ANNOTATION OF THE MATERIALS

under Art. 76 of the Regulations for Development of the academic staff
of Paisii Hilendarski University of Plovdiv

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A participant in a competition for the academic position
"Professor" of Phycology at the Paisii Hilendarski University of Plovdiv

Field of higher education:
4. Natural Sciences, Mathematics and Informatics

Professional field:
4.3. Biological Sciences (Phycology)

Announced in the State Gazette, issue 98 / 19.11.2024

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under Art. 76 of the Regulations for Development of the academic staff
of Paisii Hilendarski University of Plovdiv

of **Assoc. Prof. Dr. Ivanka Ivanova Teneva-Dzhambazova** in reference to participation in a competition for the academic position "Professor", announced in the State Gazette, Issue 98 / 19.11.2024 in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences (Phycology)

The materials submitted for participation in the competition for the scientific position "Professor" include 18 scientific publications, 2 book chapters, 4 textbooks and 4 manuals (which were not used for the acquisition of PhD and the academic positions "Chief Assistant" and "Associate Professor").

- All articles for the competition are refereed and indexed in the world-renowned databases of scientific information Web of Science and Scopus
- Total impact factor of the articles participating in the competition: 40.602
- Total number of citations (in Web of Science and Scopus) of the articles included in the competition: 96
- Total number of citations (in Web of Science and Scopus) of the candidate's articles: 455
- Scopus ID: 6506320748; h-index according to Scopus: 12 https://www.scopus.com/authid/detail.uri?authorId=6506320748
- h-index according to Web of Science: 12 https://www.webofscience.com/wos/woscc/citation-report/1a362317-2b24-4121-b6e4-203445db7b58-0144d61369
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- Google scholar: https://scholar.google.bg/citations?hl=bg&user=4ZxOEcIAAAAJ
- Researchgate: https://www.researchgate.net/profile/Ivanka-Teneva
- NACID ID: 45624; https://cris.nacid.bg/public/scientist-preview/30052
- Web-сайт: https://blogs.uni-plovdiv.bg/teneva/

I. ANNOTATION OF THE MATERIALS

INDICATOR GROUP A.
SCIENTIFIC PUBLICATIONS ON WHICH THE DISSERTATION WAS DEFENDED:

PUBLICATIONS AND PUBLISHED ABSTRACTS OF PARTICIPATION IN SCIENTIFIC FORUMS RELATED TO THE DISSERTATION FOR THE ACQUISITION OF THE EDUCATIONAL AND SCIENTIFIC DEGREE "PHD"

1. **Teneva I.** 2006. Taxonomy, phylogeny and toxic potential of some species of blue-green algae (Cyanoprokaryota). Summary of a dissertation for acquisition of the educational and scientific degree "PhD".

Anotation:

The purpose of this thesis was to investigate the morphological and molecular features used for taxonomic classification (polyphasic complex) as well as the phylogenetic relationships and toxic potential of some freshwater species of the genus *Phormidium* (*Oscillatoriales, Cyanoprokaryota*). Five freshwater species of the genus *Phormidium* (*Ph. molle, Ph. papyraceum, Ph. uncinatum, Ph.*

autumnale, Ph. bijugatum) and 6 strains of the species *Ph. autumnale* were investigated. *Phormidium* species were grown intensively under axenic conditions for 14 days. Genomic DNA was extracted according to the xanthogenate-SDS extraction protocol. The *16S rRNA* and *cpcA-IGS-cpcB* regions were amplified and sequenced. Molecular and phylogenetic analyses were carried out by using DNAStar and PHYLIP software. To study the toxic potential of the *Phormidium* species they were extracted with water-methanol-butanol solution (15:4:1). The extracts and nutrient solution in which the *Cyanoprokaryota* were cultured during the 14 days were tested for toxicity using *in vivo* and different *in vitro* methods (mouse model, histopathology, MTT, Alamar Blue/CFDA-AM, proliferation, ELISA, HPLC).

In phylogenetic analyses *Phormidium* was found to be polyphyletic. This analysis appeared to be dominated by the *cpcB* region, which is characterized by a relatively high percentage of informative substitutions. Further, alignment of informative nucleotide substitutions in the *cpcB-IGS-cpcA* sequences revealed a mosaic distribution, which may be indicative of genetic recombination events. The combination of morphological features with molecular markers as *16S rRNA* and *cpcB-IGS-cpcA* sequences can be useful tools for identification and/or phylogenetic study at the species level or higher taxonomic ranks in the order *Oscillatoriales*. Compared with *16S rRNA* sequences, *IGS* sequences may be better suited to resolve close relationships and intraspecies variability.

Performed cytotoxic tests demonstrated that: (1) investigated freshwater *Phormidium* species are potent producers of intracellular as well as extracellular cyanotoxins or other biologically active compounds; (2) suggested conditions for HPLC analysis can be used for detection of cyanotoxins from different groups; (3) in some cases, the cytotoxic effects of the cyanotoxins are temperature dependent; (4) RTL-W1 fish cell line or other fish cells could be suitable *in vitro* test system to investigate the temperature dependence of the cytotoxic effects of different xenobiotics.

2. **Teneva I.**, Dzhambazov B., Mladenov R., Schirmer K. 2005. Molecular and phylogenetic characterization of *Phormidium* species (*Cyanoprokaryota*) using the cpcB-IGS-cpcA locus. – *Journal of Phycology*, 41(1): 188-194.

(Impact factor: 2.502)

Abstract: The accurate determination of species of *Cyanoprokaryota/Cyanophyceae* has many important applications. These include the assessment of risk with regard to blooms in water reservoirs as well as the identification of species capable of producing valuable bioactive compounds. Commonly, *Cyanoprokaryota* are classified based on their morphology. However, morphological criteria are not always reliable because they may change, for example, due to environmental factors. Thus, genetic and molecular analyses are a promising additional approach, but their application has so far been limited to relatively few genera. In light of this, we present here the first characterization of species and strains of the genus *Phormidium* Kütz. based on the cpcB-IGS-cpcA locus of the phycocyanin operon. In phylogenetic analyses using deduced amino acid sequences of the cpcB-cpcA regions, *Phormidium* was found to be polyphyletic. This analysis appeared to be dominated by the cpcB region, which is characterized by a relatively high percentage of informative substitutions. The percentage of variable positions within the cpcB-IGS-cpcA locus overall was 16.5%, thereby indicating a level of divergence remarkably higher than that reported for *Nodularia* and *Arthrospira* in previous studies relying on cpcB-IGS-cpcA. Further, alignment of informative nucleotide substitutions in the cpcB-IGS-cpcA sequences revealed a mosaic distribution, which may be indicative of genetic recombination events. Finally, the length and sequences of the IGS region alone proved useful as markers to differentiate the cyanobacterial genus *Phormidium*. However, whether the IGS region *per se* is sufficiently discriminatory to differentiate between *Phormidium* species or even strains requires further investigation using newly identified *Phormidium* sequence data.

3. **Teneva I.**, Dzhambazov B., Koleva L., Mladenov R., Schirmer K. 2005. Toxic potential of five freshwater *Phormidium* species (Cyanoprokaryota). – *Toxicon*, 45 (6): 711-725.
(Impact factor: 2.255)

Abstract: Among the *Cyanoprokaryota* (blue-green algae), the genus *Phormidium* has thus far rarely been studied with respect to toxin production and potentially resulting human and environmental health effects. We here show that five previously unexplored freshwater species of this genus (*Ph. bijugatum, Ph. molle, Ph. papyraceum, Ph. uncinatum, Ph. autumnale*) are indeed capable of producing bioactive compounds. *Phormidium* extracts caused weight loss as well as neuro/hepatotoxic symptoms in mice, and in the case of *Ph. bijugatum* even death. Very low levels of saxitoxins and microcystins, as confirmed by ELISA, were insufficient to explain this toxicity and the differing toxic potencies of the *Phormidium* species. Qualitative HPLC analyses confirmed different substance patterns and in the future could aid in the separation of fractions for more detailed substance characterisation. The results in vivo were confirmed in vitro using cells of human, mouse and fish. The fish cells responded least sensitive but proved useful in studying the temperature dependence of the toxicity by the *Phormidium* samples. Further, the human cells were more sensitive than the mouse cells thus suggesting that the former may be a more appropriate choice for studying the impact of *Phormidium* to man. Among the human cells, two cancer cell lines were more responsive to one of the samples than a normal cell line, thereby indicating a potential anti-tumour activity. Thus, the five freshwater *Phormidium* species should be considered in environmental risk assessment but as well, as a source of therapeutic agents.

4. **Teneva I.**, Schirmer K., Mladenov R., Schüürmann G. 2002. Cellular responses elicited by cyanobacterial toxins in a rainbow trout liver cell line. – *12th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Vienna, Austria*, 12-16 May 2002.
5. Schirmer K., **Teneva I.**, Dzhambazov B., Mladenov R. 2003. Cyanobacteria - an in vitro approach to studying their toxic potential to mammals and fish. – *SETAC Europe 13th Annual Meeting, Society of Environmental Toxicology and Chemistry, Hamburg, Germany*, 27 April - 1 May 2003.

INDICATOR GROUP B4.
HABILITATION THESIS – SCIENTIFIC PUBLICATIONS IN JOURNALS THAT ARE REFERRED AND INDEXED IN WORLD-RENOWNED DATABASES WITH SCIENTIFIC INFORMATION (WEB OF SCIENCE AND SCOPUS)

1. **Teneva I.**, Belkinova D., Paunova-Krasteva T., Bardarov K., Moten D., Mladenov R., Dzhambazov B. 2023. Polyphasic characterisation of *Microcoleus autumnalis* (Gomont, 1892) Strunecky, Komárek & J.R.Johansen, 2013 (Oscillatoriales, Cyanobacteria) using a metabolomic approach as a complementary tool. – *Biodiversity Data Journal*, 11: e100525. **(Q2, IF-1.0)** DOI: 10.3897/BDJ.11.e100525

Absract: As a result of the continuous revision of cyanobacterial taxonomy, Phormidium autumnale (Agardh) Trevisan ex Gomont, 1892 has been transferred to the genus Microcoleus as Microcoleus autumnalis (Gomont, 1892) Strunecky, Komárek & J.R.Johansen, 2013. This transfer was based on a single strain and literature data. In the present study, we revise the taxonomic position of Microcoleus autumnalis by applying the classical approach of polyphasic taxonomy and additionally using metabolomics. Cyanobacterial strains identified as Phormidium autumnale and Microcoleus vaginatus (type species of the genus Microcoleus) were used for comparative analyses. In addition, the taxonomic relationship between the species Phormidium autumnale and Phormidium uncinatum was determined on the basis of polyphasic characteristics. Monitoring of the morphological variability of Phormidium autumnale and Microcoleus vaginatus strains showed a difference in the morphology concerning the ends of the trichomes, the shape of the apical cells, as well as the presence/absence of the calyptra and its shape. The performed TEM analysis of the thylakoid arrangement of the studied strains showed parietal arrangement of the thylakoids in the representatives of genus Phormidium and fascicular arrangement in genus Microcoleus. Molecular genetic analyses, based on 16S rDNA, revealed grouping of the investigated P. autumnale strains in a separate clade. This clade is far from the subtree, which is very clearly formed by the representatives of the type species of genus Microcoleus, namely M. vaginatus. The metabolomic analysis involving P. autumnale and M. vaginatus strains identified 39 compounds that could be used as potential biochemical markers to distinguish the two cyanobacterial species. Based on the data obtained, we suggest changing of the current status of Microcoleus autumnalis by restoring its previous appurtenance to the genus Phormidium under the name Phormidium autumnale (Agardh) Trevisan ex Gomont, 1892 and distinguishing this species from genus Microcoleus.

2. **Teneva I**., Velikova V., Belkinova D., Moten D., Dzhambazov B. 2023. Allelopathic Potential of the Cyanotoxins Microcystin-LR and Cylindrospermopsin on Green Algae. – *Plants*, 12(6): 1403. **(Q1, IF-4.0)** DOI: 10.3390/plants12061403

Abstract: Allelopathic interactions are widespread in all aquatic habitats, among all groups of aquatic primary biomass producers, including cyanobacteria. Cyanobacteria are producers of potent toxins called cyanotoxins, whose biological and ecological roles, including their allelopathic influence, are still incompletely understood. The allelopathic potential of the cyanotoxins microcystin-LR (MC-LR) and cylindrospermopsin (CYL) on green algae (Chlamydomonas asymmetrica, Dunaliella salina, and Scenedesmus obtusiusculus) was established. Time-dependent inhibitory effects on the growth and motility of the green algae exposed to cyanotoxins were detected. Changes in their morphology (cell shape, granulation of the cytoplasm, and loss of flagella) were also observed. The cyanotoxins MC-LR and CYL were found to affect photosynthesis to varying degrees in the green algae Chlamydomonas asymmetrica, Dunaliella salina, and Scenedesmus obtusiusculus, affecting chlorophyll fluorescence parameters such as the maximum photochemical activity (Fv/Fm) of photosystem II (PSII), the non-photochemical quenching of chlorophyll fluorescence (NPQ), and the quantum yield of the unregulated energy dissipation Y(NO) in PSII. In the context of ongoing climate change and the associated expectations of the increased frequency of cyanobacterial blooms and released cyanotoxins, our results demonstrated the possible allelopathic role of cyanotoxins on competing autotrophs in the phytoplankton communities.

3. **Teneva I.**, Batsalova T., Bardarov K., Moten D., Dzhambazov B. 2022. A Novel Approach for Fast Screening of a Complex Cyanobacterial Extract for Immunomodulatory Properties and Antibacterial Activity. – *Applied Sciences*, *12*(6): 2847. **(Q2, IF-2.7)** DOI: 10.3390/app12062847

Abstract: The filamentous cyanobacteria from genus *Phormidium* are rich natural sources of bioactive compounds that could be exploited as pharmaceuticals or nutraceuticals. In this study, we suggest a novel approach for assessing the immunomodulatory properties of the products derived from cyanobacteria. The influence of *Phormidium papyraceum* extract on the human leukocyte immunophenotype was evaluated by attempting to link this activity to certain putative compounds identified in the extract. By using three staining panels and flow cytometry, we found that the cyanobacterial extract affected mainly $CD4^+$ T cells upregulating activated $CD4^+CD152^+$ T cells (15.75 ± 1.93% treated vs. 4.65 ± 1.41% control) and regulatory $CD4^+CD25^+$ T cells (5.36 ± 0.64% treated vs. 1.03 ± 0.08% control). Furthermore, *P.*

papyraceum extract can modulate T cell subpopulations with a $CD4^+$ effector/memory phenotype. Extract-treated cells showed increased production of IL-2 (55 ± 12 pg/mL) and IL-6 (493 ± 64 pg/mL) compared to the untreated, 21 ± 7 pg/mL and 250 ± 39 pg/mL, respectively. No significant changes were observed in the secretion of TNF-α. In addition, *P. papyraceum* extract displayed antibacterial activity against both Gram-negative (inhibition zone from 18.25 ± 0.50 mm to 20.28 ± 1.50 mm) and Gram-positive (inhibition zone from 10.86 ± 0.85 mm to 17.00 ± 0.82 mm) bacteria. The chemical profile of the cyanobacterial extract was determined using LC–ESI–MS/MS analysis, where at least 112 putative compounds were detected. Many of these compounds have proven different biological activities. We speculated that compounds such as betulin and the macrolide azithromycin (or their analogues) could be responsible for the immunomodulatory potential of the investigated extract. More studies are needed to determine and validate the biological activities of the determined putative compounds.

4. **Teneva I.**, Belkinova D., Mladenov R., Stoyanov P., Moten D., Basheva D., Kazakov S., Dzhambazov B. 2020. Phytoplankton composition with an emphasis of Cyanobacteria and their toxins as an indicator for the ecological status of Lake Vaya (Bulgaria) – part of the Via Pontica migration route. – *Biodiversity Data Journal,* 8: e57507. (**Q2, IF-1.225**) DOI: 10.3897/BDJ.8.e57507

Abstract: As producers of biomass, cyanobacteriaare a major part of the phytoplankton in a large number of water basins. Due to the cyanobacterial b looms and cyanotoxins produced, these organisms are recognized as a threat and ecological risk for water bodies. Released cyanotoxins may cause death of many organisms including birds and fish. Vaya Lake is the largest natural lake in Bulgaria. It is located on the Via Pontica migration route of birds between Europe and Africa. Since 2003, the lake has been declared as a "Wetland of international importance" under the Ramsar Convention. According to the literature data from 2002-2006, the Lake is defined as highly eutrophied due to strong anthropogenic pressure, but regular monitoring of the cyanobacterial blooms and presence of cyanotoxins after this period is missing. Taking into account the importance of this unique, protected ecosystem, our aim was to make a complete ecological assessment of the present state of Lake Vaya by using the phytoplankton, with an emphasis on cyanobacterial blooms and the presence of cyanotoxins. As results of the study, we 1) characterized the phytoplankton composition qualitatively and quantitatively; 2) evaluated the ecological status of the western and eastern part of the Lake; 3) identified the potential producers of cyanotoxins; 4) observed cyanobacterial blooms and discussed the influence of macrophytes on their spread; 5) measured the concentrations of the cyanotoxins MCs, CYL and STXs in water samples from both parts of the Lake. Our results indicated the need for continued observation of cyanobacterial composition, blooming and the presence of cyanotoxins in Lake Vaya.

5. Batsalova T., Basheva D., Bardarov K., Bardarov V., Dzhambazov B., **Teneva I**. 2019. Assessment of the cytotoxicity, antioxidant activity and chemical composition of extracts from the cyanobacterium Fischerella major Gomont. – *Chemosphere*, 218: 93-103. (**Q1, IF-5.778**) DOI: 10.1016/j.chemosphere.2018.11.097

Abstract: Cyanoprokaryotes (Cyanobacteria/Cyanophyta) are ancient photosynthetic prokaryotic organisms with cosmopolitan distribution. They are producers of a number of biologically active substances with antitumor and antifungal activity, vitamins, antibiotics, algaecides, insecticides, repellents, hormones, immunosuppressants and toxins. So far, the cyanobacterium *Fischerella major* Gomont has not been studied regarding its impact on the environment and human health. In this study, the cytotoxic, antioxidant and antitumor activities of four extracts prepared from *Fischerella major* were evaluated *in vitro*. In addition, the total phenolic content and the potential for production of cyanotoxins were also analyzed. The conducted GC/MS analysis identified 45 compounds with different chemical nature and biological activity. Presence of microcystins and saxitoxins was detected in all *Fischerella major* extracts. *In vitro* testing on cell cultures showed a significant concentration- and time-dependent cytotoxic effect on all cell lines (HeLa, SK-Hep-1 and FL) treated at three exposure times (24, 48 and 72 h) with four extracts. A selective antitumor effect was not observed. This is the first study demonstrating biological activity of extracts from *Fischerella major*, which makes it an interesting subject for further research, including environmental risk assessments (as producer of cyanotoxins) or as a potential source of pharmaceuticals.

Highlights

- *Fischerella major* produces microcystins/nodularins and saxitoxins.
- *Fischerella major* extracts have strong cytotoxic effects on human cell lines associated with mitochondrial damage.
- The lysosomes retain their integrity and over time are able to overcome the toxic effects of the extracts.
- Forty-five components with different biological activities were found in DMSO extract from *Fischerella major.*

6. Basheva D., Moten D., Stoyanov P., Belkinova D., Mladenov R., **Teneva I.** 2018. Content of phycoerythrin, phycocyanin, alophycocyanin and phycoerythrocyanin in some cyanobacterial strains: Applications. –*Engineering in Life Sciences*, 18: 861-866. (**Q2, IF-3.165**) DOI: 10.1002/elsc.201800035

Abstract: Cyanobacteria are unique prokaryotes, which are capable to perform oxygenic photosynthesis. Within these organisms, phycobilisomes (PBS) act as an antenna of the photosynthetic pigment apparatus. Phycobilisomes contain several phycobiliproteins (PBP): phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC) and phycoerythrocyanin (PEC). The application of phycobiliproteins in the biotechnology, food industry and medicine during the last years is rapidly increasing. The aim of our study was to assess the qualitative and quantitative content of phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin in 14 cyanobacterial strains kept in Plovdiv Algal Culture Collection (PACC) and 4 strains purchased from the Culture Collection of Autotrophic Organisms (CCALA). Our data demonstrated that three strains of Microcoleus autumnalis (PACC 5505, PACC 5522 and PACC 5527) have high potential to produce phycoerythrins (0.132, 0.201 and 0.136 mg/mL, respectively). Similarly, one Microcoleus autumnalisstrain (PACC 5522) and one strain of Leptolyngbya boryana (CCALA 084) are suitable for biotechnological production of phycocyanins (0.051 and 0.264 mg/mL, respectively) as well as allophycocyanins (0.102 and 0.171 mg/mL, respectively). In addition, the data about the pigment content could be used as a biochemical marker for taxonomic purposes within the group.

INDICATOR GROUP G7.

SCIENTIFIC PUBLICATIONS IN JOURNALS THAT ARE REFERRED AND INDEXED IN WORLD-RENOWNED DATABASES WITH SCIENTIFIC INFORMATION (WEB OF SCIENCE AND SCOPUS), OUTSIDE THE HABILITATION THESIS

1. Gyuzeleva D., Batsalova T., Dzhambazov B., **Teneva I.**, Mladenova T., Mladenov R., Stoyanov P., Todorov K., Moten D., Apostolova D., Bivolarska A. 2024. Assessment of the biological activity of *Marrubium friwaldskyanum* Boiss. (Lamiaceae). – *Heliyon*, 10(11): e32599. (**Q1, IF2023-3.4**) DOI:10.1016/j.heliyon.2024.e32599

Abstract: Present scientific evidences about the biological activity and potential medical application of extracts derived from Marrubium friwaldskyanum Boiss. are limited. Therefore, our study was undertaken to define several main characteristics in this regard – in vitro cytotoxicity and antitumor properties, antibacterial activity and immunomodulatory potential. Extracts were obtained from different aerial parts of Marrubium friwaldskyanum – stems, leaves and flowers. The in vitro cytotoxicity and antitumor activity of the samples were evaluated by tetrazolium salt reduction tests and Neutral red uptake assays using four human cell lines (a normal fibroblastic and three adenocarcinoma cell lines/A549, HeLa, HT-29/) and by experiments with HT-29 tumor spheroids. Antibacterial activity toward Gram-negative (Escherichia coli) and Gram-positive (Bacillus cereus) species was assessed based on estimation of minimal inhibitory and minimal bactericidal concentrations as well as longitudinal studies on bacterial viability. Ex vivo assays with normal leukocytes were performed to define potential immunomodulatory activity of the extracts. Our results demonstrated selective antitumor activity of the extracts directed against colon adenocarcinoma HT-29 cells and cervical adenocarcinoma HeLa cell line. Metabolic activity of A549 lung adenocarcinoma cells was affected only by the sample derived from flowers. M. friwaldskyanum leaf and flower extracts showed the highest activity, which included reduction of HT-29 tumor spheroid growth and viability. The studied samples exhibited antibacterial activity against both bacterial species tested. Treatment with M. friwaldskyanum extracts affected specific leukocyte populations (HLA+, CD19+, CD11b+, CD25+ cells). These results demonstrate for the first time complex biological effects of extracts derived from M. friwaldskyanum and their potential to serve as a source of valuable compounds for the pharmaceutical industry.

2. Moten D., Batsalova T., Apostolova D., Mladenova T., Dzhambazov B., **Teneva I.** 2023. In Silico Design of a New Epitope-Based Vaccine against Grass Group 1 Allergens. – *Advances in Respiratory Medicine*, 91(6): 486-503**. (Q3, IF-1.8)**) DOI: 10.3390/arm91060036

Abstract: Allergic diseases are a global public health problem that affects up to 30% of the population in industrialized societies. More than 40% of allergic patients suffer from grass pollen allergy. Grass pollen allergens of group 1 and group 5 are the major allergens, since they induce allergic reactions in patients at high rates. In this study, we used immunoinformatic approaches to design an effective epitope-based vaccine against the grass group 1 allergens. After the alignment of all known pollen T-cell and B-cell epitopes from pollen allergens available in the public databases, the epitope GTKSEVEDVIPEGWKADTSY was identified as the most suitable for further analyses. The target sequence was subjected to immunoinformatics analyses to predict antigenic T-cell and B-cell epitopes. Population coverage analysis was performed for CD8+ and CD4+ T-cell epitopes. The selected T-cell epitopes (VEDVIPEGW and TKSEVEDVIPEGWKA) covered 78.87% and 98.20% of the global population and 84.57% and 99.86% of the population of Europe. Selected CD8+, CD4+ T-cell and B-cell epitopes have been validated by molecular docking analysis. CD8+ and CD4+ T-cell epitopes showed a very strong binding affinity to major histocompatibility complex (MHC) class I (MHC I) molecules and MHC class II (MHC II) molecules with global energy scores of −72.1 kcal/mol and −89.59 kcal/mol, respectively. The human IgE-Fc (PDB ID 4J4P) showed a lower affinity with B-cell epitope (ΔG = −34.4 kcal/mol), while the Phl p 2-specific human IgE Fab (PDB ID 2VXQ) had the lowest binding with the B-cell epitope (ΔG = −29.9 kcal/mol). Our immunoinformatics results demonstrated that the peptide GTKSEVEDVIPEGWKADTSY could stimulate the immune system and we performed ex vivo tests showed that the investigated epitope activates T cells isolated from patients with grass pollen allergy, but it is not recognized by IgE antibodies specific for grass pollen allergens. This confirms the importance of such studies to establish universal epitopes to serve as a basis for developing

an effective vaccine against a particular group of allergens. Further in vivo studies are needed to validate the effectiveness of such a vaccine against grass pollen allergens

Highlights

What are the main findings?

- A peptide consisting of 20 amino acids was designed in silico as a grass pollen allergy vaccine.
- The epitope-based vaccine has been validated by molecular docking analysis and ex vivo T-cell stimulation assay. It covers more than 80% of the European and global population.

What is the implication of the main finding?

- The immunogenic peptide can be used for treatment of patients with grass pollen allergy by triggering the T-cell response and production of competitive IgG antibodies.
- Additional studies are needed before the clinical application of the epitope-based vaccine for immunotherapy.

3. **Teneva I**., Moten D., Belkinova D., Mladenova T., Dzhambazov B. 2023. Toxic potential of *Anabaenopsis elenkinii* (Cyanobacteria) isolated from a bloom in Lake Vaya (Bulgaria). – Proceedings of 23rd International Multidisciplinary Scientific GeoConference SGEM 2023, 23(5.1.): 287-294. DOI: 10.5593/sgem2023/5.1/s20.36

Abstract: Cyanobacteria are ancient photosynthetic organisms that under certain conditions (high temperature, eutrophication) can grow rapidly and form "cyanobacterial blooms". Very often, these blooms are accompanied by production of cyanotoxins, which in most cases are dangerous to the other organisms. Knowing the producers of cyanotoxins is of utmost importance, especially today when climate change has been shown to increase the frequency of toxic cyanobacterial blooms worldwide. The aim of the present study was to characterize the toxic potential of the cyanobacterium Anabaenopsis elenkinii V.V.Miller 1923 isolated from Lake Vaia during a cyanobacterial bloom. The isolated strain (Anabaenopsis elenkinii) was identified based on a morphological analysis using light microscopy, and its taxonomic affiliation and phylogenetic position were confirmed by applying the Maximum Likelihood (ML) method for phylogenetic analysis based on the 16S rDNA sequence. The production of cyanotoxins was analyzed by applying immunological methods (ELISA) for detection of microcystins, cylindrospermopsin and saxitoxins. The toxic potential of Anabaenopsis elenkinii was evaluated in vitro by methyl-thiazole-tetrazolium (MTT) cytotoxicity and superoxide dismutase (SOD) activity assays using HT-29 cells. Our analyzes indicated that Anabaenopsis elenkinii produces microcystins (0.42 ng/mL), cylindrospermopsin (0.10 ng/mL) and saxithixins (0.05 ng/mL). The MTT cytotoxicity assay showed that the medium, in which the cyanobacterial strain was grown, significantly reduced the viability of HT-29 cells and this effect was dose- and time-dependent. In addition, 50% inhibition of the SOD activity was also observed. This is the first report of Anabaenopsis elenkinii as a producer of cyanotoxins. Our results provide valuable information about the toxin-producing cyanobacteria. They demonstrate the potential danger of "cyanobacterial blooms" where Anabaenopsis elenkinii is a dominant species.

4. Batsalova T., Georgiev Y., Moten D., **Teneva I.,** Dzhambazov B. 2022. Natural Xylooligosaccharides Exert Antitumor Activity via Modulation of Cellular Antioxidant State and TLR4. – *International Journal of Molecular Sciences*, 23(18): 10430. (**Q1, IF-5.6**) DOI: 10.3390/ijms231810430

Abstract: It has been recently proven that xylooligosaccharides (XOS) with prebiotic properties have diverse beneficial biological effects including immunomodulatory and antitumor activities. The present article focused on the chemical and biological evaluation of corn-derived commercially available XOS and aimed to elucidate their cytotoxicity and inhibitory potential against tumor cells. Spectrophotometric chemical analyses, Fourier transform infrared spectroscopy, and high-performance liquid chromatography analyses were performed. Antioxidant activity was determined by measuring the oxygen radical absorbance capacity and hydroxyl radical averting capacity. In vitro cytotoxicity assays with human cell lines derived from normal and tumor tissues, assessments of ATP production, mitochondrial membrane potential specific staining, cytokine assays, and molecular docking were used to evaluate the biological activity of XOS. The sample showed significant antioxidant activity, and it was determined that most xylose oligomers in it are composed of six units. XOS exhibited antitumor activity with pronounced inhibitory effect on lysosomes, but mitochondrial functionality was also affected. The production of proinflammatory cytokines by lipopolysaccharide-stimulated U-937 cells was reduced by XOS treatment, which suggested the involvement of Toll-like receptor 4 (TLR4)-mediated signaling in the mechanism of XOS action. Molecular docking analyses confirmed the potential inhibitory interaction between the sample and TLR4. In addition, XOS treatment had significant tumor-cell-specific influence on the glutathione antioxidant system, affecting its balance and thus contributing to the inhibition of cellular viability. The present study elucidated the tumor-inhibitory potential of commercially available XOS that could be utilized in pharmaceutical and food industry providing disease-preventive and therapeutic benefits

5. Mladenova T., Batsalova T., Dzhambazov B.,Mladenov R., **Teneva I.**, Stoyanov P., Bivolarska A. 2022. Antitumor and Immunomodulatory Properties of the Bulgarian Endemic Plant *Betonica bulgarica* Degen et Neič. (Lamiaceae). – *Plants*, 11(13): 1689. (**Q1, IF-4.5**) DOI: 10.3390/plants11131689

Abstract: Background: Extracts obtained from different Betonica species have been shown to possess important biological properties. The present study aimed to investigate the cytotoxicity, antitumor and immunomodulatory potential of the endemic plant Betonica bulgarica (Lamiaceae) and thus, reveal new aspects of its biological activity. Methods: Methanolic extract obtained from inflorescences was analyzed for cytotoxicity against mammalian cell lines. The antitumor potential of the sample was determined using human cervical and lung adenocarcinoma cells (HeLa and A549). Programmed cell death-inducing effects against HeLa cells and peripheral blood lymphocytes, as well as immunomodulatory properties of the extract were determined by flow cytometry analysis. Results: The research results demonstrated that the extract has significant inhibitory potential against HeLa cells (mean IC50 value 119.2 µg/mL). The sample selectively induced apoptotic death in tumor cells. Cytotoxic effects towards mouse cell lines were detected following treatment with high concentrations of Betonica bulgarica extract (200 and 250 µg/mL). Twenty-four-hour ex vivo incubation of peripheral blood leucocytes in growth medium containing plant extract induced prominent effects in distinct immune cell populations. They included elevated levels of CD25+ and CD56+ T cells' lymphocytes, particularly CD4+CD25+ and CD8+CD56+ cells. Conclusions: The present study demonstrates that Betonica bulgarica inflorescence extract possesses potential beneficial antitumor and immunomodulatory activity and could serve as a source of bioactive compounds with biomedical application.

6. Georgiev Y., Batsalova T., Dzhambazov B., Ognyanov M., Denev P., Antonova D., Wold C., Yanakieva I., **Teneva I.**, Paulsen B.S., Simova S. 2021. Immunomodulating polysaccharide complexes and antioxidant metabolites from *Anabaena laxa, Oscillatoria limosa* and

Phormidesmis molle. – *Algal Research*, 60: 102538. (**Q1, IF-5.276**) DOI:10.1016/j.algal.2021.102538

Abstract: The aim of the present study is to investigate the chemical composition and biological activity of polysaccharides (PSs) and secondary metabolites, isolated from poorly explored cyanobacterial species, in search of immunomodulating and antioxidant compounds. Polysaccharide complexes (PSCs) were obtained by boiling water extraction from biomasses of Anabaena laxa (AL-PSC), Oscillatoria limosa (OL-PSC) and Phormidesmis molle (PM-PSC), pretreated with 80% (v/v) ethanol and pure acetone. The total carbohydrate content in the complexes varies between 53.0% and 63.5% (w/w). The complexes contain PSs composed of neutral monosaccharides as main building blocks and the uronic acids (GalA and GlcA) are in smaller amounts (4.2–9.5%). AL-PSC and OL-PSC are rich in Glc (33.1% and 38.6%), followed by Man, Gal and Xyl as other dominant monomers. PM-PSC has the highest amount of Gal (45%), followed by Glc (21%). Additionally, a neutral exopolysaccharide (EPS) (PM-EPSC), from a (hetero)xylan type (Xyl content 70.5%), was isolated from P. molle. The weight-average molecular weights (Mw) of AL-PSC, OL-PSC and PM-PSC are estimated to be 67.0, 71.8 and 74.7 × 104 g/mol. AL-PSC (200 μg/mL) expresses the most potent ex vivo stimulating activity on human monocytes and granulocytes. AL-PSC and OL-PSC (50–200 μg/mL) manifest better in vitro growth-inhibitory effect against human colorectal adenocarcinoma (HT-29) and colon adenocarcinoma (LS-180) cells than PM-PSC, affecting their lysosomal activity. The organic extracts, obtained from the biomasses extracted with 80% ethanol and acetone, are rich in carbohydrates, followed by fatty acids and other triacylglycerol metabolism compounds, terpenes, Krebs cycle metabolites, chlorophylls and phenolics. Among the three species, the extract from A. laxa (AL-E) contains the highest phenolic content, as well as exhibiting the strongest antioxidant activity by the oxygen radical absorbance capacity (ORAC) assay. A. laxa and the other cyanobacteria should be considered valuable for isolation of immunomodulators and antioxidants with possible application in dietetics and medicine.

7. Moten D., Batsalova B., Dzhambazov B., **Teneva I**. 2021. Comparative genome analysis of *Phormidesmis priestleyi* ULC007 and some representatives of genus *Phormidium* (Cyanobacteria). – *Ecologia Balkanica*, 13(2): 125-134. (**Q4**)

Abstract: Cyanobacteria live in a wide range of habitats encompassing freshwater, marine, and terrestrial ecosystems. They are one of the most morphologically diverse groups. Techniques based on polyphasic taxonomy were introduced to cyanobacterial systematics in an attempt to overcome the shortcomings brought by the traditional emphasis on morphological features. Recent advances in genomics are greatly accelerating our understanding of cyanobacterial taxonomy. Genomes available in NCBI's GenBank were selected to comprehensively clarify the genetic similarities and differences of representatives of genus Phormidium and Phormidesmis priestleyi ULC007 (former Phormidium priestleyi). We performed a comparative genomic analysis, which includes analysis of genome characteristics and annotation of the subsystem, classification of functionally annotated common genes and automatic annotation of secondary metabolic gene clusters. Results showed that about 83% of the genes in the studied cyanobacterial genomes were identified as genes with unknown functions, and those with annotated functions were mainly involved in: (I) cofactors, vitamins, prosthetic groups, and pigments; (II) cell wall and capsule; (III) RNA metabolism; (IV) protein metabolism; (V) DNA metabolism, (VI) fatty acids, lipids, and isoprenoids; (VII) respiration; (VIII) stress response; (IX) amino acids and derivatives; and (X) carbohydrates We found that Phormidesmis priestleyi ULC007 possess seven genes involved in the chemotaxis. The in silico identification, annotation and analysis of the secondary metabolite biosynthesis gene clusters of the most promising targets within the studied cyanobacterial genomes showed presence of specific secondary metabolite genes, which need further detailed analyses.

8. Belkinova D., **Teneva I.**, Basheva D., Neykov N., Moten D., Gecheva G., Apostolova E., Naimov S. 2021. Phytoplankton composition and ecological tolerance of the autotrophic picoplankton in Atanasovsko lake (Black Sea coastal lagoon, Bulgaria) – *Applied Ecology and Environmental Research*, 19(2): 849-866. (**Q3, IF-0.816**) DOI:10.15666/aeer/1902_849866

Abstract: Atanasovsko Lake is a coastal lagoon used for salt extraction for many years. Its salinity varies within a wide range (from 6.3 to > 72‰). Despite these extreme conditions, phytoplankton blooms are often observed. Therefore, our aim was to study the key abiotic drivers and ecological optimum of the picoplankton in a 5-year period (2013-2017). The taxonomic composition, long-term dynamics and abiotic drivers of the autotrophic picoplankton (APP) has been investigated in North Saltern of the coastal lagoon in Atanasovsko Lake. The APP species were identified by using a polyphasic approach based on molecular and phylogenetic analyses of 16S/18S rDNA in combination with cytomorphological and ecological characterization. Picochlorum oklahomense (Trebouxiophyceae, Chlorophyta) and marine Synechococcus sp. (Synechococcales, Cyanobacteria) were the dominant genotypes within the APP. The relative biovolume of the APP to the total phytoplankton biovolume was strongly variable (from 36 to 99%) as the salinity and temperature were found to be the main abiotic drivers of its dynamics. Salinity between 30-59‰ and mid-summer temperature above 24°C provide an ecological optimum for the APP development at a relative biovolume of 93%. This is the first record of Picochlorum oklahomense/Synechococcus sp. assemblage within the APP composition of a European coastal lagoon. Keywords: ecology, hypersaline lakes, Synechococcus, Picochlorum oklahomense, 16S/18S DNA.

9. Moten D., Batsalova T., Mladenov R., Dzhambazov B., **Teneva I**. 2020. CP43 and CP47 proteins of photosystem II (PSII) as molecular markers for resolving relationships between closely related Cyanobacteria. – *Ecologia Balkanica*, 12(2): 141-151. (**Q4**)

Abstract: Cyanobacteria are the most primitive photosynthetic organisms on the Earth. Their classification is traditionally based on morphological characters in both botanical and bacteriological systems. Due to the enormous diversity and the lack of clear diacritic morphological features between the closely related species, for resolving the evolutionary relationships and classification of Cyanobacteria during the last years is used a polyphasic approach including sequencing data. Although many molecular markers are improved, new suitable markers for resolving relationships within cyanobacteria at species and generic level are still needed. Our objective was to examine whether the sequences of the photosystem II proteins CP43 and CP47 are suitable markers for such purposes. Phylogenetic analyses based on the CP43 and CP47 amino acid sequences showed that most of the cyanobacterial species/strains belonging to different genera are clustered in separate clades supported by high bootstrap values. The comparison between the CP43 and CP47 trees, and the 16S phylogenetic trees showed that the CP43 and CP47 proteins are more suitable markers in resolving phylogenetic relationships within Cyanobacteria at generic and species level than the conserved 16S rRNA gene sequence. The correct taxonomic classification and identification of the cyanobacterial strains is very important for all studies related to the biological activity of cyanobacteria, their biotechnological application or in the management and monitoring of water.

10. Moten D., Batsalova T., Dzhambazov B., **Teneva I**. 2020. Comparative genome analysis of some representatives of genus *Nostoc*. – *20th International Multidisciplinary Scientific GeoConference SGEM 2020. Conference proceedings*, 20(5.1): 167-174.(**SJR 0.217**) DOI: 10.5593/sgem2020/5.1/s20.021

Abstract: Cyanobacteria are a diverse group of photosynthetic bacteria found in a wide variety of habitats where they play crucial roles. They are one of the most morphologically diverse groups. The evolutionary relationships and the classification are poorly understood. The whole genome sequencing added a new dimension to our understanding of genomes of cyanobacteria and the genome-based phylogeny came into existence.
The aim of this study was to conduct a genomic comparison of phylogenetically closely related filamentous cyanobacteria (Nostoc carneum NIES-2107, Nostoc sp. PCC 7107, Nostoc punctiforme PCC 73102, Nostoc piscinale CENA21, Nostoc linckia NIES-25, Nostoc flagelliforme CCNUN1, Nostoc commune HK-02 and Tolypothrix sp. PCC7601) with a particular focus on the comparison of the genomic features and subsystem annotation, the classification of functionally annotated common genes and the automatic annotation of secondary metabolite gene clusters.
Genome sizes, N50, number of RNAs, genes and proteins were similar across all eight strains. The G+C contents were almost identical (approximately 41%). The results of the RAST Server and SEED viewer subsystem annotation showed that their genomes are considerably similar. An average of 70% of genes in the studied genomes were identified with unknown functions, and those with annotated functions were mainly involved in: (I) cofactors, vitamins, prosthetic groups, and pigments; (II) cell wall and capsule; (III) membrane transport; (IV) protein metabolism; (V) regulation and cell signalling, (VI) fatty acids, lipids, and isoprenoids; (VII) respiration; (VIII) stress response; (IX) amino acids and derivatives; and (X) carbohydrates. The results of the antiSMASH Server showed more secondary metabolite gene clusters in Tolypothrix sp. PCC7601 and Nostoc punctiforme PCC 73102 compared to other cyanobacterial strains.

11. **Teneva I**., Belkinova D., Moten D., Basheva D., Dzhambazov B. 2019. In search of new molecular markers for taxonomic classification of Cyanobacteria. – *19th International Multidisciplinary Scientific GeoConference SGEM 2019. Conference proceedings*, 19(5.2): 461-468. (**SJR 0.232**) DOI: 10.5593/sgem2019/5.2/S20.057

Abstract: Cyanobacteria are the main components of the phytoplankton in many freshwater and marine ecosystems. So far, about 150 genera and over 2000 species have been described. Although they are one of the most ancient groups of organisms, their taxonomic classification is problematic even today, due to the lack of clear diacritic morphological features between the closely related species, the high variability of the morphological features used for taxonomical purposes depending on the environmental conditions and the occupied ecological niches, as well as the possible horizontal gene transfer.
Consolidated around the idea of applying the principles of the polyphasic taxonomy, the scientists working with cyanobacteria agree that the molecular approach should be the basis for the reorganization of knowledge about Cyanobacteria. It has been accepted that it is better to have more clearly defined monophyletic genera, each of which contains relatively few species than large, poorly defined polyphyletic genera, containing many unrelated species.
In this regard, the search, development and application of new molecular-genetic markers for phylogenetic analysis and taxonomic classification of Cyanobacteria is an important task. The larger number of molecular markers would mean a more accurate and precise classification. Therefore, the goal of our study was to develop a new marker that supports and complements the known confirmed markers (16S rDNA, rpoC1, rpoB, gyrB, rbcLX, cpcBA-IGS).

Based on the results of the bioinformatics analysis, we think that Psb27 is a suitable candidate for a new molecular marker that can be used for generic and species determination of cyanobacteria. Psb27 is a small (11 kDa) lipoprotein that is part of the photosystem II (PSII) localized in the thylakoid membrane of cyanobacteria. This small protein is suitable since its surface helices III and IV are highly conserved structures responsible for binding to PSII, but the N-terminal part is species-specific.
The phylogenetic analyzes performed by various phylogenetic methods and algorithms (minimum evolution, maximum parsimony, maximum-likelihood, and neighbor-joining) based on the Psb27 gene and amino acid sequences, demonstrated its applicability as a molecular marker. The clear separation of the closely related taxonomic categories into monophyletic groups in accordance with the morphological features of the taxa as well as the good correlation with the results from the phylogenetic reconstructions based on 16S rDNA allow us to suggest the Psb27 protein as a new molecular marker that can be used in the taxonomic classification of Cyanobacteria.

12. Moten D., Batsalova T., Basheva D., Mladenov R., Dzhambazov B., **Teneva I**. 2018. Outer Membrane Efflux Protein (OMEP) is a suitable molecular marker for resolving the phylogeny and taxonomic status of closely related Cyanobacteria. – *Phycological Research*, 66: 31-36. (**Q2, IF-1.342**) DOI: 10.1111/pre.12203

Abstract: Taxonomy of Cyanobacteria, the oldest phototrophic prokaryotes, is problematic for many years due to their simple morphology, high variability and adaptability to diverse ecological niches. After introduction of the polyphasic approach, which is based on the combination of several criteria (molecular sequencing, morphological and ecological), the whole classification system of these organisms is subject to reorganization. The aim of this study was to evaluate whether the outer membrane efflux protein (OMEP) sequences can be used as a molecular marker for resolving the phylogeny and taxonomic status of closely related cyanobacteria. We have performed phylogenetic analyses based on the amino acid sequences of the OMEP and the DNA sequences of the 16S rRNA gene from 86 cyanobacterial species/strains with completely sequenced genomes. Phylogenetic trees based on the OMEP showed that most of the cyanobacterial species/strains belonging to different genera are clustered in separate clades supported by high bootstrap values. Comparing the OMEP trees with the 16S rDNA tree clearly showed that the OMEP is more suitable marker in resolving phylogenetic relationships within Cyanobacteria at generic and species level.

INDICATOR GROUP G8.
PUBLISHED CHAPTER FROM A BOOK OR COLLECTIVE MONOGRAPH

1. Belkinova, D., Mladenov, R., Dimitrova-Dyulgerova, I., **Teneva, I**., Stoyanov, P**.,** Cheshmedjiev, S. Phytoplankton of the Stouden Kladenets Reservoir (Eastern Rhodope Mountains, Bulgaria). 2012. In: PU “Paisii Hilendarski”, Jubilee Proceedings “Biological sciences for a better future”, University of Plovdiv Publishing House, 2012, 42-61. ISBN 978-954-423-780-6.

Abstract: The subject of the research was to determine the taxonomic composition,species richness and abundance of the summer phytoplankton in the Stouden Kladenets Reservo ir (the eastern Rhodope Mountains, Bulgaria). A total of 30 taxa were found, belonging to 6 Divisions: *Cyanoprokaryota* – 3, *Chlorophyta* – 14, *Zygnemaphyta* – 3, *Chrysophyta* – 1, *Euglenophyta* – 3 and *Bacillariophyta* – 6. The Divisions *Chlorophyta* (46,7%) and *Bacillariophyta* (20,0%) turned out to have the largest relative species richness in the floristic composition. The species’ richness, density and biomass of the phytoplankton increase in the direction from the dam wall towards the

tail end of the reservoir. On the basis of the species’ composition, species richness and abundance of the phytoplankton, the Stouden Kladenets Reservoiraquatory (aquatic territory) can be divided into two zones, substan tially differing in water quality. The open reservoir water is oligotrophic, with signs of mesotrophy, whereas in the tail part there is strong eutrophication and algae bloom.

2. Stoyanov, P., Belkinova, D., Mladenov, R., Teneva, I. Analysis of the water in the reservoirs Krushovitsa, Enitsa and Valchovets (Northern Bulgaria) for presence of cyanotoxins. In: PU “Paisii Hilendarski”, Jubilee Proceedings “Biological sciences for a better future”, ISSN 1312-062X, University of Plovdiv Publishing House, 2012, 237-249.

Abstract: This study presents data on the dominant species Cyanoprokaryota and results from performed toxicological analysis of the water regarding presence of cyanotoxins in three relatively small reservoirs located in Pleven district (Northern Bulgaria): Krushovitsa reservoir, Valchovets reservoir and Enitsa reservoir. Cyanoprokaryotic species *Planktothrix agardhii, Anabaena spiroides* and *Aphanizomenon flos-aquae*, reported repeatedly as producers of hepato- and neurotoxins, were dominant in the studied reservoirs with bloom concentrations from 1,03 mg/L to 10,5 mg/L. Analysis of the water samples for presence of cyanotoxins was conducted using ELISA kits for microcystins/nodularins and for saxitoxins, HPLC, as well as in vitro cytotoxicity tests. Microcystins/nodularins were detected in the water samples collected from Enitsa reservoir and saxitoxins were found in the water samples from Valchovets reservoir.

Plovdiv, 06.02.2025

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