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Reducing nutrient requirement using nitrogen-fixing bacteria for microalgae cultivation

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ABSTRACT

In environments, microalgae have been observed to coexist with bacteria. Different nitrogen-fixing bacteria (NFB) were isolated from Armenian soils and their growth was evaluated in co-cultivation with the microalga *Tetradesmus obliquus* and cyanobacteria *Synechocystis* sp. PCC 6803. The most effective mutualistic consortium was *T. obliquus*-NFB5 (*Sphingobacterium* sp. L13G8). This resulted an increase in both populations, chlorophyll fluorescence, biomass protein, carbohydrate content, an effect on lipid metabolism, without the need for external nitrogen. The findings demonstrated the significance of employing NFB for microalga growth, as they facilitate the essential nitrogen provision in N-free Bristol medium. Moreover, in mutualistic consortia, microalgae facilitate the exudation of dissolved organic carbon and O_2 to bacteria, which, in turn, become available for bacteria, thereby reducing the necessity for energy-consuming aeration processes in co-cultivation. In return, the bacteria provide the microalgae with CO_2, B vitamins and demineralize N_2, P, S, thereby further supporting the growth of microalgae.

1. Introduction

Microalgae are a class of photosynthetic organisms that are commonly associated with aquatic ecosystems. The nutrients required for proper growth include carbon dioxide, nitrogen, phosphorus, sulfur, and trace metal ions. Nitrogen is the most important nutrient for protein production, and its availability is directly related to biomass production. Microalgae utilize a variety of nitrogen sources, including ammonia, nitrates, and amino acids (Aburai et al., 2023).

As regards to nitrogen-fixing bacteria, the capability of them to exclude the use of synthetic nitrogen is well-proved (Bellenger et al., 2020; Koirala et al., 2025). The majority of these organisms are soil-dwelling, and some have been observed to coexist with leguminous plants (Pankievicz et al., 2019). These bacteria are suitable to reduce atmospheric nitrogen to NH_3 by using nitrogenase for N obsession. Nitrogenase is a complex heteroenzyme conforming of two subunits. The first element, designated as nitrogenase reductase, is a homodimer of NifH decoded by *nifH*. An alternative candidate is dinitrogenase. In its molecular form, this is comprised of two protein subunits that are linked together. The nascence chain is decoded by *nifD*, and the beta chain is decoded by *nifK*. In the context, the focus has been on three distinct structural components; notably, *nifH* genes have been employed extensively for the study of diazotroph diversity. Phylogenetic analysis based on the *nifH* gene has traditionally been used to classify *nifH* and its homologs into clusters. Nevertheless, the enhancement in the accessibility of sequence data has catalysed the evolution of progressively refined classification methodologies. The initial cluster (I) comprises NifH and VnfH from aerobic and facultative anaerobic proteobacteria and cyanobacteria. The second cluster is characterised by the presence of VnfH and NifH from a select group of archaea. The third cluster consists of NifH from pure anaerobes, whereas the fourth cluster contains all NifH homologs, including both NflH and Bch/ChlL. Despite the assertion that the nitrogenase enzyme complex remains the sole enzyme system in prokaryotes recognised as capable of fixing nitrogen, studies have

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