REVIEW

By Prof. Nelly Vladova Georgieva, PhD, Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia

Regarding: The materials submitted for participation in a competition for the academic position „Associate Professor" at the Institute of Microbiology, BAS in Professional area 4.3. Biological sciences, scientific specialty "Microbiology – Mycology", for the needs of the Laboratory "Biodiversity and molecular biology of micromycetes", Department of Mycology, announced in SG ed. 55 on 08.07.2025 with the only candidate being **Assist. Prof. Jeni Georgieva Miteva-Staleva, PhD.**

General presentation of the submitted documents

The present review is prepared on the basis of Order No I-113/04.08.2025 of the Director of the Institute of Microbiology, BAS and the decision of the scientific jury session from 17.09.2025.

Assist. Prof. Jeni Miteva-Staleva is the only applicant who has submitted documents for this competition within the deadline regulated by the law. She has worked at the same Institute in the Department of Mycology, Laboratory "Biodiversity and molecular biology of micromycetes" on a full-time position with 23 years of scientific experience in the subject of the announced competition.

The scientific interests and presented materials of the candidate are in the field of Microbiology, the subject of the competition. The submitted documents are in compliance with the requirements of the Act for the Development of the Academic Staff in Republic of Bulgaria, the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in the Institute of Microbiology, BAS. They also meet the recommended criteria for academic position of „Associate Professor" in Professional area 4.3. Biological sciences. All submitted documents are structured in a way that fully reflects the scientific research activities of the applicant.

Biographical information about the applicant

Assist. Prof. Jeni Miteva-Staleva was born on 23 March 1975 in Sofia. In 1999 she enrolled as a student in the Biological Faculty of Sofia University "St. Kliment Ohridski" and graduated in 2003 with a Master's degree in "Ecology and environmental protection". In 2005, she started working as a microbiologist at the Institute of Microbiology, BAS. Since 2010, she has held the position of Assistant at the same institute. During the period 2014 - 2017, she was a doctoral student in independent training at the Institute of Microbiology and defended her dissertation scientific specialty "Microbiology" on the topic: "Low-temperature stress and cell senescence in Antarctic fungi of the genus *Penicillium*" supervised by prof. Maria Angelova PhD. After a competition held in 2019, she has held the academic position of Chief Assistant Professor in the Department of "Mycology".

Assist. Prof. Jeni Miteva-Staleva is a participant in a total of 28 projects, of which 11 are funded by the Scientific Research Fund, 4 international projects, 2 national, 1 funded by Sofia University "St. Kliment Ohridski" and 10 funded by business organizations. Under her supervision, a thesis for the Master of Science was also defended. She completed a two-month specialization at the University of Pavia, Italy, in the Department of Earth Sciences and gained new knowledge about classical and molecular biological methods for mycetes identification. In her research activities, she uses a rich methodological arsenal, and along with well-known and widely applied microbiological, physiological and biochemical methods, she also masters some of the most modern approaches for molecular and enzymological analyses. All this has developed her as a scientist with professional experience.

Main scientific indicators

Assist. Prof. Jeni Miteva-Staleva has presented a total of 25 scientific papers, which do not include those for the acquisition of the "PhD" degree, 20 in publications with IF and 5 without IF, as in 5 publications she is the first author, chapters of books - 1 pc. The total IF for the entire scientific career is 96.835 and the H-factor is 9. The quartile distribution is the following: in Q1 – 5 papers, in Q2 – 5 papers, Q3 – 7 papers, Q4 – 3 papers. The results of the research activity were reported at many international and national scientific forums with reports and posters. According to the indicators, the evaluation determined by the Law for the Development of the Academic Staff in the RB by which Assist. Prof. Jeni Miteva-Staleva participates in the competition, convincingly shows that she meets and exceeds the requirements for position of "Associate Professor". The points of the scientific papers of the candidate for the contest are:

Indicators of A: Dissertation thesis 50 p. (min. 50)

Indicators of B: habilitation paper 125 p. (min. 100)

Indicators of G: scientific papers 241 p. (min. 220)

Indicators of D: cited papers 396 p. (min. 60)

The citation of the publications are from world - renowned scientific information (Web of Science and Scopus) and 198 p. are presented without autocytes, gathering 396 p. Regarding “Additional criteria for the growth of the academic staff in IMicB” it can be also noted that the criteria are overfilled.

Scientific contributions

The published scientific results presented in the competition are fully within the scope of the announced competition. The scientific interests reflected in the research work of Dr. Jeni Miteva-Staleva can be summarized in the following interconnected and complementary areas: Biodiversity and characteristic of the biotechnological potential of filamentous fungi isolated from habitats with extreme living conditions, characteristic of cellular response of filamentous fungi, isolated from habitats with extreme living conditions against oxidative stress, induced by various stress factors, as well as a characteristic of the antifungal and antioxidant activity of natural materials and plant extracts. The applicant's scientific contributions can be summarized as follows:

1. **Biodiversity and characteristic of the biotechnological potential of filamentous fungi isolated from habitats with extreme living conditions**

The scientific studies and publications in this area are a kind of continuation of the topic of the dissertation work in the direction of studying the biodiversity, physiological characteristics and biotechnological potential of filamentous fungi isolated from extreme habitats - Antarctica, Alaska, saline soils and environments contaminated with heavy metals, thus enriching the knowledge about their diversity in ecosystems with extremely unfavorable conditions. For the first time, information has been obtained on the biodiversity of filamentous fungi from hypersaline habitats in Bulgaria, such as the soils around Pomorie Lake. The results reveal their broad potential for the synthesis of industrially important biomolecules with applications in the food and pharmaceutical industries. Phylogenetic and morphophysiological characterization of new extremophilic/extremotolerant fungal species has been conducted, contributing to their selection as production strains in green technologies - for example, for bioremediation in saline habitats.

The creation of a collection of strains of filamentous fungi isolated from habitats with extremely unfavorable living conditions can be considered a scientific and applied contribution: permanently low temperatures (Antarctica, Alaska), soils contaminated with heavy metals (Vlaykov Peak mine, near Pazardzhik and industrial sites of Krivoy Rog, Ukraine) as well as hypersaline environments (soils near Pomorie Lake), which is a valuable bioresource with potential for future applications in biotechnology and industry.

Temperature-sensitive enzymes – sialidase and catalase – have been isolated and characterized from the Antarctic filamentous fungi *Penicillium griseofulvum* and *Penicillium rubens* III11-2, thereby new knowledge are obtaining about the characterization of temperature-sensitive enzymes produced by strains of extremophilic filamentous fungi. It has been found that enzymes exhibit high catalytic activity and stability at low temperatures and contribute to the specific adaptation of fungi to environmental stress factors. Quantitative RT-PCR has shown that low-temperature stress induces the expression of specific catalase genes involved in the antioxidant defense of Antarctic strains and in their survival at low temperatures. The role of cat1, encoding catalase-peroxidase, in a *Penicillium griseofulvum* strain has been demonstrated. For the first time, the regulatory role of catabolite repression in sialidase synthesis has been demonstrated in a strain isolated from Antarctica (*P. griseofulvum* P29), regardless of the cultivation temperature.

The purification and characterization of a temperature-sensitive sialidase, enzyme produced by the Antarctic strain *P. griseofulvum* P29 with high specific activity towards α(2→3)-linked sialic residues at low temperatures and stability in a wide range of pH values can be considered a scientific and applied contribution. A 1.7-fold increase in the enzymatic activity of CAT in the studied strain was achieved by optimizing the cultivation parameters and controlling the level of dissolved oxygen in the medium.

2. **Characterization of the cellular response of filamentous fungi isolated from habitats with extreme living conditions against oxidative stress induced by various stress factors**

In her experimental research, assist. Prof. Jeni Miteva-Staleva established the role of antioxidant enzymes as a central component in the survival of fungal cells under stressful conditions. The studies prove the importance of the enzymes superoxide dismutase (SOD), catalase (CAT) and protease as key factors for adaptation to low temperatures, high concentrations of NaCl and metals (Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}). Enzyme activity profiles were reported depending on the growth phase and the type of stress factor. Evidence was obtained for the induction of antioxidant enzyme defense in fungi isolated from habitats with extreme living conditions in response to stress factors. These data can be used for selection and development of resistant strains with potential for bioremediation and biotechnological applications.

The generation of free oxy-radicals (COP) induced by low temperatures has been established depending on the developmental phase (exponential/stationary) and the degree of the stress factor in Antarctic fungal strains with different thermal affiliations (mesophilic and psychrotolerant). The key role of the antioxidant enzyme catalase (CAT) in the adaptive response to low-temperature stress in filamentous fungi isolated from Antarctica has been demonstrated. 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 to proteins have been characterized. Strain- and phase-dependent differences in the enzymatic response have been observed.

Regularities between oxidative stress and aging in extreme environments have been established in Antarctic micromycetes. 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. A sharp decrease in temperature induces a significant increase in COP and proteolytic enzymes, regardless of the thermal affiliation of the strains.

A scientific contribution can be defined the identification of new strains of filamentous fungi with high resistance to salt stress, isolated from hypersaline extreme habitats. In this group of studies, a significant correlation was established between the origin of the strains and their adaptation to extreme levels of NaCl. New information has been obtained regarding the cellular response to oxidative stress induced by NaCl in the halotolerant strain *P. chrysogenum* P13 and its role in the increased resistance to oxidative damage. Changes in the level of stress biomarkers – antioxidant enzymes (SOD and CAT), oxidatively damaged proteins, lipid peroxidation and reserve carbohydrates (glycogen and trehalose) have been demonstrated. The role of the antioxidant defense system in adaptation to hypersaline environments has been elucidated, highlighting the importance of cellular protection by COP as a key survival mechanism.

The new information obtained regarding the development and physiological characteristics of strains of the genus *Aspergillus fumigatus* isolated from soils contaminated with heavy metals (Cd, Cu, Ni and Zn) is also considered as scientific contribution. The role of heavy metals in the cellular response to oxidative stress in strains tolerant to high concentrations of Cd, Cu, Ni and Zn has been proven. Changes in biomarkers of stress (carbonylated proteins, glycogen and trehalose) and antioxidant defense (SOD and CAT) have been identified. It has been shown that *A. fumigatus* G strain possesses higher tolerance to heavy metals, due to increased antioxidant enzyme defense, which is an indication of a strain-specific strategy for coping with heavy metal-induced stress.

3. Characterization of the antifungal and antioxidant activity of natural materials and plant extracts

The presented scientific publications on this working topic demonstrate the physiological and molecular mechanisms of antifungal activity of nanoparticles (NPs) and nanocomposites (NCs) based on chitosan (CS) and metal oxides (ZnO, CuO, SiO_2). The studies clearly show that the mechanism of action of nanoparticles and nanocomposites of chitosan with zinc, copper and silicon oxide (CS-ZnO, CS-CuO, CS-SiO_2) is associated with the induction of oxidative stress, as evidenced by the increase in SOD activity, accumulation of carbonylated proteins, lipid peroxidation and structural disorders in membranes, confirmed by laurdan fluorescence spectroscopy. The strongest effect is reported for CS-ZnO and CS-CuO. The results obtained in this direction can be considered as a contribution of a scientific and applied contribution, since the application of a nanobiotechnological approach using chitosan-based nanoparticles with fungicidal effect against phytopathogenic fungi creates new prospects for sustainable agriculture. The antifungal activity of natural and modified zeolites, as well as geopolymer coatings enriched with silver, copper and iron ions and nanoparticles, has also been established.

For the first time, the antifungal activity of individual alkaloids and alkaloid fractions from representatives of the *Amaryllidaceae* family has been studied against endophytic strains of filamentous fungi isolated from *Leucojum aestivum* L., revealing a new direction in the chemoecological interactions between plants and microorganisms. 65 strains of endophytic fungi, classified into 31 species from 11 phylogenetically distinct genera, were isolated and identified, contributing to expanding knowledge about the mycobiome diversity of *L. aestivum* L. and the relationship between the composition of the endophytic mycoflora and the geographical distribution, as well as the organospecific factors influencing plant growth.

New evidence has been obtained that alkaloids such as galantamine, epinorgalantamine, lycorine, as well as diverse fractions (homolicorine, hemantamine, tazetine and skeletal type), exhibit strain-specific inhibitory activity against endophytic micromycetes, which highlights their potential as a source of natural fungicidal compounds.

New information has been obtained regarding an extract of *Narcissus* cv. *Hawera* containing *Sceletium*-type alkaloids. The extract was identified as the most effective with broad-spectrum and highly pronounced antifungal activity. These results reveal new potential for application of the plant extract as a source of bioactive compounds in plant protection and pharmaceutical biotechnology.

New experimental evidence for a possible chemoecological function of *Amaryllidaceae* alkaloids in the regulation of plant-microbial interactions is presented, laying the foundation for future interdisciplinary research in the fields of chemical ecology, mycology, and plant physiology.

For the first time, the toxicological profile of an ethyl acetate extract of *Geum urbanum* L. administered orally to mice has been evaluated. The studies showed a lack of significant toxicity, as well as the presence of antioxidant activity, as determined by biomarkers of oxidative stress in the brain and blood of mice, confirming the potential of the extract as a safe dietary supplement.

The development of a method for using chitosan-based nanoparticles with a fungicidal effect against phytopathogenic fungi, with a perspective for application in agriculture, can be assessed as a scientific and applied contribution, in which the antifungal potential of chitosan-based nanoparticles and nanocomposites has been proven.

The biocidal properties of chitosan nanoparticles and composites (CS-ZnO, CS-CuO, CS-SiO2) against the phytopathogenic fungi *Fusarium solani* and *Alternaria solani* have been conclusively proven, which creates a prospect for the development of environmentally friendly biotechnological products for plant protection. The developed silver-containing geopolymer coatings have the potential for use as biocidal materials in construction, sanitary engineering, and sustainable water treatment.

The proven low toxicity at maximum tested doses of extract from the medicinal plant Geum urbanum supports its potential as a natural supplement with possible antimicrobial and antioxidant function in infectious conditions.

In her research activities, assist. Prof. Jeni Miteva-Staleva uses a rich methodological arsenal, and along with well-known and widely applied microbiological, physiological and biochemical methods, she also masters some of the most modern approaches for molecular and enzymological analyses. I believe that the above contributions are original in nature, lead to new knowledge in the specific field and have the potential for implementation in practice.

Conclusion

The works included for evaluation in this competition are distinguished by scientific accuracy as well as originality, and fully correspond to the scientific field of this competition.

The results obtained and the scientific contributions of assist. Prof. Jeni Miteva-Staleva, reflecting her many years of research experience, reveal opportunities and prospects for new research on current problems. All formal requirements specified in the Act for the Development of the Academic Staff in the Republic of Bulgaria, the regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and obtaining academic positions in the Institute of Micobiology, BAS, have been fulfilled. The scientific research, achievements and activities of **assist. Prof. Jeni Miteva-Staleva** allow me to strongly recommend to the esteemed Scientific Jury and the Scientific Council at the Institute to fully support the acquisition of the academic position of Assosiate Professor by **assist. Prof. Jeni Miteva-Staleva** in the Professional field 4.3 Biological sciences, scientific specialty “Microbiology - Mycology”, IMicB, BAS.

03.11.2025 Signature:……………………………….

/prof. Nelly Georgieva, PhD/