## ABSTRACT

Ciliates are significant due to their worldwide distribution, great diversity, and highly studied organism. Ciliates greatly affect the nutrients cycle along with food web in freshwater bodies because they feed upon bacteria, algae, and other small protists. Aim of this study was to isolate and characterize the freshwater ciliates based on morphology and molecular level along with estimation of their protein content. Freshwater samples were collected from Jallo lake, Lahore Canal and river Ravi. Various samples contain *Paramecium*, *Tetrahymena* and *Stylonychia* which were identified on the basis of their shape, size and movements. *Tetrahymena* MG1 was identified due to their pear shape *i.e.*, wider from one end and pointed from other end, ciliary lining, central nucleus and oral groove at pointed end.

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Pure isolates were cultured in wheat grain media at different temperatures and pH to evaluate their optimum temperature and pH. Best growth was observed at temperature 25±2℃ and at pH 7.2±0.3.

Molecular characterization was done on the basis of COX1 gene while neighbourjoining method was used to construct phylogenetic tree which shows that sample MG1 has close relations with already reported <u>Tetrahymena</u>. Single cell protein was obtained by centrifugation of pure culture and then <u>lyophilized</u> it. Biuret test was performed to estimate the total concentration of protein, <u>Ninhydrin</u> test was performed to estimate amino acids content while <u>Xanthoproteic</u> test was performed to estimate the number of aromatic acids. Protein estimation using Bradford assay shown 251.6 mg of proteins in one liter of <u>Tetrahymena</u> culture. Protein profiling of total cell protein was done on the basis of SDS-PAGE.