

## **REVIEW**

from **Prof. Maria Angelova, DSc**, The Stephan Angeloff Institute of Microbiology,  
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on the competition for the occupation of the academic position "Associated Professor" in professional field: 4.3. Biological Sciences, scientific specialty Microbiology at the Department of Biochemistry, Microbiology and Physics at Trakia University, presented to a Scientific Jury formed by order of the Rector of the TrU № I-81/01.07.2020

The only candidate announced in the State Gazette issue 61/10.07.2020 and the website of the Thracia University (TrU) for the academic position of "ASSOCIATE PROFESSOR" is Dr. Toncho Gospodinov Dinev, Assistant Professor at the same University.

### **General presentation of the procedure and the candidate**

The set of documents and materials provided by Asst. Prof. Dr. Toncho Dinev are in accordance with the requirements for holding the academic position of "Associate Professor" in the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB). The documents also meet the additional criteria of the Faculty of Agriculture (AF) at TrU.

Dr. Toncho Dinev was born in 1977 in Stara Zagora, where he graduated from the National Professional High School of Veterinary Medicine. In 2001 he received the Master's degree in Veterinary Medicine at the Faculty of Veterinary Medicine (FVM) at TrU. In the same faculty he developed a dissertation titled "Comparative studies on side effects, antimicrobial activity and pharmacokinetics of aminoglycosides and aminocyclitols in goats and microorganisms isolated from goats", which he successfully defended in 2007 and obtained the doctor degree. From 2009 until today, Dinev has been successively Asst. Prof. of Pharmacology and Microbiology at the FVM and Asst. Prof. at Microbiology at the Department of Biochemistry, Microbiology and Physics of the AF at TrU. During these 11 years of scientific experience, Dr. Dinev has formed his interests purposefully to the mechanisms of antimicrobial action of modern pharmaceuticals and extracts from new sources. His scientific career is entirely related to the topic of the competition and reflects current and promising areas of Microbiology.

Dr. Dinev actively participates in the public life of the microbiological community as a member of the Bulgarian Association of Microbiologists. He is fluent in written and spoken English and very good Russian. Dinev has also high computer competence.

### **Materials submitted for review and compliance**

Dr. Dinev is the author and co-author of 24 scientific papers. Five of them are related to his PhD degree (abstract, dissertation and 3 scientific papers), these are not subject to review under the Rules of the TrU for the application of ZRASRB. I accept for review 19 scientific papers (17 in English and 2 in Bulgarian), of which 17 are journal papers, 1 monograph and 1 textbook. Six articles and 2 poster presentations are in journals with impact factor, 8 - in journals with SJR and 1 - in a non-refereed

journal. The total IF of Dinev's works is 11.435, and for those participating in the competition it is 9.45. The results of the candidate have received a wide response among the international scientific community, they are cited 136 times and form an *h*-index 6 (Scopus).

The report on the fulfillment of the minimum requirements for the academic position "Associate Professor" shows that the candidate covers and exceeds the required points on the individual indicators, and instead of the required 400 he dials 539.67.

- Indicator A - PhD Autoreferat.
- Indicator C - monograph - 100 points.
- Indicator D - 17 scientific papers, 6 papers with IF (Q1 - 1, Q2 - 4, Q3 - 1, Q4 - 1); 2 poster presentations with IF (Q1 - 1 and Q2 - 1) and 8 with SJR. Total number of points is 237 instead of required 200.
- Indicator E - 68 citations (Scopus) - 136 points at the required 50.
- Indicator F – Asst. Prof. Dinev is an active participant in the National Scientific Program "Healthy Foods for a Strong Bioeconomy and Quality of Life" and co-author of the Manual of Microbiology - 16.67 points.

Dr. Dinev exceeds the requirements of the additional criteria of TrU, as instead of the mandatory 405 points, he presents evidence of 539.67 points. In addition, he is the first author in 2 of the papers and a single author in 2. In my opinion, this is an indication of the candidate's scientific activity, albeit in the position of Asistant Professor. I would like to note that the works of Dr. Dinev have been published in renowned specialized publications with IF, e.g. J. Vet. Pharmacol. Ther., Vet. J., Br. Poult. Sci. Fresenius Environ. Bull. etc., which allow to characterize his scientific output as topical.

The scientific work of Asst. Prof. Dinev includes also 10 participations in 4 international and 6 national scientific forums disseminating the results of his scientific research.

### **Education activity**

Teaching work is one of the main activities of the applicant. It is entirely in the field of this competition. For two years he trained students from the Department of Pharmacology at the VMF of TrU. After that, for 10 years he actively participated in the educational process of the Department of Biochemistry, Microbiology and Physics at the AF of TrU in 4 disciplines:

- "Microbiology" for the stidents of Agronomy, Zooengineering and Fish Farming (practical exercises and lectures).
- "Microbiology and microbiological methods of purification" – for the students of Ecology and Environmental Protection (practical exercises).
- "Biological control of water treatment" –for the students of Fish Farming and Aquaculture (practical exercises and lectures).
- "Microbiology of meat and meat products" – for thr students from the master's program in Zooengineering (practical exercises).

Asst. Prof. Dinev is the author of the programs by the disciplines "Biological control of water purification" and "Microbiology of meat and meat products" and co-author of two other programs - "Microbiology" and "Microbiology and microbiological methods of purification".

The academic report from TrU shows that the planned employment of the position of "Associate Professor" in the department for 2020/2021 is 399 hours, which provides the necessary workload.

The candidate is a co-author of the Manual of Microbiology, published in 2019. The Manual is well structured, compiled in accordance with the lecture courses and practical exercises included in the curriculum in "General Microbiology". It is intended for students of several disciplines of AF at TrU, but can be successfully used in other universities, by teachers of secondary school and professionals in microbiological practice.

I give a positive assessment of the overall teaching work of Dr. Dinev, including his active participation in the training of young professionals - he is co-supervisor of 9 graduates who have successfully defended their bachelor's theses.

### **Scientific activity and contributions**

Research activity of Dr. Toncho Dinev is concentrated in the field of the announced competition. Dr. Dinev participates in a studies elucidating the mechanism of action of antimicrobials, including probiotics. In this sense, the relevance of the scientific direction of the applicant are indisputable. In addition, in most cases he directs his activity to unexplored niches, which creates the preconditions for original contributions.

The scientific works of the applicant, which are presented for the competition, can be grouped and analyzed in the following directions:

#### **I. Study of the pharmacokinetics and side effects of some modern antimicrobial agents (antibiotics, fluoroquinolones, etc.) in the body of different species of animals**

Dr. Dinev's work in this direction is a purposeful study of the behavior of modern antimicrobials in animals (papers №№ 6 - 11, 13, 15, 25 – 28). Worldwide, antimicrobial agents are most commonly used as veterinary drugs for the treatment and prevention of animal diseases. They also have a positive role in providing healthy food of animal origin. Their importance is growing with the spread of the antibiotic resistance problem. Clarification of the pharmacokinetics (PK) and pharmacodynamics (PD) of antimicrobials and the relationship between them (PK/PD) are extremely important for their successful administration. The research activity of Dr. Dinev aimed to optimize the concentration and the method of administration, taking into account the type and sex of the animals. Animal species that have not been studied so far are included in the experiments. The following important contributions have been made in this section:

1. New data have been obtained on the kinetic behavior of the fluoroquinolone preparation pefloxacin and its metabolite norfloxacin in ducks depending on the form of administration. Faster absorption and higher elimination rate of pefloxacin have been demonstrated when administered orally and the low level of its metabolism to norfloxacin in both forms of administration.

2. Original data have been established on the behavior of a large group of antimicrobial compounds (sulfonamides, fluoroquinolones, penicillins, aminoglycosides and aminocyclitols) in the body of birds of different sexes. It has been proven that the rate of elimination depends on the type of organism, gender, and mode of administration.

3. Important information has been obtained on the pharmacokinetics of tobramycin following intravenous and intramuscular injection in ducks. The proven rapid absorption of the antibiotic when administered IM and its rapid elimination compared to other avian species can be very useful in the treatment of systemic diseases combined with antibiotic susceptibility.

4. Accelerated metabolism of enrofloxacin to ciprofloxacin in quails has been demonstrated for the first time. The analyzes showed low intersubject variability for danofloxacin and marbofloxacin, in contrast to that for enrofloxacin and its major metabolite ciprofloxacin. Due to their more favorable oral pharmacokinetic properties, danofloxacin or marbofloxacin are preferred over enrofloxacin for the treatment of susceptible bacterial infection in these birds.

5. For the first time, experiments on pheasants, Japanese quails and guinea fowl have shown that seabirds need higher doses of fluoroquinolones for therapeutic efficacy and microbial susceptibility.

6. An HPLC-based method has been developed to monitor the pharmacokinetics of the antibiotic spectinomycin in goats, which provides higher sensitivity and precision. The method was used successfully to study the behavior of enrofloxacin and its metabolite ciprofloxacin in the body of Japanese quails.

7. In original experiments, gentamicin and tobramycin have been shown to cause remarkable changes in goat ESR, even after treatment with therapeutic doses. Based on the results obtained, it is recommended to pay more attention to ESR data after treatment with these antibiotics.

8. The effect of the fungicide mancozeb on the development and reproduction of rainbow trout has been determined for the first time. The permitted concentrations used have been shown to be safe for consumers but they caused reproductive disorders in fish. Effective measures have been proposed to overcome these difficulties.

## **II. Antimicrobial activity of probiotic bacteria, plant species, plant extracts, foods and environmental factors**

The second direction (papers №№ 16, 17, 21 - 23, 29, 33) is focused on one of the most current problems today - antimicrobial resistance (AMR). This phenomenon has sparked a resurgence of interest in alternative antimicrobial treatment strategies. Much of the scientists' efforts are focused on microorganisms and medicinal plants, which are known as natural laboratories for production of agents unique in their structure, diversity and complex nature. Despite intensive research worldwide, scientific works is constantly growing in the direction of hitherto unexplored sources. Due to his qualification as a pharmacologist and microbiologist, Dr. Dinev actively participates in the work of research teams looking for new antibacterial and antifungal agents.

This direction includes the published monograph "Antimicrobial potential of probiotic lactic acid bacteria". This article summarized the knowledge in the field of lactic acid bacteria (LAB) and their useful properties for human health. The experience of the scientific community for studying the probiotic characteristics of LAB and the possibilities for their application in the food industry, medicine and biotechnology is also analyzed.

I consider that the more important contributions in this section concern the following:

1. Scientific information published in the last 10 years on the antimicrobial activity of probiotic species of lactic acid bacteria as *Lactobacillus plantarum*, *L. acidophilus*, *L. gasseri*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* has been collected, summarized and analyzed. The most important factors and mechanisms of antimicrobial activity (*in vitro* and *in vivo*), as well as the possibilities for biological control and production of modern functional foods on a probiotic and synbiotic basis have been considered and analyzed in detail.

2. New information has been obtained on the antibacterial and antifungal activity of seaweed extracts (*Ulva rigida*, *Cladophora vagabunda* and *Ceramium rubrum* distributed in the Black Sea) against bacteria and mycetes, including clinical isolates. Ethanol extracts from *C. vagabunda* and *C. rubrum* have been shown to be promising for use against susceptible pathogenic bacterial species causing food spoilage as well as mycotoxin-producing mycetes.

3. For the first time the fungicidal activity of ethanol and methanol extract from different parts of the plants *Amaranthus deflexus*, *A. retroflexus* and *A. hybridus* against 5 species of mycotoxigenic fungi (*Aspergillus ochraceus*, *A. niger*, *Fusarium graminearum*, *Penicillium verrucosum* and *P. expansum*) has been proven. This is a significant contribution to the fight against fungal infections in plants, as well as to reducing losses in the food and feed industry.

### **III. Microbiological studies of industrially significant processes**

This aspect of the scientific activity (papers №№ 18, 20, 30, 31, 32) includes scientific and applied developments in the field of Ecological, Veterinary and Food Microbiology. Dr. Dinev participates in interdisciplinary studies that provide new information about microbiological parameters and their compliance with the legal norms of industrial processes of regional importance. The contributions in this direction emphasize the responsibility of the candidate to the problems of society, namely:

1. Original data have been obtained on the high antimicrobial activity of *Lemna minuta* (water lentil) when it is cultivated as the only plant in an aquaponic recirculation system. The prospect of using *L. minuta* as a biological agent for purifying an aquaponic system has been proven. In addition, the biomass thus obtained can be used in Ecological Biotechnology for the purification of waters contaminated with heavy metals.

2. The microbial profile of the waters of Ovcharitsa Dam, used for cooling of TPP "Maritsa Iztok 2", has been determined. The analysis includes indicators for pollution with coliforms and *Salmonella* spp. Non-compliance with the requirements of the Bulgarian legislation to their use for irrigation of agricultural lands has been proved.

3. On the basis of microbiological analysis of sewage sludge from municipal treatment plant and poultry processing enterprises, an assessment of the vermicomposting process with the epigeic red worm *Lumbricus rubellus* has been made. The use of this method has been shown to improve microbiological parameters (total number of microorganisms, coliforms, *E. coli*, *Salmonella* spp., etc.), but does not eliminate spore-forming bacteria. The results obtained can be very useful for improving the process.

4. The compliance of the regulatory requirements for white brined cow's milk cheese to the physicochemical and microbiological parameters determined during the maturation of this product produced in a regional farm has been demonstrated. The obtained data certify the production of this farm as safe.

5. An assessment of the hygienic and ecological parameters and the corresponding microbiological indicators in a farm for intensive rearing of rabbits in the region has been made. The obtained information makes it possible to significantly reduce the content of aerobic mesophilic microorganisms by maintaining a favorable microclimate in the premises.

#### **Evaluation of the candidate's contributions**

I consider the contributions of Dr. Dinev to be significant enough. He presents achievements of obvious theoretical and methodological nature, including new information and confirmatory results in the main areas of Microbiology, as well as solutions of practical problems of regional significance. Dr. Dinev successfully combines theoretical research with specific experimental tasks and achieves real practical results.

In my opinion, Dinev has his own place in the activities of the team he works with. His qualifications and experience contribute greatly to the realization of ideas and achievement of goals that determines his personal share in the contributions.

#### **Participation in research projects**

Dr. Dinev has led the implementation of a project funded by the AF of TrU and has participated in the development of 5 projects, 1 of which is funded by the National Program and 4 - by the AF of TrU. All of them are in the field of Microbiology and concern the main directions of Dinev's research. I would also like to emphasize his activity in disseminating the results among the scientific community.

#### **Critical remarks and recommendations**

I do not have any critical remarks on the merits, but I think that the description of the contributions requires a greater summation of the results and a more careful categorization as "for the first time". In addition, I recommend Dr. Dinev to publish the full range of results included in the two poster presentations (№№ 7 and 9) in order to make them available to the scientific community.

#### **Conclusion**

Asst. Prof. Dr. Toncho Dinev is a well-established lecturer and promising scientist in the field of this competition, distinguished by his own scientific profile and modern approach to research. His teaching activity corresponds to the current requirements of higher education. He is the author and co-

author of curricula and textbooks, works actively with graduates. The published monograph of the candidate is also aimed at preparing students.

Dr. Dinev is a sought-after partner in the development of research projects and an active member of the teams he works with. The presented scientific articles define him as a professionally competent specialist in the field of Microbiology. They have been published in renowned journals and have become known to our and the international scientific community. The formulated scientific and applied contributions are the basis for further developments. His participation in specific applied tasks at the regional level is a testament to his responsible attitude to the problems of practice.

The entire activity of Dr. Dinev in terms of scientometric indicators meets the requirements of the ZRASRB, as well as the Regulations of the AF TrU for holding the academic position "Associate Professor".

In view of all mentioned above, I strongly recommend to the members of the Scientific Jury, formed by order № 2466/01.10.2020 of the Rector of the Thrakia University, Stara Zagora, to prepare a report-proposal to the Faculty Council for the selection of Assistant Professor Dr. Toncho Gospodinov Dinev to the academic position "ASSOCIATE PROFESSOR" in the professional field 4.3. Biological sciences (Microbiology).

30.10.2020

Sofia

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/Prof. Maria Angelova, DSc/