ANNOTATION

For the dissertation for Doctor of Philosophy (PhD) degree
on the speciality “6D070100 – Biotechnology” of Huma Balouch
on the theme "Study of the biodiversity of microalgae in Almaty region and prospecting for biotechnological valuable strains".

General description of the research: The overarching purpose of this Ph.D. dissertation is studying the diversity of microalgae from different freshwater habitats of Almaty region, selection, isolation and identification of the most promising strains of microalgae, which have good potential for use as source of biofuel feedstock, antibacterial compound and bio-indicators.

The relevance of the research: Heavy reliance of developing countries on fossil and imported fuels to meet the growing energy needs has become a significant impediment to their socio-economic development. Combustion of the fossil fuel is by far the largest source of atmospheric pollution, and major factor responsible for climate change and global public health concern. The need for transitioning away from fossil fuels has been on the national priority agenda of developing countries, beginning with the recognition of bioenergy as an alternate and sustainable method for producing carbon-neutral energy source. This is further viewed as a crisis because of the recognition that bioenergy, which includes both biomass and biofuel, will exert pressure come of exploiting agricultural lands for growing biofuel crops. Research is being directed towards exploring ways and harnessing alternate, local or indigenous sources for biomass and biofuel without negatively impacting food security. With the current trend towards conservation and sustainable resource management, there is renewed interest in exploring microalgae for biofuel production, bioremediation, value-added industrial and biodegradable products.

Antimicrobial resistance (AMR) poses a severe, ongoing, and global risk to population and environment health. Recent estimate provided by World Health Organziation (WHO) suggest that more than a half a million deaths globally are attributable to the drug-resistant bacterial infections. Extensive efforts are underway to find novel and unusual antibacterial compounds which expand beyond the known antibacterial motifs. Owing to their extraordinary physiological, ecological, molecular and regulatory mechanisms, microalgae can be found in number of environments and capable of surviving in extreme conditions. Thus, the novel natural products with diverse biological activities, notably antibacterial, are expected to be obtainable from microalgae, thriving in extreme conditions.

Ecological monitoring is fundamental to our understanding of how the above mentioned human interventions, particularly persistent metal pollution or contamination, impact key species and ecosystem functions. Understanding the specific changes to freshwater biota following exposure, bioaccumulation and

toxicity of heavy metals pollutant is vital to assess the functional status of ecosystem. The identification of specific molecular, biochemical, physiological and behavioral changes in highly sensitive species can be used as tool to build the resilience of degrading ecosystem. Recent decades have witnessed an increased appreciation of the role of microalgal diversity in ecosystem function and their significant value as source for bioindicators of water quality. However, thus far there has been limited success with restricted set of indicator species.

The comprehensive understanding of the afore mentioned ecological role and deeper exploration of biotechnological potential of microalgae in areas of basic, applied and industrial research requires the accurate identification of species. There have been few systematics investigations of microalgae species from unusual or extreme habitats thereby limiting our knowledge of their biodiversity, function and their potential as source of biotechnologically important bioactive compounds. The lack of basic information on microalgal species diversity at different taxonomic levels has significant implications for many aspects of ecosystem monitoring, conservation biology, and evolutionary biology. Recent advancements in molecular biology, biomolecular engineering, informatics, and other related sciences has greatly facilitated scientists for tapping the maximum potential of microalgae with applications ranging from drug development, agriculture, industry, to bioenergy and sustainable environment.

The purpose of the research: In the present study, investigations were focused on finding promising strain of microalgae from freshwater habitats of Almaty region, able to demonstrate biofuel potential, antimicrobial activity and bioindication.

The main tasks of the research:

1. To investigate the diversity of microalgae species in the Almaty region with the aim of providing baseline information needed for the assessment of changes in microalgae biological diversity in the Almaty region.
2. To develop approaches for accurate identification of isolated monoculture microalgae strains based on light and scanning microscopy, ITS amplicon sequencing, and rbcL-gene primer.
3. To isolate microalgae species with high biomass, high lipid content and suitable fatty acids, desired for producing biofuel.
4. To study the antimicrobial potential of microalgae isolates and to determine the zone of inhibition of methanol extracts against different pathogenic bacteria.
5. To assess the utilization of microalgae strain as bioindicators of heavy metal pollution in freshwater bodies.

The research objects and materials: Microalgae isolates including *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02,

Ankistrodesmus falcatus ZBD-03, *Parachlorella kessleri* ZBD-04, and *Desmodesmus pannonicus* ZBD-05, *Monoraphidium sp*. ZBD-06, and *Ankistrodesmus sp*. B-11.

Research methods: Cultivation methods, lipid extraction, trans-esterification, Gas Chromatography Mass spectrometry - Fatty Acids Methyl Esters (FAMEs) analysis, agar disk-diffusion assay, minimum inhibitory concentration (MIC), DNA extraction, Polymerase Chain Reaction, DNA cloning, sanger sequencing, fluorescence assays.

The scientific novelty of the research: As a result of the research, 7 new isolates of microalgae were isolated and identified: *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Ankistrodesmus sp*. B-11, *Parachlorella kessleri* ZBD-04, *Desmodesmus pannonicus* ZBD-05, including a psychrophilic new strain *Monoraphidium sp.* ZBD-06, for which no information was available in GenBank.

The potential of isolated strains of green microalgae as valuable sources of fatty acids methyl esters for biodiesel fuel has been studied for the first time. Strains *P. kessleri* and *A. falcatus* were selected as species with high biomass and lipid productivity, while the study of fatty acid composition demonstrated the dominance of palmitic, stearic and oleic acids, which are known to be the most dominant components of biodiesel fuel. The presence of higher quantities of saturated fatty acids (C16 - C18) and high productivity of biomass confirms the prospects of their use in the bioenergy sector as a raw material.

The potential efficacy of a crude extract of isolated microalgae isolates as an antibacterial agent has been tested for the first time. Their ability to suppress the growth of some pathogenic gram-positive and gram-negative bacteria has been established. In the previous literature, very few studies have successfully reported the efficacy of antimicrobial activity of green microalgae against gram negative bacteria. However, in this study, it is noteworthy that the green microalgae isolates have good potential in screening bio-control agents and discovery of natural products with new structures.

The selected strain *Ankistrodesmus sp*. B-11, showed high sensitivity to cadmium ions, which can be used as a test object in assessing the ecological state of aquatic ecosystems contaminated with heavy metal ions. It was established for the first time that cadmium reduces the rate of electronic transport to the membrane of thylakoids, inhibits the transport of electrons and protons at the level of plastoquinone (PQ) during the transfer of electrons from PS II to PS I. It was found that the concentration of cadmium 0.01 mg/l causes a change in the ultrastructure, which primarily affect the photosynthetic apparatus, in particular, the modifications concerned the location of thylakoids in the stroma: an increase in interthylakoid spaces, resulting in a decrease in photosynthetic activity. A certain significant

increase in the vacuolization of cells for calculating structural changes in the cytoplasmic membrane under the control of cadmium ions.

Theoretical and practical significance of the research: The main conclusions and provisions of the work expand the theoretical basis of this direction of research, deepen our knowledge associated with the basics of the influence of heavy metals on the photosynthetic activity and ultrastructure of microalgae cells. Also, the theoretical significance of this study lies in the fact that its results significantly supplement the currently available scarce data on the species biodiversity of the algal flora of aquatic ecosystems in the Republic of Kazakhstan, in particular, 5 lakes in the Almaty region. The results obtained expand the data on the properties of new strains of microalgae that are promising for use in various fields of biotechnology, including bioenergy. The study of the metabolic characteristics of lipid-producing strains of microalgae and their antibacterial properties is of fundamental nature, revealing individual features of the processes of lipid accumulation in microalgae cells. The data obtained suggest lines for further work on improving the accumulation of lipids by strains of microalgae using the synthetic and industrial biotechnology techniques. The data obtained on the antibacterial effect of the isolated isolates of microalgae and their fatty acid composition significantly expand the understanding of the mechanisms of the antagonistic action of microalgae against pathogenic bacteria.

The practical significance of this research lies in obtaining pure microalgae cultures with great biotechnological potential and which, accordingly, can be used as raw materials in bioenergy, pharmaceuticals and medicine. In addition, the revealed changes in the induction curves of rapid and delayed fluorescence, being one of the first rapidly recorded parameters of microalgae cells after the action of cadmium, could be used to diagnose the condition of objects. The relative changes of chlorophyll fluorescence parameters indicated in this study can also be used for biotesting water quality in natural and artificial reservoirs.

The main provisions for the defence:

This study reveals that the majority of species, making up the phytoplankton community in freshwater bodies of Almaty region, were diverse and showed significant variations among different taxa.

In this study, six microalgal species based on parameters for fast growth were cultivated and their total lipid and FAME profiles indicated their high potential of two strains *Parachlorella kessleri* ZBD-04, and *Ankistrodesmus falcatus* ZBD-03 for use as feedstock for biofuel based on their calculated biodiesel properties (Cetane number 50 and 48, Iodine Value 103.6 and 83.4), more than the required limit (CN >47.0) in accordance with the EN14214 biodiesel standards.

The study showed high potential of microalgal isolated strains for biotechnological application in different aspects and their utilization as raw-material for the production of high-value products such as antibiotics.

The accumulation of heavy metals in cells of *Ankistrodemus sp*. and the observed ultrastructural changes indicated their sensitivity to the presence of potentially toxic heavy elements in the environment, thus indicative of water pollution.

Key research findings and conclusion:

These results obtained in this study have increased the scope of finding industrially important microalgae from the freshwater habitats of Almaty region and these isolates, which have been maintained as stock cultures, could be vital source for the discovery of industrially useful bioactive compounds.The results of the study gave an idea of the detailed morphological characteristics and biochemical nature of the isolated strains which would aid in the identification and characterization of the isolated microalgal strains in future.

The ITS region and rbcL gene was examined in six species namely, *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Parachlorella kessleri* ZBD-04, and *Desmodesmus pannonicus* ZBD-05, *Monoraphidium sp*. ZBD-06, which has successfully provided additional sequence data in Genbank databases and has resolve better the debate on their taxonomic status.

The fatty acid compositions of four isolates were studied and the major fatty acids were palmitic acid, oleic acid and stearic acid comprising of 10-45%, 5- 34% and 5-30% of the total fatty acids. Collectively these fatty acids occupied up to 30-70% of the total fatty acids in two strains *Parachlorella kessleri* ZBD-04 and *Ankistrodesmus falcatus* ZBD-03 with Cetane number more than 47, which is an ideal component and number to be considered for biodiesel, hence, this study suggest that the studied isolates represent valuable resources for future research for microalgae-based biofuels.

The present study has shown the promising antimicrobial activities of methanol extracts of four strains against gram positive and gram negative bacteria. The preliminary (agar disk-diffusion) and secondary (minimum inhibitory concentration (MIC)) antimicrobial assays displayed significant antibacterial activity of methanol extract of *Parachlorella kessleri* against *Bacillus subtilis* (maximum zone of inhibition 0,8 mm), *Staphylococcus aureus and Klebsiella pneumoniae*, *Nephrochlamys subsolitaria* against *Bacillus subtilis* (maximum zone of inhibition 0,6 mm), *Pseudomonas aeruginosa* and *E. coli, Monoraphidium griffithii* against *Klebsiella pneumoniae* and *E. coli*, and *Ankistrodesmus falcatus* against *Klebsiella pneumoniae* and *E. coli*.

Study of the effect of low concentrations of cadmium ions on growth, photosynthetic activity and ultrastructure of cells of the microalga *Ankistrodesmus sp. B-11* revealed a high sensitivity of the strain to cadmium concentrations of 0.005-0.02 mg / l in the nutrient medium.

The levels of research organization: The research described in this dissertation was performed on genetic, biomolecular, cellular, and organism level.

Relationship of the research with the scientific project. The dissertation research work was undertaken under the framework of three projects including 'AP08052402 Development of technology for obtaining bio-fertilizers based on nitrogen-fixing cyanobacteria'; AR08052481 Development of a technology for producing biodiesel based on active strains of microalgae'; 'AP05131743 Development of scientific and methodological foundations for biomonitoring technology and forecasting the state of polluted aquatic ecosystems using phototrophic microorganisms.

The contribution of author for the results described in the dissertation: All the main results described here are performed, and collected by the author. In addition, main research results, analyses, data and figures are generated by the author, and all the new observations and conclusions are made from the results derived from Ph.D. candidate's work and research.

Research approbation: The main results and observations are presented and discussed at international conferences and symposiums:

- VI International Farabi Readings, Almaty, Kazakhstan, 2-12 April 2019, on the sideline -International Scientific Conference of Students and Young Scientists Almaty, Kazakhstan, April 9-10, 2019, Oral presentation.
- European Biotechnology Congress -2020 September 24-26, 2020, Prague.
- International scientific and practical conference on 'Aspects and Innovations of Environmental Biotechnology and Bioenergy' Al-Farabi Kazakh National University, Almaty, Kazakhstan, 12-13 February, 2021.

5th Symposium on EuroAsian Biodiversity (SEAB-2021) – Online –jointly by Al-Farabi Kazakh National University, Kazakhstan, and Muğla Sıtkı Koçman University, Turkey, on July 01-03, 2021.

Publications: The majority of this dissertation was published in 6 scientific works, including 1 research article with impact factor, indexed in Web of Science (WoS) and SCOPUS, 4 articles in scientific journals recommended by Education and Scientific monitoring Committee of Ministry of Education and Science of the Republic of Kazakhstan (CCESF MES RK), 4 abstract in the materials of international conferences. Two articles are under process of publication in reputable international peer reviewed scientific journals.

Dissertation structure: This dissertation is written in 104 pages, containing notations and abbreviations, and describes, in detail, introduction, literature review,

materials and methods, results and discussion, conclusion, supported by 287 citations to technical literature (references), and contains 11 tables and 18 figures.