GENETIC MANIPULATION OF MICROBIAL STRAINS FOR IMPROVED PERFORMANCE IN ENVIRONMENTAL BIOTECHNOLOGY

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Use of pesticides and other chemicals are the inevitable evils of modern agricultural and industrial development. Along with attaining self-sufficiency in food production and making life more comfortable through industrial development man created a monster called environmental pollution, which has already started showing its ugly teeth. Most of the pesticides particularly the organochlorine compounds and other chloroorganics are highly recalcitrant and persist for long periods in the environment, get bioconcentrated through the food chain and ultimately reach human body. Indians have the highest loads of pesticide residues in their adipose tissue. Almost all food articles, why even breast milk in this country contains alarmingly high levels of residues of BHC, DDT and other OC pesticides. Environmental pollution is a global phenomenon in one or other way. This is a very serious situation and it is highly imperative that action be taken immediately to alleviate or abate the pollution problem. In recent years it has been well recognized that bioremediation of contaminated sites and industrial effluents through microbial degradation is very efficient and cost effective. However, it has been found difficult to obtain potent microbes from nature that can effectively degrade many of these xenobiotics. And also the laboratory-developed microorganisms do not perform well in the field conditions due to various reasons, such as the presence of heterogeneous mixtures of chemicals, heterogenous groups of native microbial populations and a variety of physico-chemical conditions. Hence, it is necessary to improve the microbial strains through adaptation/acclimation and/or through genetic manipulations for attaining increased degrading ability, substrate range and tolerance to co-substrates. Also the strains should be able to overcome the "biochemical incompatibility" so as to utilize compounds belonging to different groups that are generally catabolized through different biochemical pathways. There are several examples in literature on genetic improvement of microbial strains that are meant for deployment in treatment technologies. Expansion of the substrate range of a *Pseudomonas* strain to utilize chlorobenzoates, methyl

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benzoates, chloro and methyl phenols, through recruitment of genes from different sources and assembling them to form an *ortho*-cleavage pathway, is a crowning example. Japanese workers have cloned and got expressed in *E.coli* the genes of *Sphingomonas paucimobilis* that are involved in the biodegradation of γ- hexachlorocyclohexane (γ-HCH or lindane). Cloning of a few other genes of pesticide degradation have also been reported. There are several reports of improvement of the degrading ability and broadening of the substrate range through acclimation or "plasmid-mediated molecular breeding". Isolation of the 2,4,5-trichlorophenoxyacetic acid (2,4,5,-T)- degrading *Burkholderia cepacia* (then *Pseudomonas cepacia*) AC1100 by A.M.Chakrabarty's group, development of *P.putida* strain able to degrade mixtures of chloro- and methyl substituted aromatics through a common pathway, drastic improvement of HCH, DDT and phenol degradation by different microbes, and evolution of a chlorobenzoate degradative pathway in a column reactor in our laboratory are good examples of these techniques. Conjugal transfer of the catabolic plasmid pJP4 encoding the 2,4-dichlorophenoxyacetic acid (2,4, -D)- degradative pathway from *Alcaligenes eutrophus* JMP134 in to the 2,4,5-T degrading *B.cepacia* AC1100 and enabling it to degrade simultaneously both 2,4-D and 2,4,5-T- is yet another glorious example of the role of molecular biology in environmental biotechnology. It can be concluded that recombinant DNA technology has a key role to play in the improvement of microbial strains meant for deployment in effluent treatment and bioremediation technologies.