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Tardigrades in Wastewater Biomonitoring: Microscopic Organisms with Exceptional Environmental Resilience

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Tardigrades are microscopic, multicellular invertebrates with a cosmopolitan distribution across terrestrial and aquatic ecosystems. Due to their exceptional physiological adaptations, particularly their capacity to endure extreme environmental stressors, they have emerged as model organisms in comparative physiology and extremophile biology. To date, over 1200 species have been documented from a broad range of ecological niches, including bryophytes, lichens, soil substrates, and both ephemeral and permanent aquatic habitats. A defining characteristic of tardigrades is their ability to undergo cryptobiosis—a reversible ametabolic state triggered by adverse environmental conditions such as severe desiccation, extreme temperature fluctuations ranging from 90 °C to −196 °C, hyperbaric pressures (up to 7.5 GPa), exposure to organic solvents, and ionizing radiation[1]. During cryptobiosis, metabolic activity is virtually suspended, and the organism contracts into a desiccated, barrel-shaped form known as a "tun," wherein the cellular water content is reduced to as little as 2–3%, and body volume diminishes by approximately 85–90%. This anhydrobiotic state facilitates survival in environments that exceed the tolerances of most known metazoans. Notably, tardigrades have demonstrated viability even after a 10-day exposure to the vacuum and radiation of outer space[2-4].

In recent years, tardigrades have received increasing attention as potential bioindicators for assessing environmental quality in contaminated ecosystems, particularly within wastewater treatment facilities, owing to their exceptional physiological resilience under harsh environmental conditions. Species such as *Thulinius ruffoi*, observed in activated sludge, demonstrate the capacity to persist in environments characterized by high nutrient loads and complex chemical pollutants. The elevated abundance of such taxa during early stages of wastewater treatment—typically associated with peak pollutant concentrations—underscores their potential utility in monitoring effluent quality dynamics[5]. Nevertheless, the relatively limited distribution and low population densities of tardigrades in certain wastewater habitats, in comparison with more commonly employed indicator taxa such as protozoans and rotifers, have posed constraints on

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their broader application as routine bioassessment tools[6, 7]. Consequently, while tardigrades may serve as complementary bioindicators within integrated environmental monitoring frameworks, their effective utilization necessitates the development of standardized sampling protocols and the execution of comprehensive studies to elucidate species-specific sensitivities to environmental perturbations[5, 6] .

Given the extraordinary stress tolerance of tardigrades and emerging findings—such as the determination of LC_{50} values for *Macrobiotus hufelandi* upon lead exposure (94.651 mg/L at 24 hours and 8.048 mg/L at 96 hours)—demonstrating the necessity for expanded research into their environmental and biomedical applications has become increasingly evident[8]. The discovery of unique proteins such as the unique damage suppressor protein (Dsup), which binds to nucleosomes and protects DNA from hydroxyl radical-induced damage, and its demonstrated efficacy in enhancing human cell resistance to ionizing radiation and oxidative stress, has opened new avenues for engineering stress-tolerant microbial strains[9]. The heterologous expression of tardigrade-derived genes in target microorganisms offers the potential to develop innovative bioremediation systems capable of maintaining pollutant-degrading functionality under extreme contamination conditions. Furthermore, leveraging tardigrades or their molecular biomarkers for monitoring water and soil quality—due to their pronounced tolerance to toxicants—may significantly enhance the sensitivity and reliability of environmental surveillance tools.

A particularly promising application involves the genetic simulation and transfer of tardigrade-specific genes into beneficial bacteria used in wastewater treatment processes, with the aim of increasing their resistance to toxins and environmental stressors. This strategy could improve the performance of treatment systems operating in industrial or heavily polluted settings. In addition, investigating the resistance mechanisms of tardigrades to various wastewater contaminants—such as heavy metals and pharmaceutical compounds—may yield insights into their survival strategies under extreme conditions and inform the development of next-generation wastewater treatment technologies. However, current challenges related to the large-scale production of these proteins underscore the need for advances in synthetic biology platforms and optimization of gene expression using state-of-the-art biotechnological methods. Achieving these objectives will require integrative approaches that synergize molecular biology, genetic engineering, and environmental science[2, 8].

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