REVIEW

for holding the academic position of Associate Professor
presented to the scientific jury appointed by Order No РД-38-570 / 03.12.2020 of the Rector of Sofia University "St. Kliment Ohridski"

Subject: competition for the academic position "Associate Professor" in the professional area 4.3. Biological Sciences (Microbiology – General microbiology and Biology of extreme microorganisms), announced in SG No 88 / 19.10.2020 for the needs of the Faculty of Biology of Sofia University - Department of General and Industrial Microbiology.

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An application for participation in the announced competition has been submitted by a single candidate - Ch. Assistant Dr. Anna Atanasova Tomova from the Department of General and Industrial Microbiology at the Faculty of Biology.

All documents for the competition are prepared precisely and accurately and are presented according to the requirements set out in the Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions at Sofia University "St. Kliment Ohridski".

1. General information about the professional career of the candidate

Ch. Assistant Professor Dr. Anna Atanasova Tomova graduated from the National High School of Natural Sciences and Mathematics in 1993 and graduated from Sofia University with a Master's degree in Molecular Biology, specializing in Microbiology at the Faculty of Biology in 1999.

In the period 2000-2002 she works as a biologist at the Institute of Microbiology and after few years became PhD student, graduated in 2011, received her educational and scientific degree and since 2012 she has been a senior assistant.

From 2015-2016 he was a senior assistant at Reacher Center (NIS) at Sofia University.

From 2017 to present he is a senior assistant in the Department of General and Industrial Microbiology at the Faculty of Biology of Sofia University.

In the course of her education and professional development, Chief Assistant Dr. Anna Tomova acquires professional experience and skills in two main areas: research and teaching activities.

The professional realization of the candidate so far is entirely related to the topic of the competition and reflects current and promising areas of microbiology and in particular in the field of molecular biology of microorganisms, biology of extreme bacteria and biodiversity of extreme habitats.

Chief Assistant Anna Tomova is developing as a scientist at the Institute of Microbiology of the Bulgarian Academy of Sciences where she works in the laboratory of Extremophilic bacteria where she defends her PhD thesis. In this laboratory she receives high methodological competence in various modern problems of molecular biology, microbiology, molecular taxonomy, study of various enzymes and others. She specialized in two renowned European laboratories and was a member of the teams of several national and international projects.

After entering the Faculty of Biology, Department of General and Industrial Microbiology Anna Tomova is involved in the research of the Laboratory of Industrial Microbiology and thanks to the teamwork skills to participate in few national and international projects. He grew up as a teacher very quickly. She is assigned practical classes and cycles of lectures in basic courses for the Department in the bachelor's degree such as General and Soil Microbiology (Bachelor's degree, specialty Agrobiotechnology), Microbiology (Bachelor's degree, Biomenagment and Biotechnology), Genetic Engineering (Bachelor's Degree, Agrobiotechnology) as well as those in the Master's degree as Organoleptic Analysis, Genetically Modified microorganisms in Food (Master's Degree, Food Quality and Safety), Biology of Extreme Microorganisms, Molecular Biology of prokaryotic and eukaryotic microorganisms, Antibiotics and Antibiotic Resistance (Master's Degree Microbiology and Microbiological Control).

Since 2017 she is responsible for organization of Master program Microbiology and microbiological control and permanent member of the working group of the Council of Specialties of the Faculty of Biology.

1. **Analysis of the materials submitted for participation in the competition**

A comparative analysis of the materials submitted for participation in the competition, in accordance with the state requirements for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application, and the additional requirements in accordance with the Regulations of Sofia University "St. Kliment Ohridski" is presented in the table below.

Indicators group	**Content**	**Requirements for "associate professor" position**	**Applicant data**
A	Indicator 1	**50**	**50**
B	Indicator 2	**-**	**-**
C	Indicators 3 or 4	**100**	**100**
D	Indicators 5 to10	**200**	**205**
E	Indicator 11	**50**	**448**
F	Indicator 12	**-**	**290**

The applicant presents a total of 20 research papers, 17 of which are in journals with impact factor and SJR (according *Scopus* database) and 3 are in proceedings from scientific forums.

The total impact factor of the candidate is 26,055. Hirsch index (h-index) according to Scopus - 10.

A reference to the citations of scientific papers is presented - 448 and for the period 2000-2021 the total number of citations is 356.

Ch. Assistant Professor Dr. Anna Tomova has worked on 19 scientific and educational projects, in 18 of which she was a member of the teams and one of them is a leader. Nine of these projects are international and 10 nationals.

The supervision of the applicant includes 7 graduates.

Conclusion on item 2:

The applicant fully meets the criteria of state criteria for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application for the academic position of associate professor and in indicators E and F significantly exceeds its requirements.

3. Analysis of the research work and scientific contributions of the candidate

The most important research contributions of the candidate presented for participation in the competition can be systematized in three main directions as follows:

3.1. Biodiversity of microbial communities in extreme habitats and biology of extreme microorganisms (papers: B4-4; G7-2; G7-7, G7-8, B4-2, B4-5, G7-4, G7-5)

The research is related to the isolation and cultivation of bacteria and archaea and analyzed their diversity in extreme habitats as well as investigation on their metabolism and possibility for their use for biotechnology application. Such a combined approach to biodiversity assessment of microbial populations, with the addition of a metabolic gene, provides important practical information on the metabolic characteristics of non-cultivated microorganisms in a modern way and provides important information for biotechnological use. The object of research are several extreme habitats - Vlasa hot spring, Velingrad, Varvara hot spring, Levunovo hot spring, Vetren hot spring, hot spring in the village of Dolni Bogrov, Magurata cave. The microbial community of cultivated and non-cultivated bacteria and archaea is analyzed. Biodiversity has been analyzed by different molecular methods. Interesting results of a fundamental value have been obtained. The most significant contributions of the research are:

- The biodiversity of an archaeal community from the Vlasa hot spring, Velingrad, has been studied through metagenomic analysis. It was found that the archaea in the community are dominated by representatives of hyperthermophilic, anaerobic heterotrophic species, belonging to two orders of the *Creanarcheota* division (86.9%) - *Desulforococcales* and *Thermoproteales.* More than half of the 16S rDNA sequences analyzed were attributed to the *Thermosphaera aggregans.* The presence of five new phylogenetic units in the archaeal community has been proven, based on the low percentage of homology of their 16S rDNA sequences with those deposited in GenBank. The new 16S rDNA and GH4 sequences were deposited with GenBank (Accession number FN650703-FN650707; FN424089-FN424092). The only representative of the *Korarchaeota* division - *Korarchaeum cryptofilum* - has been identified.

A total of 35 archaeal operational taxonomic units (OTUs) belonging to three divisions of the *Archaea* domain - *Crenarchaeota, Eutyarchaeota* and *Korarchaeota* have been identified from water and sedimentary samples from the Varvara hot spring. The studied archaeal community is dominated by representatives of uncultivated archaeal groups and new phylotypes, not described so far (Hot Spring Sediment group).

A large number of sequences are grouped into four heterogeneous groups belonging to the *Crenarchaeota* division (23), three of which show no association with cultivated organisms. The presence of a group of sequences (Hot Water Crenarchaeotic Group, HWCG III), which are phylogenetically related to the thermophilic species *Candidatus Nitrosocaldus yellowstonii*, has been demonstrated.

Biodiversity of bacteria and archaea in two Bulgarian hot springs - Levunovo and Vetren dol. The analysis of microbial diversity was done by studying the genes for 16S rDNA and GH 57 family of glycoside hydrolases in bacteria and archaea. Greater 16S rDNA archaeal diversity has been proven in the Levunovo hot spring and 28 different phylotypes assigned to five archaeal groups (I.1b, *Methanosarcinales,* MCG, *Methanobacteriales and* I.3b) of *the Crenarchaeota* and *Euryarchaeota* divisions. The formation of a thermophilic archaeal group in the order M*ethanosarcinales* has been proposed.

- In the spring Vetren dol, characterized by a lower water temperature (67 ° C), a greater diversity in the bacterial community was found. It has been shown that the identified 47 rDNA

sequences from both sources belong to the groups *Bacteroidetes, Proteobacteria, Cyanobacteria* and *Chloroflexi.* The conducted phylogenetic analysis reveals the presence of a large number of new archaeal and bacterial sequences. The major part of the identified GH-57 sequences in both sources are related to the bacterial divisions *Bacteroidete*s, *Delta- proteobacteria* and Candidate Saccharibacteria, as well as to unclassified representatives of the division *Crenarchaeota*.

A new type of thermophilic Gram-positive bacterium has been isolated from a Bulgarian hot spring in the area of the village of Dolni Bogrov. A new species of *Anoxybacillus bogrovensis sp. nov*. (5DSM 17956T, 5NBIMCC 8427T) is recognized by the International Committee on Systematics of Prokaryotes and is included in the latest edition of Budgie.

- Biodiversity of the bacterial community in the Magura Cave - the gallery of prehistoric drawings was studied using molecular methods. 68 bacterial taxa have been identified, which reveals an extremely high degree of diversity for this type of extreme niche. The taxonomic affiliation of the obtained sequences has been established. They belong to 8 bacterial groups - *Proteobacteria* (40%), *Nitrospirae* (22.5%), *Acidobacteria* (21.5%), *Actinobacteria* (6.4%), *Chloroflexi* (3.2%), *Planctomycetes* (2.2*%), Firmicutes* (2.2%), and *Gemmatimonadetes* (2.2%). It has been shown that about 1/3 of the isolated sequences show a weak relationship with the nearest bacterial sequences, which suggests the existence of new taxonomic units. The grouping of the studied 16 S rDNA sequences mainly with sequences of non-cultivated bacteria confirms the fact that a significant part of the microorganisms in different natural habitats remain undetected.

A total of 46 aerobic heterotrophic bacteria were isolated. Gram-negative heterotrophs have been shown to be the dominant group in the bacterial community, with the species composition being divided into nine genera: *Serratia, Pseudomonas, Enterobacte*r, *Sphingobacterium, Stenotrophomonas, Commamonas, Acinetobacter, Obesumbacterium* and *Myroide*s. Gram-positive isolates are phylogenetically assigned to three genera: *Bacillus, Arthrobacter* and *Micrococcus.*

A new species *Myroides guanonis sp.* nov. (= DSM 26542T = NBIMCC 8736T) has been isolated and accepted as a new bacterial species by the International Committee on Systematics of Prokaryotes.

3.2. Microbial enzymes and exopolysaccharides of biotechnological importance. (Papers: B4-1, B4-3, G7-1, G7-3, G7-6, G7.0-1, G7.0-2)

Various enzymes and exopolysaccharides isolated from extreme bacteria have been studied. The research has been carried out at a high scientific level and significant results have been obtained, both fundamental and scientifically applied. The most significant of them are as follows:

- Thermostable inulinase from thermophilic strain *Bacillus sp. 11*. A thermophilic bacterial strain of *Bacillus sp.* 11, producer of thermostable inulinase have been isolated. The kinetics of enzyme production, the mechanisms of action as well as purification of the enzyme have been achieved.
- Thermostable gelan lyase synthesized by a thermophilic strain of *Geobacillus stearothermophilus* 98. The physicochemical properties of the first reported thermostable gelan lyase degrading the microbial polysaccharide gelan molecule are characterized. It was found that the purified gelan lyase forms two types of crystal structures that are well reproducible and can serve as a basis for future study of the tertiary structure of the enzyme.
- Thermostable lipase produced by thermophilic *Bacillus stearothermophilus MC7* Extracellular thermostable lipase produced by thermophilic bacterial strain *Bacillus*

stearothermophilus MC7 was isolated and purified. An effective approach for purification of thermostable lipase to electrophoretically homogeneous state has been developed.

- Thermostable β-amylase and α-glucosidase produced by thermophilic strains. Two bacterial producers of thermostable starch - degrading enzymes - α-glucosidase and β-amylase - have been isolated from Bulgarian hot springs. 16S rDNA analysis was performed to determine the affiliation of both isolates to the species *Bacillus stearothermophilus* (reclassified as *Geobacillus stearothermophilus*, Nazina et. Al., 2001). *Bacillus stearothermophilus* α-glucosidase was found to have the highest temperature stability ever described. The enzyme is purified to an electrophoretically homogeneous state and its physicochemical values are determined - molecular weight, temperature and pH optimum of action, substrate specificity. The studied β-amylase from *Bacillus stearothermophilus 233* has the highest thermal stability among the described other enzymes produced by representatives of this genus. A protocol for purification of the enzyme β-amylase has been developed, as a result of which a high yield (53%) of the purified enzyme and a significant increase in its specific activity has been achieved.
- The thermophilic exopolysaccharide producer, taxonomically identified as *Brevibacillus thermoruber*, was isolated from a hot spring in the Rupi region. As a result of the created technological scheme for polysaccharide production, three times higher biopolymer production was achieved.

3.2.Yeasts Saccharomyces cerevisiae as a model system for studying the Go state (Papers: G7-9, G7-10, G7-11, G7-12, G7.0-3)

- The role of the cellular antioxidant enzymes SOD and catalase for the entry and survival of cells at rest in two different cell lines - mouse and human fibroblasts - was studied. An *in-silico* approach has been developed based on bioinformatics analysis of genes encoding SOD and catalase in human and mouse cells, which are responsible for the entry and survival of cells in the Go state. Both types of cells, as well as a number of directing signals in the protein molecule of SOD and catalase, providing plasticity in their subcellular localization. The data obtained reveal that the enzymatic antioxidant protection in human and mouse cells is characterized by a high adaptive potential. The role of both enzymes in maintaining redox homeostasis in Go cells has been shown. The redox status of cells at Go state was characterized by comparative characterization of intracellular concentrations of reactive oxygen species (ROS) and NADH / NADPH.
- The effect of different concentrations of four drugs (menadione, hydrogen peroxide, ibuprofen and zeocin) on the viability of logarithmically growing and Go cell populations of S. cerevisiae BY4741 was studied. Lethal concentrations of test compounds (IC50) were determined. observed 50% inhibition of growth. A difference in the sensitivity of proliferating and Go cells to the IC50 of the studied toxic compounds was found. A large-scale proteome analysis of untreated and treated with menadione, hydrogen peroxide, ibuprofen and zeocin (IC50) proliferating cells and cells at rest was performed and the level of expression of various proteins in yeast cells of S. cerevisiae in the Log growth phase and in Go state.
- In response to treatment with toxic agents, a higher level of expression of certain proteins was found in Go cells. Seven of the proteins involved in the cellular response of Go cells to toxic substances have been identified. Protein expression profiles of treated proliferating and Go cells show that in both cell types the cellular response to H2O2, menadione, UPS and zeocin is different. Electrophoretic methods have shown that Go cells are characterized by decreased expression of RNA pol I and RNA pol III and increased expression of pol II RNA. Menadione was found to

cause more significant proteomic changes in S. cerevisiae Go cells than the thiol-oxidizing agent H_2O_2.

Conclusion on item 3

The scientific papers submitted for review are in the scientific field of the competition. The results of research reflected in publications, collections of reports and participation in scientific forms have a wide resonance in the international scientific community. In these areas, the candidate has serious scientific contributions of an original nature, scientific and theoretical contributions of a confirmatory nature, as well as those of a methodological nature.

It is obvious that the candidate has established himself as a good researcher and expert in the biology of extreme microorganisms, expert in microbiological techniques for isolation, cultivation and phenotypic characterization of microorganisms, techniques for isolation, purification and characterization of enzymes and peptides, electrophoretic and hybridization techniques. analysis of proteins, peptides and nucleic acids, PCR techniques, metagenomic analysis, creation of genomic libraries, bioinformatics.

There is a potential for the development of a new research direction in the Department by the candidate, related to the biology of extremophilic bacteria and opportunities for future basic research and biotechnological application.

4. Evaluation of the teaching activity of the candidate

The candidate has significant teaching activity as follows:

Practical classes at the Bachelor's Degree: Methods for obtaining strains of super-producers, specialty Biotechnology, General and soil microbiology, specialty Agrobiotechnology, Genetic Engineering, Legislation and Control. specialty Agrobiotechnology, Fundamentals of industrial microbiology - elective course, specialty Molecular Biology.

Practical classes in the Master's degree

Molecular biology of prokaryotic and eukaryotic microorganisms, Applied microbiology, Biology of extreme microorganisms, Genetically modified organisms in food, Antibiotics and antibiotic to MP Microbiology and microbiological control, Organoleptic analysis, Genetically modified microorganisms in food.

Lectures Bachelor's degree - General and soil microbiology, specialty Agrobiotechnology, Microbiology, specialty Biomanagement and sustainable development, Methods for obtaining strains of overproducers, specialty Biotechnology

Lectures Master's degree - Biology of extreme microorganisms, MP Microbiology and microbiological control, organoleptic analysis, MP Quality and safety of food

Average teaching activity of the candidate for the period 2016/2021 is up to 400 hours yearly. In general, this is a significant activity exceeding considerable requirements. Unfortunately, this activity has not yet found a proper place as a criterion in the requirements for the development of the academic potential of universities. T

Teaching materials have been developed by the candidate in English and Bulgarian (Textbook folder), which are provided for students of bachelor's (Fundamentals of Industrial Microbiology, General and Soil Microbiology) and master's degree, in order to upgrade the knowledge and skills acquired in the basic courses.

Chief Assistant Dr. Anna Tomova was the research supervisor of 7 graduates Analysing the materials for this competition, I considered my assessment of the applicant's abilities for research and training fully correct.

Conclusion on item 4

All lecture courses and practical classes in which the candidate participates are in the direction of the competition. It is obvious that the candidate has a certain affinity for teaching, presents to students, especially from the Master's degree, the latest achievements in the relevant fields, introduces innovative teaching methods, strengthens the discussion element in presenting problems, which leads to increased interest in students and high appreciation of her teaching activities.

General Conclusion

In terms of volume, content and quality, the presented scientific production and the active teaching activity of the only applicant in the announced competition for the academic position "Associate professor " Dr. Anna Tomova fully meets the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application, and the additional requirements of Sofia University "St. Kliment Ohridski".

The complex evaluation of the submitted materials, as well as the overall activity of the applicant allows me to convincingly propose to the scientific jury and the esteemed Faculty Council of the Faculty of Biology at Sofia University to elect Chief Assistant Dr. Anna Tomova as an **Associate professor** in professional area 4.3. Biological Sciences (Microbiology - General Microbiology and Biology of extreme microorganisms) for the needs of the Department of General and Industrial Microbiology of the Faculty of Biology at Sofia University.

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Sofia