreactor. Cold-active catalase is of great interest due to its enormous potential at the medical, commercial and biotechnological level (14,16). In a molecular biological study conducted to identify catalase genes in strain *P. griseofulvum* P29, five catalase genes were sequenced. By applying quantitative real-time PCR (qPCR), the expression level of each of them was studied under low temperature stress. The results showed that 4 of the genes are induced by low temperature in response to oxidative stress. The most pronounced is the increase in the expression of the *cat1* gene, encoding the catalase-peroxidase enzyme. The available data indicate that *cat1* is mainly involved in the mechanism that allows the growth of *P. griseofulvum* P29 under conditions of oxidative stress induced by low temperature. (8).

Extracellular catalase is relatively uncommon, and there is very little information available about it. This enzyme participates in the cellular response to low temperature stress, which aids the survival of filamentous fungi in extremely cold habitats. In this context, 62 Antarctic filamentous fungal strains were studied for their potential ability to synthesise intracellular and extracellular catalase. The Antarctic mesophilic strain *Penicillium rubens* III11-2 was identified as the best producer of extracellular catalase. In this study, an efficient purification scheme for the enzyme was developed. The purified enzyme showed maximum enzymatic activity in the temperature range of 5 - 40 °C and temperature stability between 0 and 30 °C, therefore it was characterised as cold-active enzyme. This is the first purified extracellular preparation of cold active catalase from an Antarctic filamentous fungus (4).

The *Penicillium griseofulvum* P29 strain isolated from Terra Nova Bay, Antarctica, already mentioned as a producer of cold-active catalase, was also analyzed for sialidase activity. It was found that it produces a sialidase classified as cold-active and different from known bacterial enzymes. The enzyme is more active towards $\alpha(2. \rightarrow 3)$ sialyl bonds than towards those containing $\alpha(2 \rightarrow 6)$ bonds, which suggests a relatively narrower substrate specificity than bacterial enzymes. This study presents an effective purification scheme and structural characterisation of the enzyme. The results were achieved using a combination of a number of physical and chemical methods: electrophoretic analysis (SDS-PAGE), MALDI-TOF/MS, high-performance liquid chromatography, etc. Based on the established amino acid sequence, a 3D structural model of P29 sialidase was created. Fluorescence spectroscopy has shown that the enzyme is conformationally stable in the neutral pH range, but is more stable in an alkaline environment. The purified cold-active enzyme is the only sialidase produced and characterized from Antarctic fungi to date (11). The involvement of carbon catabolite repression (CCR) in the regulation of the biosynthesis of cold-active sialidase by the psychrotolerant strain *P. griseofulvum* P29 was investigated. The drastic reduction of sialidase activity in the presence of glucose in the growth medium is consistent with downregulation of sialidase gene expression. Despite the psychrotolerant nature of *P. griseofulvum* P29, the sialidase it produces is a cold-active enzyme, and the growth temperature does not affect the degree of catabolite repression.

The second section of the scientific contributions is entitled "*Characterization of the cellular response of filamentous fungi isolated from habitats with extreme living conditions against oxidative stress induced by various stress factors*". The results presented in the publications that make up this section (2, 3, 4, 5, 12, 13, 16, 20, 21, 23, 25) are directly related to the contributions described above.

Characterizing of the cellular response is a key aspect in revealing the fundamental adaptive mechanisms in microorganisms. These studies provide an opportunity for application in various fields of biotechnology, such as increasing the resistance of industrial strains or in developing strategies for controlling pathogenic species.

The most significant contributions from the above sections can be summarized as follows::

- New information has been obtained regarding the relationship between protease activity and oxidative stress in Antarctic filamentous fungi. The dynamics of synthesis of intracellular and extracellular proteases under conditions of low-temperature shock and their participation in adaptive processes related to protection from oxidative damage of proteins have been characterized. Strain- and phase-dependent differences in the enzyme response have been observed. It has been established that the activity is comparable to the accumulation of biomarkers of oxidative damage.
- For the first time, data have been obtained on the relationship between the growth phase of the culture and changes in the cellular response of strains from different temperature classes isolated from Antarctica. The established patterns are a contribution to clarifying the relationship between oxidative stress and aging in extreme environments.
- The role of heavy metals in the cellular response to oxidative stress of strains tolerant to high concentrations of Cd, Cu, Ni and Zn has been proven. Changes in stress biomarkers (carbonyl proteins, glycogen and trehalose) and antioxidant defense enzymes (SOD and CAT) have been established.
- New strains of filamentous fungi with high resistance to salt stress, isolated from hypersaline extreme habitats, have been identified.
- The role of the antioxidant defense system in adaptation to hypersaline environments has been elucidated, highlighting the importance of cellular protection from reactive oxygen species as a major survival mechanism

The third section of the scientific contributions is entitled "*Characterization of the antifungal and antioxidant activity of natural materials and plant extracts*".

Dr. Jeny Miteva-Staleva's research activities included in this section are related to her expertise in working with fungi and her high methodological training. She participated in testing the antifungal activity of silver-based geopolymer paints (GP-TiO2 +NZ-Ag) by diffusion method, in which a strain of *Aspergillus niger* from the collection of the Department of Mycology at the Institute of Microbiology - Bulgarian Academy of Sciences was used. An antifungal effect was proven on both uncarbonized (raw) and carbonized geopolymer paint. The results of the antimicrobial tests identify geopolymer paints based on modified zeolites as promising for future developments in the search for new solutions to limit the invasion of pathogens (1).

In connection with the growing need to find effective and environmentally friendly alternatives to controlling fungal infections in potatoes, a study was conducted to evaluate the antifungal properties of nanoparticles (NP) of chitosan (CS) and zinc oxide CS-ZnO, copper oxide CS-CuO and silicon dioxide CS-SiO2 as potential fungicides against the plant pathogens *Alternaria solani* and *Fusarium solani*. The results showed that all types of NPs exhibited pronounced antifungal activity against both pathogenic strains, with CS-ZnO nanoparticles being the most effective. It has been established that the activity of NPs includes the induction of oxidative stress in fungal cells and changes in the molecular organization of their membranes. Similar studies show the potential of these nanoparticles as effective and universal antifungal agents. (5, 7). A method for using chitosan-based nanoparticles with fungicidal effect against phytopathogenic fungi has been developed, with a perspective for application in agriculture, etc.

The effect of ethyl acetate extracts of Geum urbanum L. in vitro on provoking acute and subacute toxicity by daily oral administration (per os) in healthy male and female H-albino mice was investigated. Certain biochemical parameters (superoxide anion radical, hydrogen peroxide, superoxide dismutase, lipid peroxidation, and damaged proteins in blood plasma and brain) and complete hematological parameters were studied. In conclusion, the studied extract is proposed for use as a nutritional supplement for various infectious diseases (17).

For the first time, the ability of some alkaloids from the Amaryllidaceae family to inhibit the growth of endophytic fungi has been studied. Sixty-five species capable of growing in laboratory conditions have been identified and taxonomically determined. Species from the genera Fusarium, Trichoderma, and Talaromyces predominate. It has been found that various alkaloids, the main components in the different genera, exhibit different growth-inhibiting effects depending on the specificity of the fungal strain. The results show that alkaloids from the Amaryllidaceae family may play a role in the interactions between plants and endophytes (18).

CONCLUSION

The developments included for evaluation in this competition are distinguished by originality, relevance and social significance. They fully correspond to the scientific field and scientific direction of this competition. They are united not only by the desired direct connection between fundamental and applied research, but also by the skillful application of modern research methodologies. The results obtained reveal opportunities and prospects for new research on current problems, aimed at solving global challenges in the field of medicine and the production of biologically active compounds with potential application in various sectors of industrial production.

Senior Asst. Prof. Dr. Jeny Miteva-Staleva fulfills the requirements for the academic position of "Associate Professor" in the Professional Direction 4.3. Biological Sciences. and significantly exceed the minimum national and additional requirements of the ADAS RB, and the relevant regulations of the BAS and the Institute of microbiology - BAS.

All this gives me reason to support the candidate and to confidently recommend to the esteemed members of the Scientific Jury to evaluate her positively and propose to the Scientific Council of the Institute of Microbiology - BAS to award to Senior Assistant Professor Dr. Jeny Georgieva Miteva-Staleva the academic position of "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences

Sofia
07.11.2025 г.

Assoc. Prof. Maria Gerginov

**На основание
чл. 2 от ЗЗЛД**