

## **Review**

by: prof. Iskra Vitanova Ivanova,

member of the scientific jury, according to the decision of the FC of Faculty of Biology, Plovdiv University "P. Hilendarski", protocols № 254/16.07.2020 and №255/11.09.2020 ON THE COMPETITION FOR THE ACADEMIC POSITION "ASSOCIATE PROFESSOR " In the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty Cell Biology, announced in the State Gazette, issue no. 57 of 26.06.2020

## **Brief biographical reference and assessment of the publishing activity**

Ch. Assistant Dr. Tsvetelina Georgieva Batsalova was born on December 26, 1983. She graduated with honors from the University of Plovdiv (PA) "Paisii Hilendarski" (bachelor and master), and in 2007 she obtained a professional qualification master in cell biology. In 2009-2011 she was a full-time doctoral student and successfully defended a dissertation for ONS "Doctor" on "The role of MHC class 2 and post-translational modifications of collagen type 2 for intercellular communication in rheumatoid arthritis." The work experience of the candidate in the specialty with his doctoral studies exceeds 9 years and she has acquired the following positions - as a biologist (2005-2006), full-time doctoral student (2009-2011), lecturer at the University of Plovdiv "P. Hilendarski". assistant (2011-2012) and as chief assistant from 2013 to the present. She has been a visiting researcher at the Karolinska Institute, Stockholm and Lund, Sweden. Ts. Batsalova is a member of the USB, sections Immunology, Biochemistry, Biophysics and Molecular Biology and BAC (Bulgarian Association of Cytometry)

## **General description of the submitted materials in the competition.**

The scientific works of assistant prof. Ts. Batsalova can be distributed in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations thereto, as well as the Regulations on the terms and conditions for obtaining degrees and holding academic positions at Faculty of Biology, Plovdiv University. For participation in the competition for the academic position of "Associate Professor" in the scientific specialty of Cell Biology, a total of 21 scientific papers were presented, which were not used in the procedures for obtaining the educational and scientific degree "Doctor" and for the academic position "Chief Assistant".

The documents and materials submitted by assistant prof. Ts. Batsalova, meet all the requirements of ZRASRB, the Regulations for application of ZRASRB and the relevant Rules of the Faculty of Biology at Plovdiv University "Paisii Hilendarski":

1. Criteria "A" - an abstract of a dissertation for awarding the educational and scientific degree "Doctor" - 50 points
2. Criteria "B" - 4 articles are presented (equivalent to a monographic work), which do not repeat the ones presented for acquiring the educational and scientific degree "Doctor", and for holding the academic position "Chief Assistant" (100 points). All of them are in specialized journals with impact factor, referenced and indexed in world databases and fall into category Q1. 4
3. Criteria "D" includes 12 publications in publications that are referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus), which are in categories Q1 - Q4 (214 points).
4. Criteria "E" includes 76 citations in scientific journals, monographs, collective volumes and patents, referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus) (152 points). According to the additional requirements of FB, 19 articles are presented, 14 of them with IF and in 10 Ts. Batsalova is a leading author (for required 14 with impact factor, 10 of them as a leading). Attached is a list of 76 citations for the required 10, defended graduates 7 (required 5), 11 participations in projects (required 2). To these must be added participation in Commissions, reviews for international journals, participation in editorial boards and participation in international scientific forums.

**The contributions of the submitted materials for participation in the competition for the academic position "Associate Professor" in the scientific specialty of Cell Biology can be grouped in several areas:**

***1. Contributions to the analysis of the autoimmune responses and elucidation of the cellular-molecular mechanisms of autoimmune diseases.***

The presented scientific publications on this issue include elucidation of the cellular-molecular mechanisms in the autoimmune disease rheumatoid arthritis and the development of methods and means for treatment and diagnosis of this disease. They are presented in publications under numbers 1,2,3,4,15. The results obtained in these studies may find application in the treatment of patients with rheumatoid arthritis, as the glycosylated form of the peptide CII259-273 binds to human MHC II molecules (DR1 and DR4). In addition, T cells specifically responsive to this peptide have been identified in patients with rheumatoid arthritis. The unmodified CII259-273 epitope of type II collagen was found to play a major role in activating CII-specific pathogenic T cells in the development of collagen-induced arthritis when the *in vivo* expressed MHC II haplotype is DR4. It was observed that

the activation of DR4-restricted CII-specific T cells depend mainly on the lysine residue at position 264 of the CII259-273 epitope, which is recognized in both unmodified and hydroxylated forms. Posttranslational modifications of proteins are extremely important for the induction and pathogenesis of a number of autoimmune diseases, including RA. The contribution is related to the development of a methodology for studying the interactions between glycosylated peptides and receptors based on a combination of structural-based virtual screening, ligand-based statistical molecular design and biological research. Structural analysis of the antigens presented by MHC class II proteins is extremely important for clarifying the pathogenesis and the possibilities for treatment of RA. The contribution is related to the development of a methodology for studying the interactions between glycosylated peptides and receptors based on a combination of structural-based virtual screening, ligand-based statistical molecular design and biological research. An analysis of the CII-specific humoral immune response in Bulgarian patients with rheumatoid arthritis has been published for the first time. Significantly elevated levels of anti-CII antibodies have been demonstrated in RA patients compared to healthy individuals and patients with other types of autoimmune disease.

## **2. Contributions towards the field of *in vitro* cytotoxicology and analysis of biological activity.**

Clarification of the cytotoxicity and biological activity of extracts, essential oils and purified new molecules from various plant species and microalgae is progressively developing scientific field with high potential for identifying new candidate agents and establishing nutritional supplements with beneficial effects on the immune system and general state of the organism. Publications № 6, 7, 8, 9, 11, 13 contribute to this field. The antioxidant activity of the essential oil from Southern Bulgaria and its *in vitro* cytotoxicity to human cell lines was studied for the first time. For the first time was proved an insight to the immunomodulatory properties of pectins isolated from the flowers of silver linden (*Tilia tomentosa*). New knowledge on the chemical and biological activity of three polysaccharide samples (PPS) isolated from the aerial parts of common purslane (*Portulaca oleracea* L.), from lavender (*Lavandula angustifolia* Mill.) and silver linden (*Tilia tomentosa* Moench) were performed. The obtained results prove the immunomodulatory properties of the studied polysaccharides and provide a perspective for the application of these substances in therapies that stimulate and support the activity of the immune system. Plants of the *Lamiaceae* family are often used as a source of new pharmaceuticals due to their broad-spectrum biological activity. In was clarified the *in vitro* effects on a panel of cell lines treated with acidified, alkalized or lipophilic extract of the medicinal plant *Clinopodium vulgare* L. Steroid saponins have a number of biological activities such as hemolytic activity, antidiabetic, anticoagulant and antitumor activity, as well as the formation of cholesterol complexes. The biological activity of two new furostanol saponins isolated from *Smilax aspera*

(*Liliaceae*) were examined. The compounds were studied with *in vitro* cytotoxicity assays on human normal amniotic cells and human lung cancer cells. The results show significant cytotoxicity with dose-dependent effect and IC<sub>50</sub> values in the range of 32.98-94.53 µM. Microalgae, including *Cyanoprokaryota*, are known to be a rich source of biologically active peptides, macrolides, alkaloids, fungicides, and enzyme inhibitors for therapeutic use. Recently, more and more attention has been paid to cyanoprokaryotes as potential sources of pharmaceuticals with different structure and biological activity, including cytotoxicity, immunosuppression, antiproliferative activity, and antitumor activity.

### ***3. Contributions towards elucidation of the role of different types of chemokines in pollen allergies out of the pollen season.***

Pollen allergy is one of the most common allergies worldwide and it is associated with cross-reactivity of IgE. This type of allergy can also cause the development of hypersensitivity to plant foods, inducing primary sensitization of sensitive individuals, which in turn leads to cross-reactivity and secondary allergy to foods of plant origin. Chemokines play a major role in the regulation of cellular trafficking during immune responses. In individuals with pollen allergy, the importance of chemokines has been studied mainly during active allergic reactions. Chemokines play a major role in the regulation of cellular trafficking during immune responses. In individuals with pollen allergy, the importance of chemokines has been studied mainly during active allergic reactions. Little is known about chemokine levels and their effect on the immune response out the pollen season, when allergic individuals show no clinical symptoms. Information on this scientific issue is provided in Publication №5 and 6. The obtained data provide a basis for the development of new therapies and strategies for monitoring pollen allergy.

### ***4. Contributions to the resolution of the taxonomic status of cyanobacterial species.***

For a long time, the taxonomy of cyanobacteria has been a significant scientific problem due to their simple morphology, high variability and adaptability to a variety of ecological niches. Publication №12 contributes to the elucidation of new taxonomic biomarkers based on the polyphasic approach. The paper presents phylogenetic analyzes based on the amino acid sequences of the outer membrane efflux protein (OMER) and DNA sequences of the 16S rRNA gene of 86 cyanobacterial species with fully sequenced genomes and proves that OMER is a more appropriate marker for resolving the taxonomic status of different cyanobacteria.

### ***5. Contributions towards evaluating the effect of prebiotics on the immunomodulatory and adhesion properties of different species and strains of the genus *Lactobacillus*.***

The positive effects of probiotics and prebiotics are mainly due to modulation of the composition and activities of the intestinal microbiota, as well as modulation of immunological reactivity in autoimmune diseases. Publication №14 proves that the immunomodulatory properties of probiotic bacterial strains are species-specific and prebiotic-dependent. New information is provided on the role of metabolic products from *Lactobacillus brevis* strains cultured in the presence of different prebiotics. Publication №17 contributes to elucidating the influence of different types of prebiotics on the adhesion abilities of three *Lactobacillus* species.

**6. Contributions towards the field of bionanotechnology - *in vitro* analyzes of the cytotoxicity of iron oxide nanoparticles (ION); biofunctionalization of nanoparticles and development of methods for diagnosis of autoimmune diseases.**

Iron oxide (II, III) nanoparticles have wide biomedical and industrial applications. Therefore, there is a need for more information on the potential toxic effects of ION. Publication №18 defines the *in vitro* cytotoxicity of iron oxide (II, III) nanoparticles towards different types of human and microalgae cells. ION provoked higher total protein production in algal cells, suggesting activation of a specific response to nanoparticle-induced cytotoxicity. The results presented in this article define the cyto- and ecotoxicological potential of ION and prove the need for a more detailed study of their biological activity.

Autoimmune diseases affect approximately 3 to 5% of the population worldwide and represent significant social and economic burden for modern society, similar to that of cancer and cardiovascular disease. A new perspective in this regard are the biofunctionalized nanoparticles. Publication №19 contributes to the development of a new type of rapid and sensitive diagnostic tests for rheumatoid arthritis. The obtained modified nanoparticles specifically bind and detect the presence of RA diagnostic markers in serum samples.

**Teaching activity**

Dr. Batsalova conducts laboratory exercises in "Cell Biology", "Ecology and Environmental Protection", "Biology", "Medical Biology", "Bioinformatics" and "Molecular Biology". She was the supervisor of 7 graduates and co-supervisor of one PhD student. Lecture courses for students from bachelor's programs and lecture courses for students from master's programs (for the specialty Biodiagnostics and for the specialty Reproductive Biology) have been developed. Two practical guides are presented for students from different biological specialties of Plovdiv University "Paisii Hilendarski", which can be used by students in related specialties from other universities (Papers №20 and 21). The textbooks are intended for use by students majoring in "Biology" and "Medical Biology"

at Plovdiv University "Paisii Hilendarski". In the practical textbook in Cell Biology the basic structural elements of the cell and their functions are presented theoretically and demonstrated in practice. Classical and new methods for studying the cell structure and processes are presented. The textbook on Animal Cell Cultures introduces students to the methods for maintaining sterility when working with *in vitro* cultures, as well as the basic techniques for establishing cell lines, methods for manipulating and maintaining animal and human cells in culture.

### **Critical notes and recommendations**

I have no critical remarks to the materials presented by assistant prof. Ts. Batsalova. They correspond to the theme of the competition, both in volume and quality. In addition, the documentation is designed precisely and allows to get a complete picture of all areas of the applicant's activity. Summarizing all the above I can summarize that my assessment of the research and teaching activities of Ch. assistant Tsvetelina Batsalova is emphatically positive.

### **Conclusion**

The documents and materials submitted by assistant prof. Ts. Batsalova, meet all the requirements of ZRASRB, the Regulations for application of ZRASRB and the relevant Rules of the Faculty of Biology at Plovdiv University "Paisii Hilendarski". She is already an established scientist in the field of cell biology, has the ability to focus on current issues and to present innovative ideas for specific therapy of severe, socially significant diseases such as osteoarthritis and allergies. From the analysis it is clear that ch. assistant professor Ts. Batsalova participated in the competition with scientific production, which in terms of scientometric indicators exceeds the requirements for the academic position of "Associate Professor": publications in journals with high IF (falling into categories Q1 and Q2) and citations in renowned international journals. To these must be added the educational activity: defended graduates, teaching activities, as well as participation in numerous projects. I strongly recommend to the members of the esteemed scientific jury, formed by a decision of the FC of the Faculty of Biology, protocols № 254 / 16.07.2020 and №255 / 11.09.2020 to propose to the FC to elect ch. assistant professor TSVETELINA GEORGIEVA BATSALOVA as a "ASSOCIATE PROFESSOR" in the professional field 4. Natural Sciences, Mathematics and Informatics, 4.3. Biological sciences, scientific specialty Cell biology.

Sofia, 16.11.2020

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prof. Iskra Ivanova