SUMMARY AND FUTURE PERSPECTIVES
SUMMARY

- A custom multibacterial 60-mer oligonucleotide microarray (BiodegPhyloChip) was developed for the detection of functional (biodegradation) and bacterial 16S rRNA (phylogenetic) genes from chemically contaminated habitats.

- The array contains 14327 unique 60-mer oligonucleotide probes derived from 1057 biodegradative genes and 880 unique probes embodying 110 phylogenetic genes representing diverse bacterial communities.

- The validation of the developed array using pure bacterial strains and mixed cultures having known genomic content established that the array was highly sensitive and specific to detect the target genomes in complex habitats.

- The minimum detection limit of the array was 50 ng of environmental DNA, and replicability of the hybridization results was 90%.

- Evaluation of the developed microarray using DNA extracted from five different contaminated sites, viz., chloroaromatic contaminated habitats, river bed, heavy metal contaminated habitat, common effluent treatment plant and a petroleum contaminated habitat, revealed the presence of bacteria similar to well characterized genera involved in biodegradation of diverse pollutants.

- Twenty five bacterial strains were found to be commonly present in all five contaminated sites, and belonged to two major groups actinobacteria and β-proteobacteria. Among 25 bacterial strains, highest signal intensity was observed in the bacteria present in petroleum contaminated site. The major strains detected were Azoarcus sp. EbN1 (ethyl benzene), Gordonia sp TY5 (propane), Paenibacillus sp Ao3 (dibenzofuran), Pimelobacter simplex
(benzoquinol), *Rhodococcus rhodochrous* (crude oil) and *Rhodococcus* sp HDN3 (dibenzofuran).

- Several site specific bacterial strains were detected at each of the five sites.
- In petroleum contaminated site, mercury accumulator *Anoxybacillus* sp HT14 (fircmutes), arsenic accumulator *Bacillus* sp CDB3 (fircmutes), toluene degrading *Pseudomonas putida* (γ-proteobacteria) and 4-chloronitrobenzene degrading *Pseudomonas putida* ZWL73 (γ-proteobacteria) were detected.
- In chloroaromatic contaminated site, 3-hydroxybutyric acid degrading *Pseudomonas mendocina* D3 (γ-proteobacteria), dibenzo para-dioxin degrading *Sphingomonas yanoikuyae* (α-proteobacteria), aromatic aldehyde degrading *Sphingomonas* sp 14DN-61 (α-proteobacteria) and polychlorophenol degrading *Sphingomonas* sp MT1 (α-proteobacteria) were detected.
- Genes involved in complete degradation pathways for hexachlorocyclohexane (*lin*), 1,2,4-trichlorobenzene (*tcb*), naphthalene (*nah*), phenol (*mph*), biphenyl (*bph*), benzene (*ben*), toluene (*tbm*), xylene (*xyl*), phthalate (*pht*), salicylate (*sal*) and resistance to mercury (*mer*) were detected with highest intensity from the five sites studied.
- The most abundant genes belonged to the enzyme hydroxylases, monooxygenases and dehydrogenases, which were present in all the five samples.
- The developed microarray was employed successfully to profile the biodegradation and bacterial 16S rRNA genes in five highly contaminated sites, viz., Nandesari GIDC (Mini River), Damanganga river, Vapi CETP, Vatwa GIDC (Khariked canal) and Vatwa CETP in the State of Gujarat.
Summary and future perspectives

➢ Site specific bacterial strains were detected at each of the site, and their expression was highly dependent on the nature and abundance of pollutants existing at each site.

➢ Site specific factors played a pivotal role to effect the microbial community structure as the type of microorganisms varied drastically from one site to another. The highly expressed genes were indicative of active biodegradation of different groups of compounds at each site of Gujarat.

➢ A *Bordetella* sp. strain IITR02 capable of growing on four different chlorobenzenes viz. 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,3-trichlorobenzene (1,2,3-TCB), 1,4-dichlorobenzenes (1,4-DCB) and 1,2-dichlorobenzenes (1,2-DCB) as the sole source of carbon and energy was isolated by selective enrichment from soil samples of a pesticide industry waste site.

➢ 1,2,4-TCB was degraded up to 81% upon growth on 3.2 mM concentration while, 1,2,3-TCB 59%, 1,4-DCB 58% and 1,2-DCB 61% were degraded upon growth on 3.5 mM concentration of the respective compounds during 11 days fermentation.

➢ The *tcb* genes (*tcb*Aa, *tcb*Ab, *tcb*Ac, *tcb*Ad *tcb*B, *tcb*C, *tcb*D, *tcb*E, *tcb*F, *tcb*G, *tcb*R, *IS*1066) coding for the enzymes involved in 1,2,4-TCB biodegradation pathway were present in strain IITR02, and their sequences were 97-99% similar to the previously reported *tcb* genes

➢ The genes for 1,2,4-trichlorobenzene were present in two different gene clusters, and along with upper pathway genes, one insertion element (*IS*1066) was present at the end.
The organization of upper pathway \textit{tcb} genes in IITR02 was: IS1066, \textit{tcbAa}, \textit{tcbAb}, \textit{tcbAc}, \textit{tcbAd}, \textit{tcbB}, forming the first cluster. The lower pathway genes \textit{tcbC}, \textit{tcbD}, \textit{tcbE} and \textit{tcbF} constituted the second cluster in the strain IITR02.

Contrary to earlier reports, both the \textit{tcb} gene clusters were located on chromosomal DNA in IITR02 despite the presence of three large plasmids of molecular size 31, 36 and 154 kb.

All the three plasmids were stable even after several generations of growth on nutrient rich medium.

The results explicitly demonstrate that the developed 60-mer microarray (\textit{BiodegPhyloChip}) can be successfully employed to understand the genetic capabilities, and microbial communities in real hazardous environmental samples. The studies on biodegradation of di- and trichlorobenzenes using strain IITR02 provide basic framework to understand the possible mechanism underlying the degradation of chlorinated benzenes.
Future Perspectives

High-throughput technologies are needed for monitoring the formidable biodiversity and functional capabilities of microorganisms in the environment. Microarray technology has offered unparalleled potential to simultaneously determine the dynamics and/or activities of most, if not all, of the microbial populations in complex environments. The microarray technique can be used for profiling of microbial populations having biodegradation potential from real hazardous polluted environments. It can be applied to monitor the active biodegradation process at any site by expression analysis of the functional genes occurring in the environment. Looking at the high quantum of pollution existing in various parts of the world, such tools shall greatly facilitate as a tool to understand the degradation potential of diverse polluted environments to find practical solution for polluted soil remediation.

Further technological advances leading to enhanced sensitivity and specificity of the microarray technology to make it applicable in complex environmental conditions are required. Also, improvement in the format of the microarray can be done to accommodate higher number of genes to make the array more comprehensive to enable its application for diverse chemical contaminants. The information generated by the microarray studies can be utilized to develop a national database of the type of contamination and genetic pool existing at various sites in India. World wide effort by researchers in the area of ‘environmental genomics’ may soon help microarray technology to achieve its promise for comprehensive high-throughput, near real-time monitoring of microbial populations within ecological communities. This will help to develop better and cost effective strategies for bioremediation of contaminated environments for a safe and better health to human kind.