High cadmium tolerance in *Stichococcus*-like microalgae (*Tetratostichococcus* sp. P1) from Malaysia

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High cadmium tolerance in *Stichoccocus*-like microalgae (*Tetratostichoccocus* sp. P1) from Malaysia

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Abstract. Cadmium (Cd) is a common industrial pollutant that has become a global issue due to its toxicity to living creatures, particularly aquatic organisms. Algal-based treatment offers cost-effective and environmentally friendly solutions for heavy metal removal. In this work, we studied the acid-tolerant microalgae *Tetratostichoccocus* sp. P1 isolated from a tropical peatland in Malaysia for its potential for Cd removal. The objective was to study the growth of *Tetratostichoccocus* sp. P1 strain cultivated in high-level Cd concentrations at pH 3.0. *Tetratostichoccocus* sp. P1 grew best in 20 μM and could survive in a concentration up to 100 M, according to the specific growth rate ($\mu = 0.36 \pm 0.05$ d^{-1}) and the chlorophyll content (28.24 μg mL^{-1}). This strain was also highly resistant to Cd, evidenced by its half-maximal inhibitory concentration (IC_{50}) value, which was determined at 125 μM (14.8 mg L^{-1} Cd). This is the first study of its kind to demonstrate *Tetratostichoccocus* sp. P1's ability to absorb Cd at elevated concentrations under acidic conditions.

Keywords: *Tetratostichoccocus* sp. P1, cadmium uptake, heavy metal removal, acid-tolerant microalgae, acidic pH

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1. Introduction

Industrial and human activities substantially impact the environment, resulting in the release of heavy metals into the environment. Only copper, zinc, manganese, and iron are utilized in trace amounts by the biota; others are regarded as non-essential and harmful [1]. Cadmium (Cd) is a highly toxic heavy metal that enters the environment through mining, battery disposal, and wastewater treatment. Cd is also commonly used in the galvanoplasty industry and semiconductor alloys for household products. Samadani et al. [2] estimated that wastewater treatment plants contribute 15% of the 6.4 tonnes of Cd discharged into the aquatic environment. Cd concentrations in severe ecosystems, such as acid mine drainages (AMDs), range from 300 g L^{-1} to 5000 g L^{-1}, with sulfur ions predominating [3]. In Malaysia, Cd levels in aquatic species were reported to range from 29.33 µg g^{-1} to 148.01 µg g^{-1} [4].

Pycoremediation can be defined as the use of microalgae to recover hazardous heavy metals from the contaminated medium. The use of microalgae in remediation is superior to conventional physicochemical techniques due to its advantages, such as fast recovery, low cost, high efficiency, and eco-friendliness [5]. For instance, in treating wastewater, phycoremediation produces less sludge, uses less energy, and minimizes greenhouse gas emissions [6].

In general, microalgae have high sorption capabilities, strong metal ion sorption selectivity, large surface areas, and high binding affinity to remove metals from aqueous solutions. However, not all microalgae can live in acidic and cadmium-contaminated environments (e.g., AMD, peatland, geothermal hot springs) [7]. Only acidophilic and acidic-tolerant eukaryotic algae can survive in such environments [8]. Among acidophilic microalgae that have been used for Cd and other heavy metals removal were *Spirulina platensis* (cultured in pH 1) [9], *Rhizoclonium hookeri* (pH 2) [10], *Desmodesmus* sp. and *Heterochlorella* sp. (pH 3.5) [11][10], and *Oscillatoria laete-virens* (pH 5) [12]. In terms of Cd removal percentage, acidophilic microalga *Euglena gracilis* was found to be capable of accumulating up to 50–75% of 2–25 μM CdCl at pH 3.5 [13]. *Desmodesmus* sp. MAS1 and *Heterochlorella* sp. MAS3 accumulated more than 58% of Cd in the presence of 2 mg L^{-1} in the medium of pH 3.5. It was also reported that at pH 7, *D. armatus* accumulated up to 341 μg g^{-1} Cd from 93 μM CdCl [14].

It is important to understand how microalgae respond to heavy metals in acidic conditions to help identify suitable strains for bioremediation purposes. In our preliminary work, *Stichocococcus*-like microalgae have been identified as a promising candidate for Cd removal. *Stichocococcus*-like microalgae are divided into seven genera [15], widely distributed, short-lived, and tolerant to a wide range of pH [16]. This green microalga was isolated from Malaysia's peatlands and has been registered as *Tetratostichoccocus* sp. P1 [GenBank: MT053478]. The objective of this study was to determine the algal strain's ability to live in various Cd concentrations at pH 3.0. This paper will be useful for future research on the cultivation of *Tetratostichoccocus* sp. P1 and its applications in highly acidic and Cd-contaminated conditions.

2. Materials and methods

2.1. Cd treatment and cultivation

Tetratostichoccocus sp. P1 was originally isolated from an acidic peatland in Selangor, Malaysia [17]. The acid-tolerant strain was cultivated in BG11 culture medium at pH 3.0 in a test tube under continuous illumination (70 µmol photons m^{-2} s^{-1}), aerated with 2% CO_2 (v/v) at a temperature of 30 ± 1 °C. A Cd stock solution (100 mM) was prepared by dissolving $CdCl_2$ in ultrapure water and filtered through a 0.22 µm sterile disposable syringe. Four different concentrations of Cd solutions were made: 20 μM (2.2 mg L^{-1}), 50 μM (5.6 mg L^{-1}), 100 μM (11.2 mg L^{-1}), and 200 μM (22.4 mg L^{-1}). Each Cd solution was mixed with 50 mL of BG11 in a 100-mL flask. There were triplicates of each sample.

2.2. Analysis of algal growth and chlorophyll content

Algal growth was determined by measuring the optical density (OD) at 730 nm using a UV-Vis spectrophotometer (UV-1900, Shimadzu, Japan) every 2 days. The specific algal growth rate, μ was determined at the exponential phase. For chlorophyll content analysis, cultures were pelleted by centrifugation at 15000 rpm for 3 minutes. The chlorophyll was extracted by 90% methanol. The content of chlorophyll-a and chlorophyll-b was determined at 655.2 nm and 652 nm, respectively, by using the UV-Vis spectrophotometer. Each measurement was conducted three times. The OD results were presented as averages with standard deviations, while the total chlorophyll was a sum of chlorophyll-a and chlorophyll-b. IC_{50} was examined by using OD values taken on day 9 (final OD).

3. Results and discussion

3.1. Effect of Cd concentrations on the growth and chlorophyll content

To investigate the effect of Cd concentrations on algal growth, Cd concentrations were ranged between 0 μM (control) and 200 μM. **Figure 1** shows that the growth of *Tetratostichoccocus* sp. P1 increased over time for all concentration levels except for 200 μM which was detrimental for this strain.). On day ninth, the strain reached 2.76 and 2. 61 at OD_{730} under 20 μM and 50 μM. At the same time, the control goes OD_{730} 2.31. The best growth was found at 20 μM (specific growth rate, μ: 0.36 ± 0.05 d^{-1}), followed by 50 μM (μ: 0.33 ± 0.06 d^{-1}), 0 μM (μ: 0.23 ± 0.05 d^{-1}), 100 μM (μ: 0.16 ± 0.03 d^{-1}), and 200 μM (μ: 0.02 ± 0.01 d^{-1}). These results indicate that *Tetratostichoccocus* sp. P1 was tolerant to Cd up to 100 μM and had an adverse effect at 200 μM.

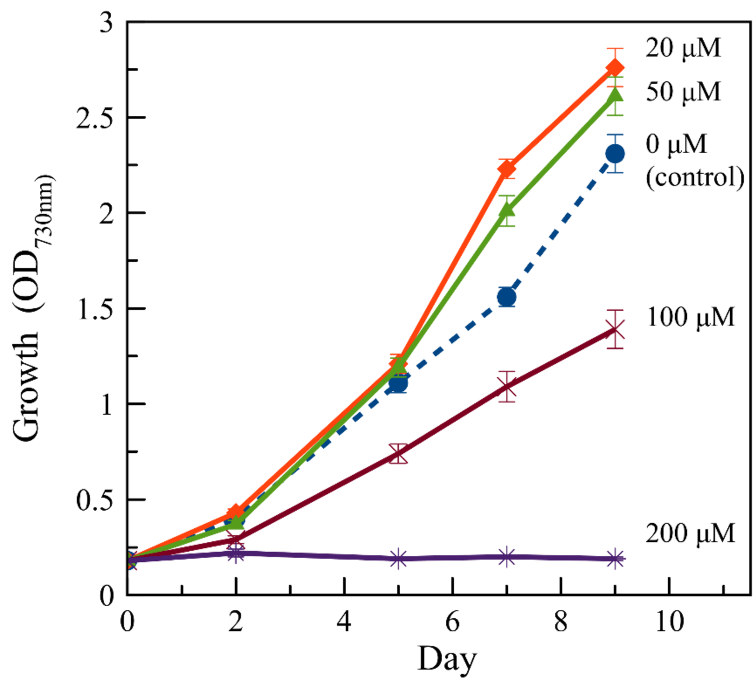

Figure 1. Response of *Tetratostichoccocus* sp. P1 in terms of optical density at 730nm to the stress imposed by different concentrations of Cd at pH 3.0.

The chlorophylls, a and b, are the pigments of photosynthesis. The primary role of chlorophyll is to absorb light energy into chemical energy for algal growth. **Figure 2** shows the total chlorophyll content for 20 μM and 50 μM Cd was comparable with the control (0 μM), implying that at this range (20–50 μM), Cd may not affect the strain's metabolism. In contrast, at 200 μM, the chlorophyll content in

Tetratostichoccocus sp. P1 was negatively affected, as evidenced by the decreasing chlorophyll content after day 2.

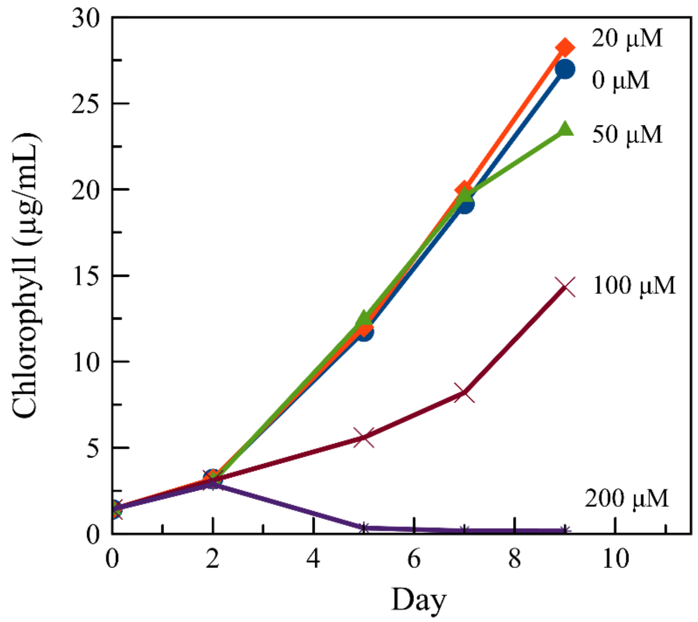

Figure 2. Total chlorophyll content in *Tetratostichoccocus* sp. P1.

3.2. IC_{50} of Tetratostichoccocus sp. P1

The tolerance of Microalagae to Cd can be assessed by measuring the IC_{50} (the concentration of Cd causing cell inhibition by 50%) or LC_{50} (lethal concentration 50%). After nine days of cultivation, results showed that the IC_{50} of *Tetratostichoccocus* sp. P1 was around 125 μM (14.8 mg L^{-1}), as shown in **Figure 3**. The algal growth was inhibited by only 39% compared to the control at 100 μM Cd. Interestingly, the growth of *Tetratostichoccocus* sp. P1 did not seem to be affected by the 20 μM and 50 μM Cd. In fact, the growth was better than the control, as mentioned earlier in Figure 2. The IC_{50} determined in this study was higher in comparison to similar past studies. For example, the IC_{50} for *Chlorella* sp. was found to be at 8 mg L^{-1} [1], *Chlorella sorokiniana* was 0.3 mg/*L* [18], IC_{50} for *Scenedesmus incrassatulus* was 7.36 mg L^{-1} [19], LC_{50} for *Ankistrodesmus* sp. was 5.43 mg L^{-1} [1], and LC_{50} for *Chlamydomonas acidophila* was 1.94 µ M [20]. Our findings have indicated that *Tetratostichoccocus* sp. P1 has a better potential for withstanding high concentration levels of Cd compared to other strains. It is noteworthy that environmental factors such as pH, temperature, light, cellular density, and culture medium are among the key factors for growth, uptake, and tolerance. In fact, experimental results can be different even for the same algal species and heavy metal types [21]. For instance, the pH of the medium is possibly the most critical factor affecting metal adsorption by microalgae. It has been reported that the tolerance of Cd increased from 12% to 51% when medium pH was decreased from 7 to 4 [2].

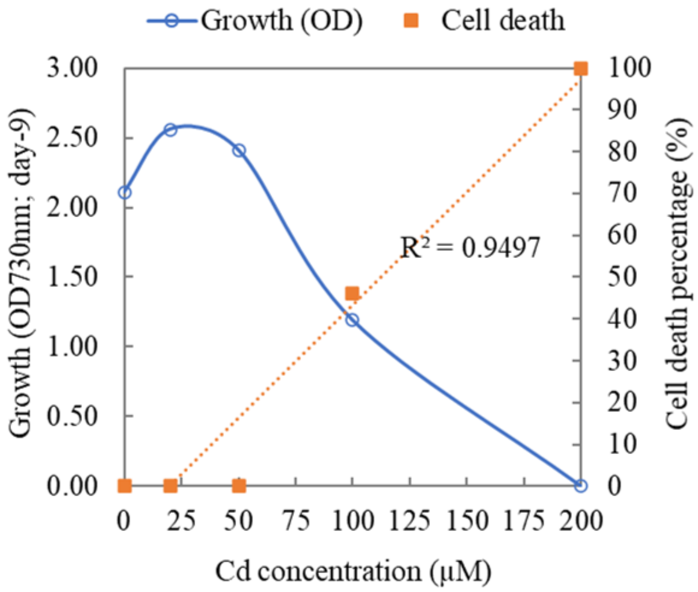

Figure 3. IC_{50} and mortality percentage for *Tetratostichococcus* sp. P1.

Cd removal or accumulation by microalgae is generally conducted at neutral pH, and not all strains can grow under acidic and toxic conditions. According to Yu et al. [22], Cd concentrations in the contaminated-environment range between 5–50 μg L^{-1}. This study has demonstrated that *Tetratostichoccocus* sp. P1 can tolerate up to 11.2 mg L^{-1} (100 μM), which is 224 times higher than the typical concentrations found in the environment. Our findings showed that *Tetratostichoccocus* sp. P1 is a great candidate for Cd removal in aqueous conditions, given its high tolerance to Cd. Thus, it is also suggested that *Tetratostichoccocus* sp. P1 can be promising for other heavy metal removals as well.

3.3. Microalgae bioremediation mechanism

Tetratostichoccocus sp. P1 involves some particular mechanism to deal with Cd pollutants since it has high tolerance by showing fast growth and high IC_{50}. The inhibition indicated that high Cd concentration was accumulated in the cell and affected metabolism. At the same time, some Cd might be pumped out from the cell. *Tetratostichoccocus* sp. P1 may involve two possible mechanisms to deal with Cd pollutants: (i) Cd ion binding at cell surfaces and intracellular levels and (ii) metal ion transport. It has long been believed that the high metal-binding abilities of microalgae are attributed to their cell walls [23]. It has been suggested that when the cell walls or membranes block the metal cation uptake, this is the first step in heavy metal accumulation. The proteins, carbohydrates, and lipids found on the cell walls and membranes could react with metallic species [24]. Polyelectrolytes with charged groups such as carboxyl, phosphate, hydroxyl, or amine might be found in algal cell walls. The amphoteric characteristics of the algal cell walls are due to anionic and cationic sites. The functional groups are essential in the uptake of heavy metals by both living and non-living microalgal cells. Furthermore, these groups are protonated or deprotonated [5].

Metal ion transport into the cytoplasm is vital. Metal transporters in the plasma membrane help algae interact with their environment. These membrane transporters protect algal cells from being exposed to heavy metals. The study of algal metal transporter repertoires could shed light on a critical element of algal life [25]. When the concentrations of heavy metals are substantially higher than those in the intracellular, activated transport through the plasma membrane allows the movement of metal cations between the extracellular and intracellular compartments [26]. Due to the difference in concentration levels, Cd is transported and accumulates in the cells.

4. Conclusions

Tetratostichococus sp. P1, which was isolated from a tropical peatland in Malaysia, has been shown to be highly resistant to Cd under acidic conditions. Algal growth was seen to be significantly greater in the presence of Cd than in the absence of Cd, showing that this strain can survive in a Cd environment. *Tetratostichoccocus* sp. P1 grew best at 20 μM, with a specific growth rate (μ) of 0.36 ± 0.05 d^{-1} and chlorophyll content of 28.24 μg mL^{-1}, respectively. The growth of *Tetratostichoccocus* sp. P1 was inhibited at 200 μM, indicating that Cd was toxic to the strain at this concentration level. The IC_{50} for this strain was determined to be 125 μM (14.8 mg L^{-1} Cd), showing that it is very resistant to Cd when compared to other strains described in the literature. This is the first study of its kind to demonstrate the potential of *Tetratostichoccocus* sp. P1 to absorb Cd at different concentrations under acidic environments.

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