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Biodegradation and rapid removal of methyl parathion by the paddy field cyanobacterium *Fischerella* sp.

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ABSTRACT

A paddy field cyanobacterial isolate that is capable of degrading and utilizing the organophosphorus pesticide methyl parathion (MP) as a phosphate source has been characterized as *Fischerella* sp. To investigate the MP removal and degradation capabilities of this cyanobacterium along with the mechanism it has adopted to combat the pesticide's toxicity, different doses of MP (0, 5, 10, 20 and 30 mg L^{-1}) were applied to the cyanobacterial culture. At 20 mg L^{-1} of MP, the cyanobacterium efficiently modulated its antioxidative defense system and its fatty acid and hydrocarbon profiles to support growth. The initial rapid removal of methyl parathion (~80%) was due to the adsorption of the pesticide onto the cyanobacterial surface. Fourier transform infrared (FTIR) spectral analysis revealed that MP interacts with the −OH group on the cell surface, and this chemical interaction may lead to chemisorptions. The initial removal pattern has followed the pseudo-second-order kinetics model of biosorption that also defines the chemisorptions mechanism. The appearance of *p*-nitrophenol in the medium coupled with modulation of the physiological indices of this cyanobacterium has indicated that biosorption followed by the simultaneous bioaccumulation and biodegradation of MP led to its complete removal from the medium. Under phosphorus-deficient conditions, MP exposure induced the growth and intracellular alkaline phosphatase activity of the cyanobacterium, which both support the view that the organism can use this pesticide as a phosphorus source. Thus, due to its tremendous efficiency in degrading and removing the organophosphorus pesticide MP, the isolated cyanobacterium *Fischerella* sp. can be used as a potent bioremediation agent.

1. Introduction

The extensive use of organophosphorus compounds in agricultural fields and household systems in the forms of insecticides, nematicides and molluscicides has contaminated different environments all over the world. These compounds cause a number of human and animal diseases by inhibiting the acetylcholinesterase enzyme, which is found in neuromuscular junctions [1]. Even after being prohibited in several countries, many OP compounds are still being used in India. Methyl parathion is one of these OP compounds, and it is normally applied to agricultural fields as an insecticide. The WHO has classified MP as an extremely hazardous pesticide [2]. Only 0.1% of the total insecticide that is applied to the field targets insect pests, and the remaining 99.9% is disseminated throughout the environment, which affects other non-target organisms such as fish, reptiles, birds, mammals and beneficial insects [1,3–6]. Thus, the removal of these remaining pesticides from the environment is necessary to protect non-target organisms from pesticide exposure.

Because physical and chemical methods are exorbitantly expensive and usually fail to provide the desired results, the use of living organisms is a better way to remove organic pollutants from the environment [7]. A number of microorganisms have been reported to degrade and consume organophosphorus pesticides by using these compounds as phosphorus or carbon sources [1]. Of these organisms, the cyanobacteria are unique because of their impeccable capacity to fix nitrogen, perform photosynthesis, and grow in all possible extreme and highly polluted environments and wastewater [8,9]. Using cyanobacteria as bioremediation agents lowers the maintenance cost because they can easily grow in the absence of additional carbon and nitrogen sources [10], and their use is also convenient for environmental sustainability [11,12].

Due to their larger surface area and high organic content, cyanobacteria readily sorb heavy metals and organic contaminants [13]. The components of the cyanobacterial cell wall contain both hydrophilic and hydrophobic portions to which heavy metals and organic pollutants are adsorbed by different types of interactive forces, which is also

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known as biosorption. Although numerous studies related to the biosorption of metal contaminants by cyanobacteria have already been performed, much less attention has been paid to the sorption of organic contaminants, especially organophosphorus pesticides (OPs). Megharaj et al. [14], Barton et al. [15] and Fioravonte et al. [16] reported the transformation and degradation of MP by different cyanobacterial strains, but in comparison to bacteria, they were not to efficient at degradation. The role of biosorption in the cyanobacterial removal of MP has also not been explained by these authors. Therefore, this study has been conducted to isolate a cyanobacterial strain that is efficient at degrading and removing the organophosphorus pesticide methyl parathion (MP) and to investigate the role of biosorption in the rapid removal of OP pesticides. Cyanobacterial tolerance to MP and its protective mechanisms have also been assessed through this work.

2. Materials and methods

2.1. Medium and culture conditions

A cyanobacterial strain isolated from the paddy field of Ghazipur, Uttar Pradesh, India (25° 48′ 2″ North, 80° 45′ 3″ East) was used to study the biodegradation of MP (purchased from Sigma with 99.7% purity). The isolated cyanobacterial strain was maintained in BG11 medium [17], pH 7.4 at 27 °C ± 1 °C temperature under cool white light (with an intensity of 50 $\mu mol^{-1}\ m^{-2}\ s^{-1}$). To test the cyanobacterial ability of using MP as a phosphate source, the BG11 medium was modified by replacing K_2HPO_4 with K_2SO_4 and using MP as a phosphate source.

2.2. Morphological characterization

To study the morphology of the filaments and cells, a light microscope (Dewinter Optical Inc. India) with an attached camera was used. A measurement of the cell dimensions was performed using Dewinter Biowizard 4.1 software.

2.3. Molecular characterization

Genomic DNA from cyanobacterial isolates was purified using a HiPuraA™ Bacterial Genomic DNA isolation kit MB505. The 16S rDNA sequences were amplified using primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGA TCCAGCC-3′), which were derived from the conserved sequences of the bacterial 16S rRNA gene [18]. PCR was performed in a 25 μL reaction mixture containing 20 ng DNA, 2.5 μL of 1 × Taq buffer B, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.5 μM of each primer and 1 unit of *Taq* DNA polymerase. The first step of the PCR reaction was started by pre-heating at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min. The last cycle was a final extension at 72 °C for 5 min. The *rbcL* gene was amplified using the primer pairs rbclf (5′-GACTTCACCAAA-GAYGACGAAAACAT-3′) and rbclr (5′-GAACTCGAACTTRATYTCTTTC-CA-3′) [19] as indicated in Singh et al. [20].

2.4. Effect of methyl parathion on pigments and protein

Cyanobacterial growth was measured in terms of dry weight. The total cellular chlorophyll and carotenoid contents were measured according to the protocol by MacKinney [21]. Cyanobacterial pigments were extracted in methanol, and chlorophyll and carotenoid absorbance measurements were taken at 663 and 420 nm, respectively. The phycocyanin contents were determined using the protocol in Singh et al. [22]. For the phycocyanin extraction, each cell pellet was resuspended in 50 mM phosphate buffer (pH 7) containing lysozyme (1 $g\ L^{-1}$) and maintained at 37 °C in a water bath for 1 h. The cyanobacterial suspension was again washed and resuspended in phosphate buffer. After 10–12 freeze-thaw cycles, the absorbance of the supernatant was recorded at 615 nm. The protein contents of the cyanobacteria under different methyl parathion treatments were measured according to the protocol by Lowry et al. [23]. Each cyanobacterial culture (2 mL) was centrifuged, and 1 mL of 1 N NaOH was added to each pellet. The resulting mixture was kept in a water bath at 65 °C for 10 min. In addition, 5 mL of reagent A (2 mL of l% Na-K tartrate solution containing 0.5% $CuSO_4$ with 50 mL of 5% Na_2CO_3 solution) was added and left to stand for 15 min at room temperature. After the addition of 1 mL of 1 N Folin reagent, the absorbance of each blue-colored sample was recorded at 650 nm against the blank. The protein content was determined by preparing a standard BSA curve.

2.5. Measuring MP using HPLC

Methyl parathion uptake experiment was performed in 250 mL Erlenmeyer flask containing 100 mL of BG11 medium supplemented with 20 $mg\ L^{-1}$ MP. About 50 mg of exponentially growing cyanobacterial culture was inoculated in the flask covered with cotton plug for aeration and kept on the shaker at 110 rpm in a 12 h light and dark condition. Ten milliliters of cyanobacterial cultures were withdrawn at the regular time interval of 1 h, 6 h, 12 h, 24 h, 48 h and 96 h and centrifuged at 1000 rpm. The supernatant was extracted with *n*-hexane for three times and the methyl parathion (MP) in the extract was determined at 25 °C using a high-performance liquid chromatograph (HPLC) (Waters, USA). The HPLC was equipped with a reversed-phase Nova-Pak C18 column (4.6 mm × 150 mm, 5 mm particle size) and a PDA detector set at 220 nm. The mobile phase used for the process was methanol: water (70%:30%, v/v), and the flow rate was maintained at 1 $mL\ min^{-1}$. The MP was quantified by external standard method using Eq. (1) as obtained from the standard plot of the concentration Vs peak area.

$$x = \frac{(y + 8206.4)}{10,\ 903,\ 525.9} \quad (1)$$

To determine the surface level of the MP, the cell pellets of MP-treated cyanobacterium were washed using BG11medium and extracted with *n*-hexane. Finally, the extracted samples were injected into the HPLC column.

2.6. Measurement of p-nitrophenol

Level of *p*-nitrophenol (pNP) produced in the medium was estimated by using colorimetric method. The cyanobacterial culture (3 mL) treated with or without MP was taken out of the flask at regular time interval of 2 days and centrifuged. The supernatant was collected and optical density (OD) was recorded at 410 nm. The amount of pNP present in the medium was calculated from standard plot of pNP (concentration vs. OD).

2.7. Biosorption kinetics

The sorption capacity at apparent equilibrium (q_e) was calculated using the following equation: [24].

$$q_e = \frac{(C_o - C_e)V}{m} \quad (2)$$

where C_o and C_e are the initial and equilibrium concentrations of MP in the culture medium, respectively. V is the volume of the medium and m is the cyanobacterial biomass that is used as a sorbent.

For the pseudo-first-order kinetic study, a linearized form of the Lagergren model was used

$$\log(q_e - q_t) = \log q_e - \frac{K_1 t}{2.303} \quad (3)$$

where K_1 is the Lagergren rate constant for the first-order biosorption