

R E V I E W

on the set of documents for the academic position “**professor**”, scientific direction 5.11. Biotechnology (Technology of biologically active substances), for the needs of the Department of Biotechnology (Laboratory of Metabolomics) at The Stephan Angeloff Institute of Microbiology – Bulgarian Academy of Sciences

Reviewer: Prof. **Albert Ivanov Krastanov**, DSc, University of Food Technology – Plovdiv

Assoc. prof. Milen Ivanov Georgiev, PhD is a candidate in the announced competition for the academic position “**professor**” (State Gazette No 47 from 22.05.2020). The candidate has submitted all required documents, according to the current Act for the Development of the Academic Staff in the Republic of Bulgaria. The evaluation of the documents revealed that the scientific subject of the candidate fully corresponds to the scientific direction, announced in the competition call. Assoc. prof. Milen Georgiev graduated a MSc degree in Biotechnology, in 2001, at the University of Food Technology – Plovdiv (UFT – Plovdiv). In 2006 he was awarded the degree “Doctor of Philosophy” at the Stephan Angeloff Institute of Microbiology – BAS (IMicB-BAS) and the year after has been appointed as a research fellow at the IMicB-BAS (division Plovdiv). The academic career development of Mr. Georgiev went through the Institute of Food Technology and Bioprocess Engineering, Technical University of Dresden, Germany, and the Institute of Biology, Leiden University, The Netherlands as a postdoctoral researcher, assoc. prof. at IMicB-BAS, guest-researcher at the Institute of Botany, Technical University of Dresden, Germany, department leader at the Center of Plant Systems Biology and Biotechnology in Plovdiv and currently a head of the Laboratory of Metabolomics, IMicB-BAS. Along with that he coordinates the SuSMAPWaste project at the University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania and, as highlighted above, he had a *Marie Curie Postdoctoral Fellow* in prestige international research organizations. These trainings definitely contributed to the progress of Milen Georgiev as a highly erudite and well-respected scientist, among the international scientific community, in the field of plant biotechnology. I would like to underline, that these are not short-, but rather long-term prestigious trainings with in-depth scientific studies, which are also confirmed by the publication activity of the candidate.

The current research of Assoc. prof. Georgiev is focused on the biosynthesis and the metabolic engineering of pharmaceutically-relevant plant-derived molecules and their sustainable mass production. The main emphasis is on natural compounds with anti-inflammatory and anti-obesity activity. Assoc. prof. Georgiev introduced for first time in Bulgaria the contemporary platform for metabolomics, based on nuclear magnetic resonance (NMR). Currently, the NMR-based metabolomics platform is successfully applied in the fields of biotechnology, life science and pharmacy.

Milen Georgiev is author/co-author of 130 scientific publications cited 2500+ times. In the “professor” competition he takes part with 57 publications, of which 28 in exponent B and 29 in exponent G. With regards to the thematic distribution, scientific contributions of assoc. prof. Milen Georgiev are in the field of plant biotechnology (*Publications No 23, 28, 50, 53, 55*), chemistry of natural compounds (incl. metabolomics and metabolite profiling) (*Publications No 5, 12, 31, 33, 34, 38, 42, 43, 52*) and pharmacology (*Publications No 10, 15, 17, 19, 20, 21, 25, 27, 29, 32, 35, 37, 54, 56*).

The candidate has delivered reports in excess of 60 scientific conferences in 20 different countries, as in 30 of the meetings he was an invited speaker. The total impact factor of the publications is **well over 390 (!)** and the citations currently, most likely, exceed **2500** with *H*-index - **31** (Google scholar) and **25** (Scopus). These impressive numbers are a real measurement for the significance of the scientific production of assoc. prof. Milen Georgiev and the impact, which his scientific investigations have for the development of the science in this particular research area. When judging the merits of the candidate, we have to start from the fact that over 2500 scientists around the world have relied to the scientific investigations of assoc. prof. Milen Georgiev and hence applied his achievements to continue successfully with their studies. In this case, when we evaluate if the candidate should receive the academic title “professor”, we should consider not only the number of the publications (which is by far above the requirements of IMicB-BAS), but also their impact over the progress of the science in his research field and the added value of these publications and their attached significance over the scientific investigations all over the world. The high impact factor mentioned above reveals that the publications of the candidate are published in high impact factor journals after profound peer review process from leading scientists in the respective research area. In all publications it is visible the contribution of the candidate as a specialist in the field of plant biotechnology and that the core ideas and leading role during the experiments, concerning the theme of the competition belongs to assoc. prof. Milen Georgiev.

In all publications provided for the current competition are clear the contributions in respect to obtaining and proof of new facts, confirm of hypothesis and establishment of new classification, technologies, methodologies and preparations. The scientific contributions of assoc. prof. Milen Georgiev are in the field of plant biotechnology, the chemistry of natural products (incl. metabolomics and metabolite profiling) and pharmacology. After analyzing the delivered set of publications, the following contributions of the candidate could be underlined:

- Establishment of new complex knowledge for metabolite regulation of the plant secondary metabolism.
- Metabolomics platform (NMR, GC-MS and LC-MS) for complex analysis of the metabolite fingerprints in the cells as a result of different biochemical reactions and its application for quality and quantity control of complex pharmaceutical products and food supplements.
- Isolation and purification of biologically active compounds by liquid-liquid chromatography.
- Antineoplastic potential and mechanism of action of extracts and pure molecules.
- Assessment of the anti-inflammatory and immunoregulatory activity of plant extracts and pure molecules.
- Assessment of the anti-virus activity of plant extracts and pure molecules.
- Development of new approaches for obtaining new phytochemicals based on the “green” chemistry concept.

For instance, so far unknown regulatory mechanisms for the biosynthesis of plant secondary metabolites (*i.e.*, iridoids and phenylethanoid glycosides) have been investigated, hence creating an effective instrument for regulation and better “exploitation” of the secondary metabolism in plants, further leading to the establishment of “plant cell/tissue factories” for sustainable production of pharmaceutically-relevant molecules.

A coherent model of the spatial repression of rutin biosynthesis by jasmonate-responsive transcriptional factors has been proposed. An efficient protocol for the establishment of transformed root culture of *Verbascum xanthophoeniceum* using sonication-assisted *Agrobacterium rhizogenes*-mediated transformation has been reported. A design of glass-column bioreactor with pulsed aeration, suitable for hosting Devil's claw (*Harpagophytum procumbens*) cell suspension cultures and biosynthesis of pharmaceutically-important verbascoside has been developed. These results confirm the complexity of the

biosynthesis of secondary metabolites in plant *in vitro* systems for the mass production of value-added molecules.

Special interest and high appreciation are gaining the publications dedicated to the multi-metabolite analysis of intact plants and different *in vitro* systems, as well as, the polar and volatile metabolites of plant cell suspension cultures. Metabolomics in combination with transcriptomics and proteomics in a holistic approach is forming systems biology, which synergism opens new avenues for complete investigation of the biochemical processes in plants, aiming to accelerate the process for discovery of new pharmaceutically-relevant molecules. At present, the complete analysis of the “chemical fingerprints” created by the metabolic processes start to play an important role in the personalized medicine. Thus, the application of the metabolomics (NMR and HPLC) has been developed for a phytochemical characterization of different *Rhodiola* species, recognition of unique metabolites between them and identification of adulterated commercial products. In particular, these are the first publications in the field of plant metabolomics not only in Bulgaria, with regards to the combination of NMR-based metabolomics with principal component and hierarchical analysis for the investigations of metabolite differences in plant systems.

The NMR-based metabolomics combined with principal component and hierarchical analysis proved significant biochemical changes of the metabolites during plant growth and development. The established analytical platform is useful for investigation of the phytochemical variety in different *Rhodiola* species, the recognition of unique metabolites between them and the identification of adulterated products.

Beside the fundamental contribution, the presented publications have an applied contribution as well through the development of new classifications, technologies, methodologies and preparations. Two new minor “Valeriana type” iridoid glycosides from the leaves of *S. ebulus* L. have been isolated. One of the molecules is a rare representative of iridoid diglycosides, containing uncommon ribohexo-3-ulopyranosyl sugar moiety. A scheme for isolation of individual compounds from the fruits of *S. ebulus* has been developed and quercetin-3-*O*-laminaribioside, isorhamnetin-3-*O*-laminaribioside have been identified for first time in the genus *Sambucus*. Cimifugin was isolated for the first time from *Peucedanum schottii* fruits, and an efficient and rapid protocol for the isolation by using high-performance counter-current chromatography was established.

The fact that I frequently repeat “for the first time” unambiguously shows that the research of assoc. prof. Milen Georgiev and his respective scientific publications are paving new roads in science and technologies for obtaining new biologically active substances from

plant *in vitro* systems. The estimation of the anti-virus and immunoregulatory potential of extracts and pure molecules has not only scientific importance, but also a social impact. For instance, pure molecules from *Rhodiola rosea* L. appeared to modulate effectively the TNF-related apoptosis-inducing ligand (TRAIL), which might be supposed as an innovative approach to rescue the resistance to apoptosis in auto-immune diseases and cancer. Extracts and preparations of *H. procumbens*, as well as, verbascoside reveal significant anti-inflammatory activities, while forsythoside B, leucosceptoside B, and verbascoside, isolated from *Verbascum xanthophoeniceum* proved to be effective radical scavengers and cholinesterases inhibitors. In this regard the contributions are too many and essential, and hence cannot be summarized in a single review, but clearly emerged the significance of the obtained results and their impact for the development of science not only in Bulgaria, but also around the Globe.

The development of methods to obtain new materials with antimicrobial properties, based on green chemistry principles has been a target of research over the past few years. Assoc. prof. Georgiev has described a method for plant-based synthesis of nano-materials through ethanol extraction of *Melissa officinalis* L. The cytogenotoxicity of the synthesized nano-materials was evaluated. By utilizing an eco-friendly route, three types of nanoparticles were obtained, *i.e.* silver and gold nanoparticles, and bi-metallic silver/gold nano-architectures. It can be concluded that the bi-metallic nanoparticles seem to be the most promising materials for further applications.

Many young scientists are already walking through the steps made by assoc. prof. Georgiev. He is supervisor of three PhD students, six diploma students and nine trainees. A new scientific school is being created! And this is among the most valid things that should be taken into consideration when evaluating the merits of the candidate for the academic position “professor”! When summarizing the research activity of assoc. prof. Milen Georgiev I would like to highlight that it is featured with **actuality**, extremely **good methodology** of the studies, characterized with utilization, creation and exploitation of suitable contemporary methods and approaches; **significant results** for the science and practice, as well as, potential for future fundamental and applied research; **impressive scientific production** at very high level.

However, no matter how good they are, only publications cannot fulfil the complete profile of a contemporary scientist worthy of the prestigious academic position “professor”. A comprehensive scientific activity is required, which the candidate unambiguously possess. Assoc. prof. Milen Georgiev took part in 12 scientific projects with total amount over 33.5 million BGN, five of which he is coordinating, amounting 6.77 million BGN for the basic

organizations. All projects correspond to the scientific profile of the candidate, which is eloquence enough criteria for the high scientific value of the research publications of assoc. prof. Georgiev. Besides that, he is an invited reviewer for many scientific journals with high impact factor. Assoc. prof. Milen Georgiev serves as an associate editor of the journal *Phytomedicine* (IF 4.268; Q1; Elsevier), *Food and Chemical Toxicology* (IF 4.679; Q1; Elsevier) and *Food Frontiers* (Wiley). He is also a member of the editorial boards of *Biotechnology Letters* (Springer), *Chinese Medicine* (Springer) and *Molecules* (MDPI). These facts reveal the appreciation of the international scientific community to the merits of assoc. prof. Milen Georgiev as an established scientist. At the same time, the candidate holds several awards as Pythagoras awards for established scientist in the field of life science and engineering, Pythagoras award for established scientist in the field of technical science, Pythagoras award for a young scientist, Diploma for excellent scientific achievements from the Executive Board of BAS, Marin Drinov award of BAS for young scientist in biological science, seven awards for the SusMAPWaste project, coordinated by assoc. prof. Milen Georgiev and etc.

Another fact that deserves to be mentioned and dignifiedly valued is the teaching activity of assoc. prof. Georgiev – a specialized course “Metabolomics” for MSc students of the Biological Faculty at the Plovdiv University “Paisii Hilendarski”, as well as, the PhD students and the post-graduates, which I already mentioned above. Along with that, assoc. prof. Milen Georgiev is a co-founder and a member of the executive board of Bulgarian Phytochemical Society, chairman of the Pythagoras awards committee and a member of the executive board of Center of Excellence of Plant Systems Biology and Biotechnology.

I know personally assoc. prof. Milen Georgiev, since he was a student, and the scientific directions that he works on, hence I dare to state that in the field of plant biotechnology he is among the leading scientists in Bulgaria and through his ambitions, profound knowledge, pragmatism and broad international collaborations he has reached a very high level as a scientist and a teacher. This broad international reputation is decisive in that the candidate would hold the academic position “professor” with merits.

Conclusion: Assoc. prof. Milen Georgiev is very well trained and erudite scientist already appreciated by the international scientific community. He has presented an impressive scientific record with essential scientific and applied contributions and along with that he has vast opportunities for the multiplication of all achieved so far in new scientific directions. At the same time, he established and lead research groups, deliver lectures in Bulgaria and abroad, and is an editor of reputable journals in Europe. The submitted materials for the call

are exceed the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADAS) and the Rules for ADAS application, as well as specific Rules of BAS and that of BAS. Based on the evaluation of submitted set of documents, their significance and the content of their scientific and applied contribution, I found reasonable to suggest assoc. prof. Milen Georgiev to take the academic position “**professor**” in the scientific area of Technical Science, in the direction of 5.11. Biotechnology (Technology of biologically active substances), for the needs of the Department of Biotechnology (Laboratory of Metabolomics) at The Stephan Angeloff Institute of Microbiology – Bulgarian Academy of Sciences.

31.07.2020

Plovdiv

Reviewer:

(Prof. Albert Krastanov, DSc)