



REVIEW

According to the documents of the competition for the academic position "Professor" in the field of Higher Education 4. Natural sciences, mathematics and informatics, professional area 4.3. Biological sciences, (Microbiology and antibacterial effect of nanomaterials), announced in SG, no. 103 of 12.12.2023

REVIEWER: prof. Dr. Petya Koycheva Hristova

1. General presentation of the procedure:

The competition for " Professor " in the area of Higher Education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty " Microbiology and antibacterial effect of nanomaterials" has been launched for the needs of the Department of "General and Industrial Microbiology", at BF, Sofia University in SG no. 103 of 12.12.2023. The scientific jury was formed in accordance with order № ПД-38-93/14.02.2024, based on Art. 4 and Art. 29a of the Act for the Development of the Academic Staff in the Republic of Bulgaria, art. 60 of the Regulations for its implementation and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in Sofia University. The procedure for the disclosure and announcement of the competition provided for in the law and the Regulations has been followed and all the necessary documents have been provided in a timely manner. The competition documentation reflects the candidate's research and educational activities..

2. Biographical data, career growth and professional qualities of the candidate

The only candidate who submitted documents for participation in the competition for the academic position "Professor" in Professional Field 4.3. Biological Sciences, specialty Microbiology and antibacterial effect of nanomaterials is **associate professor Ilyana Atanasova Ivanova PhD.**

The candidate graduated with a degree in "Molecular and Functional Biology" in 1986 at the Faculty of Biology. In the Autobiography, Prof. Ivanova incorrectly indicated the information that she graduated with a "Master's degree in Biochemistry and Microbiology", which does not correspond to the data from the attached diploma. In the period 1986-1988, she worked as a biologist at the Institute of Genetics at the BAS and at the Department of Plant Physiology at the Faculty of Science of the SU. From 1989 to 1992, Iliana Ivanova was a full-time doctoral student at the Department of General and Industrial Microbiology. In 1994, she acquired the educational and scientific degree "PhD" after successfully defending a dissertation on the topic: "Microflora of the Tyulenovo oil field and opportunities for its practical application". In the course of developing the dissertation, Iliana Ivanova studied the biology and taxonomy of various physiological groups of bacteria (oil-degrading, sulfate-reducing, methanogenic, etc.), which have the potential for practical application in technologies for cleaning soils and waters contaminated with oil, as well as for stimulating of oil production. For the next three years (1994-1997), the candidate worked as an ecologist-biotechnologist at the "Exploration and extraction of oil and gas" enterprise, where she continued the subject of his dissertation on the bioremediation of oil-contaminated soils. In 1997, the candidate won a competition for an assistantship in microbiology at the Department of General and Industrial Microbiology in the Faculty of Science of SU. According to Certificate No. 15/19.01.2024 of the Human Resources Department, Iliyana Ivanova has successively held the following positions at the University: senior assistant since 1999, chief assistant since 2002 and associate professor since 2014.

Therefore, Associate Professor Dr. Iliyana Atanasova Ivanova complies with Article 56 of the Regulations on the Terms and Conditions for Holding Academic Positions of SU, as she occupied the necessary time for each academic position, which guarantees mastering the specific duties and responsibilities listed in their respective job descriptions.

From the presented resume and scientific works, it is evident a deep interest and accumulated professional experience in various current problems of applied microbiology. For several years, the candidate's professional growth has been related to the expansion and upgrading of knowledge in the field of bioremediation of water and soil, in the development of prokaryotic tests for the assessment of the toxicity of heavy metals in water, as well as with the introduction of fluorescence assessment methods of microbiota vitality in different ecological niches.

The establishment of a Bionanotechnology laboratory at the Department of General and Industrial Microbiology directs Dr. Ivanova's research activities in the field of the antibacterial

activity of nanoparticles and the possibilities for their practical application. With the results achieved in this area, the candidate fully meets the theme of the current competition.

3. Evaluation of the scientific production and the scientometric indicators of the candidate

Assoc. Dr. Iliyana Ivanova has submitted all the documents necessary for the competition in compliance with the requirements of the ZRASRB and the Rules of the SU. Unfortunately, there are a number of inaccuracies or omissions in some of the documents, which lead to a reduction in the marks for the individual criteria, but these do not affect the final positive mark. For example, I find a discrepancy between the total list of publications and the summary data in the CV and contributions, not all articles included in the minimum requirements document are indexed with the indicated points, not all project participations are presented correctly, and others are omitted.

3.1. Characteristics and evaluation of scientific and publishing activities

The total scientific research activity of the candidate is represented by a total of 67 scientific publications, 33 participations with posters and reports in national and international forums. The attached evidence presents the articles that meet the minimum requirements, but no evidentiary material (abstracts) from the participation in the scientific forums is found. It makes a good impression that the publications on the competition are mainly in three main directions, affecting various current problems of nanomaterials and are strictly in accordance with the theme of the competition.

Publications in specialized refereed and indexed foreign and our scientific journals with IF/ SJR and quartiles Q1-Q4 are a total of 30 and book chapters - 4. Scientific and educational publications in journals without IF and SJR are 33. The total IF of all publications is 31.61 .

3.2. Evaluation of citations of scientific works

The candidate's published scientific results according to the Scopus database have been cited 133 times (without self-citations) and the citation index h factor is 8 (according to Scopus). The candidate presents citations in other databases as well, which increases the number of proven citations to 165.

3.3. Evaluation of the implementation of the minimum national requirements for the relevant scientific field and requirements of SU "St. Kliment Ohridski"

The minimum national criteria according to the RASRD have been met according to the presented scientometric indicators:

According to Indicator A - Dissertation work for the award of the educational and scientific degree "doctor" - 50 points, proof of diploma No. 23088/25.05.1994.

According to Indicator B, the requirement of 100 points is fulfilled by the presentation of a monograph on the topic "Biological effects of nanomaterials".

According to Indicator G, evidence is presented for 240 points out of the required 200 minimum points, which are formed by 14 publications, of which 6 - Q2, 7 - Q3 and 1 chapter in books. I do not find evidence of indexing of articles №13 and №14, therefore I exclude the points indicated by the candidate from the final evaluation for this indicator.

According to Indicator D, evidence is presented for 140 citations after habilitation for associate professor, which give 280 points and exceed the minimum criterion of 100 points.

According to Indicator E In this paragraph, the candidate forms 165 points with a minimum required of 150 points. Some of them are obtained as a participant in 1 national research project (10 points), 3 projects with national co-financing under the international COST program (60 points.) and as a participant in 3 educational projects (30 items). I do not accept the candidate as the head of the educational project BG05M2OP001-2.09-0013 under the operational program "Science and education for intelligent growth 2017-2019", where as a participant she developed a new course for training doctoral students on the topic "Nanomaterials and interactions with cells". Dr. Ivanova is co-scientific supervisor of a successfully defended doctoral student (25 points) and author of a textbook on "Ecotoxicology and nanotechnologies" (40 points).

3.4. Evaluation of teaching and learning activities, scientific guidance students and administrative experience

Dr. Ivanova, as a teacher in the Faculty of Biology, actively participates in the training of students, graduates and doctoral students. The academic report from the Department of Educational Activities for the last five academic years shows a serious classroom workload of an average of 445 hours per year, which is almost 1.5 times more than the required hours. Prof. Ivanova gives lectures on two mandatory courses in Microbiology and Virology at the "Bachelor" University of Applied Sciences for the majors Geography and Biology, Biology and English, Biology and Chemistry (full-time study) and for Special Biology (part-time study). Also, she conducts practical classes in Microbiology for special Biotechnologies and special Pharmacy (in English) at FCHF. Assoc. Prof. Ivanova has developed two elective courses in the OCS

"Bachelor": "Prokaryotic tests for ecological control of the environment" and "Bioethics" for special BMUR. In the Master's degree, she gives lectures and exercises in two courses - in Sanitary Microbiology for MBMBK and Physicochemical Food Control for KBH. The MP MBMBK has developed an elective course "Ecotoxicological tests for environmental assessment and control". In the CV, there are again inaccuracies regarding the names of elective subjects.

To date, under her guidance, 24 students (17 masters and 7 bachelors) have successfully defended their theses. She is the supervisor of one successfully defended doctoral student and is currently teaching one full-time doctoral student. Also, she has been actively involved as an academic mentor in the Student Internship program.

Assoc. Prof. Ivanova does not participate in the administrative activities of the faculty and department, but participates in the evaluation of candidate student exams, which, according to the attestation cards, is counted as an administrative activity.

3.5. The project activity of the candidate was evaluated

The submitted reference shows the candidate's participation in national and international projects. Evidence of participation in one national project financed by the Ministry of Education and Science, in three projects with national co-financing to the European scientific network COST, five projects financed by the FNI at SU in support of doctoral students and three educational projects are presented.

Conclusion under item 3: *The candidate for the current competition for the academic position "Professor", Assoc. Ph.D. Iliyana Atanasova Ivanova, meets the minimum criteria of ZRASRB and the Regulations for its application and even exceeds them (835 total points).*

4. Evaluate the original scientific contributions of the candidate

The monograph presented by the candidate on the topic "Biological effects of nanomaterials" collects and summarizes the synthesis methods of nanomaterials and nanocomposites developed so far, with the main attention being paid to the relationship between their composition and their biological properties. The main contributions of the monograph are related to the in-depth analysis of the data published so far regarding the physico-chemical and molecular mechanisms for the implementation of the antimicrobial activity of the nanomaterials, establishing the specific mechanisms of toxicity, as well as the genetic mechanisms of protection and reactions of the tested pathogens under the action of nanocomposites with low content of

copper and silver nanoparticles. This study proves the existence of a general plan for the effect of nanomaterials on cells with different structural organization. According to this plan, hydrophobic nanomaterials interact with cell membranes and cause breakthroughs in them. Destruction of cell walls and destruction of cell membranes occurs. Metal nanoparticles, metal-oxide and graphene materials cause the formation of reactive oxygen radicals when interacting with macromolecules in the cell cytoplasm, disrupt basic metabolic pathways in cells by changing the configurations of enzymes, prevent DNA replication and induce apoptosis. A significant fundamental contribution is the described effect of copper and silver nanocomposites on the gene expression of the industrial strain *Escherichia coli* NBPMKK 3548. The monograph discusses etc. "green" methods of synthesis of nanoparticles with extracts of microorganisms and plants, which are alternative possibilities to obtain new nanocomposites and stabilizing substances that can enhance and improve the antimicrobial effect of nanomaterials. Substantial attention is given to the problems related to the incomplete assessment of the safety of newly synthesized materials, which have been confirmed by own research. The information presented in the monograph is an important basis for improving the existing standards in determining the risk loads in the production of nanomaterials and their release into the environment.

The research work of Prof. Iliana Ivanova, reflected in the attached publications, is characterized by scientific developments grouped in three main directions:

- ✓ study of the antimicrobial effect of newly synthesized nanomaterials and nanocomposites,
- ✓ prevention of microbial adhesion and biofilm formation;
- ✓ creation of biologically active thin films with antimicrobial action.

I. Investigation of the antimicrobial effect of newly synthesized nanomaterials and nanocomposites

Five scientific articles, one book chapter and nine participations in scientific conferences have been published in this direction. The object of research is the antibacterial activity of monometallic, metal oxide and non-metallic newly synthesized nanomaterials. The obtained data have an applied contribution as they prove that silver, copper, zinc-oxide, niobium, selenium and gold nanoparticles are the most cytotoxic to the tested bacteria. Graphene-oxide nanoparticles also show a stand-alone cytotoxic effect. It has been confirmed that metal nanoparticles conjugated with small polymer molecules are more active antimicrobial agents than pure metal nanoparticles due to delayed agglomeration. For the first time, the antimicrobial and pro-oxidant activity of newly synthesized zinc oxide nanoparticles, nanoclusters and those coated with silica have been

compared. The pro-oxidant activity of all nanomaterials was investigated according to free radical oxidation reactions in chemiluminescent model systems.

Research in this direction leads to the fundamental conclusion that the structure of nanoparticles and the approaches to their preparation are of crucial importance for their properties.

II. Prevention of microbial adhesion and biofilm formation

11 articles have been published in this direction, which suggests a thorough study of the antimicrobial effect of nanocomposites preventing microbial adhesion and biofilm formation. The object of the studies are nanocomposites of nanoparticles embedded on biological (collagen, fucoidan) or chemical polymers (siloxane, graphenes) and combinations between them. The antibacterial properties of collagen nanocomposites linked to graphene, graphene oxide, (GO), zinc-oxide and metal nanoparticles were investigated and their pro-, antioxidant and biological activities were evaluated by luminescent and standard microbiological methods. An important applied result is the found higher sensitivity of tumor cell lines compared to non-tumor cells to the graphene composites, proving their antitumor activity.

The obtained new collagen/(ZnTiO₃ - zinc titanate) composite biomaterials, by using cryogenic drying technology, have broad-spectrum antimicrobial activity against Firmicutes (*Staphylococcus epidermidis*, *Bacillus cereus* and *Candida lusitanae*) and Gracilicutes (*Escherichia coli*, *Salmonella enterica* and *Pseudomonas putida*) microorganisms and are cytotoxic to model eukaryotic cells (osteosarcoma, fibroblast and keratinocyte cells). Both activities depend on the concentration of zinc titanate, and the optimal balance between antimicrobial activity and cytotoxicity can be achieved by varying its concentration.

New biofilm-preventing anti-adhesive composites with fucoidan, antioxidants and siloxane polymers have been developed for the first time. Complete elimination of biofilm formation has not been reported so far. For the first time, the ability of a non-toxic, natural, biologically active substance such as fucoidan to inhibit bacterial growth on medical coatings of catheters and implants has been investigated. It was investigated how different amount of fucoidan affects the surface characteristics and bioadhesion as well as bacterial cell growth.

The first report on the antibacterial activity of medical siloxane coatings containing fucoidan is also referred to in this group of publications. The biological activity of the studied siloxane coatings is attributed to the formation of a weakly adhesive, biologically active surface top layer consisting of siloxane oil and dispersed water-soluble fucoidan particles.

Another important applied contribution is the development of a collagen-based composite biomaterial with improved antimicrobial properties and activity using a combination of zinc titanate embedded in a silane matrix (ZnTiO₃/SiO₂) and fucoidan at different concentrations. A broad-spectrum antimicrobial activity of the studied porous collagen /(ZnTiO₃/SiO₂)/fucoidan composite was established, specific for the different test microbial strains and dependent on the concentration of fucoidan.

An important contribution in the research of new anti-adhesive coatings is the study of their action against the formation of biofilms. The use of antioxidants in protective coatings to inhibit marine biofilm formation has been unexplored to date. The effectiveness of low-adhesion siloxane composite coatings equally loaded with different antioxidants against *Marinobacter hydrocarbonoclasticus* biofilm formation was characterized. Not all antioxidants were found to reduce monospecies biofilm formation.

III. Creation of biologically active thin films with antimicrobial action

In this direction, six articles and three reports have been published in conference proceedings. Data on the preparation of thin films by magnetron sputtering are presented and over 200 different nanoparticle compositions and ratios are investigated. A synergistic effect of copper and silver nanoparticles encrusted on a titanium dioxide coating and an enhancement of the antimicrobial effect when replacing the copper nanoparticles with those of silicon dioxide were found. This applied contribution relates to the production of an antimicrobial film that can cover critical contact surfaces in hospitals, public facilities, in the food and pharmaceutical industries.

A contribution to academic education is the compilation of the textbook "Ecotoxicology and Nanotechnology" (2018) of the University Publishing House St. Kliment Ohridski for students from all biological majors and degrees of education.

In view of the above data, the following conclusion can be made:

CONCLUSION

I give a positive assessment of the research, teaching and project activities of Assoc. Prof. Iliyana Atanasova Ivanova and consider that she fulfills the minimum requirements of ZRASRB and the Rules for its implementation for occupying the scientific position "Professor" in

professional direction 4.3. Biological Sciences, (Microbiology and antibacterial effect of nanomaterials).

Sofia

REVIEWER:

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Prof. Petya Hristova