

Abstract

Ctenopharyngodon idella, commonly known as grass carp, serves a dual purpose as a vital food source and an effective natural solution for weed control in aquatic ecosystems. Due to these remarkable attributes, *C. idella* has earned its status as one of the most extensively cultivated fish species in Asia. This research endeavor was undertaken to isolate and meticulously characterize six distinct biofilm-forming pathogens and six beneficial probiotic strains derived from *Ctenopharyngodon idella* specimens harvested from diverse fish ponds situated in Sheikhpura and Lahore, within the Punjab region of Pakistan. The isolated strains underwent a comprehensive characterization process, encompassing evaluations of their morphological, physiological, and biochemical attributes. The pathogenic strains were unequivocally identified as *Vibrio* sp., *Pseudomonas* sp., *Enterococcus* sp., *Mammaliococcus sciuri*, *Bacillus* sp., and *Streptococcus* sp. In contrast, the probiotic strains were classified as *Staphylococcus* sp., *Lactobacillus* sp., *Enterococcus durans*, *Bacillus pumilus*, and two separate strains of *Streptococcus* sp. To gauge their potential for biofilm formation, all pathogenic strains were subjected to various assessment methods, including the Congo red assay, test tube assay, and liquid interface coverslip assay. The results revealed that *Vibrio* sp., *Enterococcus* sp., and *Clostridium* sp. displayed the highest optical density (OD) at day 3, while *Pseudomonas* sp., *Bacillus* sp., and *Streptococcus* sp. exhibited peak activity at day 5. Under optimal growth conditions, characterized by a pH level of 7 and a temperature of 37°C, all strains flourished. Furthermore, the probiotic strains demonstrated substantial antibacterial activity against specific pathogenic strains when assessed using the cross-streaking method. Notably, their efficacy surpassed that of the control group (*Bioflor*), highlighting their potential in combating biofilm-forming pathogens. In summary, this study provides invaluable insights into the isolation and characterization of biofilm-forming pathogens and probiotics sourced from *Ctenopharyngodon idella* in the examined region of Pakistan. It underscores the promising role of probiotic strains in mitigating the adverse impacts of biofilm-forming pathogens. These findings significantly contribute to our comprehension of microbial ecology and biofilm-related infections within aquaculture systems. Consequently, this research lays the foundation for the development of targeted control strategies and enhanced management of fish health.