Abstract

Pesticide consumption for good agronomical husbandry is increasing at an unprecedented rate in the world, but unmanaged use of pesticides in the third world countries including Pakistan is creating serious environmental concerns. Pesticides are often persistent in soils, enter into food chain, and ultimately reach to humans causing various illnesses. So pesticide remediation bears prime importance. At the current time, biostimulation and bioaugmentation are considered as the most reliable techniques of pesticide biodegradation being eco-friendly. The present study deals with the biodegradation of chlorpyrifos (CP), an organophosphate insecticide. In the 1st phase of experimentation, chlorpyrifos resistant 56 microbial strains from cotton growing agricultural soils, and 24 from sludge of industrial wastewater drain (carrying effluents of pesticide factory) were isolated. These isolates were resistant at 175mgL^{-1} of CP. In the 2nd phase of study, growth potential of these isolates was tested, and 3 isolates (Ct3, Ct27 and WW7) were selected based upon their highest level of CP resistance and growth potential. These isolates were identified as *Bacillus cereus* (Ct3), *Klebsiella oxytoca.* (Ct27) and *Pseudomonas aeruginosa.* (WW7), based on 16S rRNA (ribotyping). Thirdly, biodegradation potential of these isolates was investigated in liquid media and soil. Number of factors like, CP concentration, temperature, pH, carbon sources and inoculum densities were optimized, in order to enhance the percentage and rate of biodegradation. In liquid media, best degradation was exhibited by *Bacillus* sp. The order of degradation ability was as follows:

Bacillus cereus > *Pseudomonas aeruginosa* > *Klebsiella oxytoca*

Bacillus sp. degradaed 84% of 300mgL^{-1} CP with inoculum density of 10^6 CFUml^{-1} at 30°C and 8.5 pH in 6 days. By increasing inoculum density up to 10^8 CFUml^{-1}, 100% degradation was achieved in the same time. Whereas, maximum degradation by *Pseudomonas* sp. was 81% in 15 days (initial concentration, 300mgL^{-1}). Optimum conditions for *Pseudomonas* sp. were 8 pH, and 30°C. Conversely, maximum degradation efficiency in *Klebsiella* sp. was up to 79% in 15 days. Among all the 3 carbon sources, glucose proved to be the best in enhancing CP degradation. The order of effectiveness of tested carbon sources is as follows:

Glucose > yeast extract > starch ≥ no added supplement

The CP degradation ability of selected isolates was also tested in soil to explore in-situ bioremediation possibilities. It was noted that the strain which was potent in liquid media also showed good result in soil. The degradation percentages were 93, 86 and 84 by *Bacillus* sp., *Pseudomonas* sp. and *Klebsiella* sp., respectively. Enhancement potential of different organic amendments was studied and the results revealed the following order:

Farmyard manure > green compost > rice husk > no added organic amendment

Lastly, the biodegradation kinetics in liquid media and in soil was calculated. The kinetic data revealed that all the 3 selected strains have the potential for bioremediation and can be used for rapid CP degradation. The present study was thus innovative and highly successful as it provided the eco-friendly solution using indigenous bacteria for countering CP pollution. These strains (*Bacillus* sp., *Pseudomonas* sp. and *Klebsiella* sp.) can be used for soil and ecological restoration.