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I am writing this reference letter in support of my student, Ms. Huma Balouch, and her Ph.D dissertation as requisite for the degree of Doctor of Philosophy (Ph.D) in the specialty: 6D070100 – Biotechnology, titled "Assessment of Biodiversity of Microalgae in Almaty region: Morphological and Metagenomic Exploration".

Microalgal biotechnology has been increasingly studied due to its potential industrial applications and for obtaining infinite number of high-value products. In the recent alarming scenario of industrialization and exploitation of non-renewable resources, microalgae seem to be a promising solution. Hence, the study by Ms. Balouch aimed to highlight the potential of microalgae strains as an alternative and most sustainable source of biomass for next-generation biofuels, novel antibiotic compounds, and bioindicators for water quality assessment.

Her research involved multi-level analyses of microalgae from technical, economic and environmental points of views. Her study highlights that isolation, identification of indigenous microalgal strains with promising properties is a key to improving the feasibility of bioprospecting for microalgal-derived high value products.

The multi-level analyses of microalgal biodiversity of this study will provide a system to understand the mechanism contributing towards assessing the microalgal diversity and conferring the value it may have to the structure and function of entire community in a given area. This is particularly important for microalgal species and strains of economic value or environmental concern. The molecular analyses of this study aim to highlight the on-going challenge in identification of microalgae strains due to unavailability of taxonomically curated DNA databases. For improving success in accurate specific or infra-specific identification of microalgae strain, this systematic study enables deposition of more taxonomically accurate reference barcodes in curated databases using combined approach of morpho-taxonomy and metabarcoding using different ITS region and *rbcL* molecular markers.

In this study, Ms. Balouch's efforts were focused on identification, isolation, and characterization of microalgae from the under investigated freshwater environment. Green microalgae were isolated from the freshwater reservoirs of Almaty region, and algologically and bacteriologically pure form were obtained for microalgae strains with potential biotechnological significance. The taxonomy and phylogeny of the green microalgae was examined based on morphological (light and scanning electron microscopy) and sequence-based approach using universal molecular markers of ITS region and *rbcL* gene). In her present work, native species of microalgae were evaluated as a source of feedstock for biofuel production by fatty acids profiling. The antibacterial activity of the isolate was also determined by disk diffusion assay and minimum inhibitory concentration, using eleven different types of pathogenic bacteria. To unfold the bioindication potential of microalgae, ultrastructure of microalga cells treated with various concentrations of cadmium ions in the medium were examined for changes in growth,

photosynthesis, and to select the most sensitive chlorophyll fluorescence parameters for their prospective use in bio-testing.

This thesis helps advance knowledge in the field of microalgae biodiesel research. The work has developed a methodology for screening microalgae species and for ensuring that the biomass is suitable for biodiesel production. The obtained results of biofuel properties and fatty acid composition enhanced our understanding of the multifaceted role of microalgae from the extent of microalgal bioindication function to and microalgal biomass productivity for sustainable feedstock for biofuel.

The thesis work by Ms. Balouch helps develop a rich understanding of the available literature, a well-thought-out structure. She has connected her study well with the existing knowledge, as well as advance knowledge for future research to maximize the use of microalgae, and offered technological solutions to health, natural resource, and ecosystem sustainability issues.

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