PUTATIVE EFFECT OF XENOBIOTICS ON ESTERASES OF BACTERIAL FLORA FROM *CHIRONOMUS CIRCUMDATUS* (BLOODWORMS

Chironomids (Diptera: Chironomidae) are the most abundant insects found in freshwater. Daily, these insects are exposed to different phthalates as they are used as plasticizers in many plastic materials and do not form covalent bond with the polymers therefore being as one of the major pollutants in the aquatic habitats and soil. Therefore, in most of the studies evaluations on the alterations in the morphology as signs of phthalate toxicity on these Chironomus species have been studied, but through our study we tried to research on another aspect of the problem wherein we have tried to find out the role of microbiota present in the Chironomus species in helping to adapt to these phthalate mediated stress. Chironomus circumdatus are ubiquitously found in almost all Asian countries and even in India they are readily available in different aquatic habitats. As they spend maximum of their lifespan in larval stages, in the present work, we explored the microbiota of C. circumdatus larvae and tried to evaluate the role played by these microorganisms in the host in adaptation to phthalate mediated stress. Also, we aim to evaluate the effects of the phthalate mediated stress on the esterase activity of bacterial flora found in the C. circumdatus.

In the first chapter, studies related to metagenomic analysis using next generation sequencing has been described which is used to find out the unculturable and culturable bacteria found in the living organism. At the genus taxonomic level, 437 taxons were present in the whole larvae of C. circumdatus, which included some of the pathogenic as well as useful members of bacterial species.

In the second chapter, through culture-dependent isolation of bacterial flora, two bacterial isolates (BI1244 and BI1245) showed a clearance zone on tributyrin agar plates indicating the presence of esterase enzyme within them. Based on molecular characterization, the bacterial strain BI1245 was found to be another strain of Enterobacter mori therefore the strain was named as E. mori BI1245. Further studies were carried on using the bacterial strain E. mori BI1245. From substrate specificity studies, it was observed that the crude extract obtained from E. mori BI1245 had more specific activity towards p-nitrophenyl acetate as compared to p-nitrophenyl butyrate and p-nitrophenyl palmitate indicating that it possesses more of esterase activity. Maximum crude activity was observed at pH 8 and at 60ºC with p-nitrophenyl acetate as substrate.

In the third chapter through experiments it was found that bacterial isolate BI1245 could grow fast in the culture medium added with shorter alkyl-chains phthalate esters such as diethyl phthalate, dibutyl phthalate than in that with longer alkyl-chains dioctyl phthalate, indicating that the side chain of the substrates has a significant effect on the utilization pattern of phthalic acid ester by the bacterial isolate. Characterization of esterases from the crude extracts obtained from diethyl, dibutyl and dioctyl phthalates was also done. The crude extracts samples showed high activity towards p-nitrophenyl acetate as compared to p-nitrophenyl butyrate and p-nitrophenyl palmitate indicating that the crude extracts had high esterases activity. The order of affinity of crude extracts towards substrate p-nitrophenyl acetate based on Km values was as follows: diethyl phthalate > dibutyl phthalate > dioctyl phthalate. Degradation was studied by using the first-order kinetics for dibutyl phthalate, diethyl phthalate and dioctyl phthalate by using high performance liquid chromatography and gas chromatography mass spectrometry.

In the fourth chapter expression studies of enzymes carboxylesterases (carEW), dialkyl phthalate ester hydrolase (dpeBH), monoalkyl phthalate ester hydrolase (mpeH) and phthalate 4, 5- dioxygenase (oph

A1) genes was carried out wherein it was found that in dibutyl phthalate sample, gene expression of CarEW and oph A1 was seen, while in

the diethyl and dioctyl phthalate sample, along with CarEW and oph A1, expression of mpeH gene was also seen. Thus it can be hypothesized that enzyme carboxylesterases is capable of hydrolyzing diethyl phthalate, dibutyl phthalate and dioctyl phthalate to phthalic acid while phthalate 4, 5- dioxygenase enzyme catalyzes the incorporation of two hydroxyl groups on the phthalate ring to yield phthalate dihydrodiols and initiates the process of biodegradation of phthalate into smaller compounds. Thus the study done gives us preliminary insights that the bacterial strain E. mori BI1245 can completely mineralize the diethyl, dibutyl, and dioctyl phthalates to smaller compounds. Present investigation is important for the bioremediation of widespread environmental pollution caused by phthalates.