Summary
Salient features:

* Surface water samples were collected from different sites of west coast of India viz. Bombay High Oil field area and Goa Ship yard and physicochemical characteristics of water samples were determined as temperature 28°C- 30°C, pH 8-8.3 and salinity 33-35 %.

* Viable count of bacteria in these water sample revealed that majority of natural bacterial population (68%) can grow up to 0.4mM of TBTC.

* The five potent TBTC resistant bacterial strains were selected after continuous sub-culturing in MSM in presence of TBTC and were designated as 25W, 25B, 5Y2, 3(4Sub) and 9(3A).

* These five bacterial strain have been identified as *Pseudomonas aeruginosa* strain USS25 (25W), *Pseudomonas aeruginosa* strain 25B, *Pseudomonas aeruginosa* strain 5Y2, *Pseudomonas fluorescens* strain 3(4Sub) and *Pseudomonas stutzeri* strain 9(3A).

* All these isolates were found to grow up to 2mM of TBTC in MSM broth as well as MSM agar as they utilize TBTC as carbon source.

* The optimum temperature, pH and salinity for growth of all five isolates were found to be 28°C, pH-7.2 and 2.5 % (25%) respectively.

* All the five isolates were cross tolerant to heavy metals viz. Hg, Zn and Cd and common antibiotics such as Penicillin, Ampicillin, Tetracyclin, Chloramphenicol and Streptomycin including some other antibiotics viz. Neomycin, Spectinomycin, Rifampicin, Kanamycin, Nalidixic acid etc.

* On the basis of faster utilization of TBTC as carbon source, the strain *Pseudomonas aeruginosa* strain USS25 was selected for further biological characterization with reference to TBTC degradation and its molecular mechanisms.

* It was observed that MSM was best medium for growth and TBTC degradation by
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*Among the various tested carbon sources viz. glucose, succinate and glycerol, glucose was found to be the best for growth of \textit{Pseudomonas aeruginosa} strain USS25, whereas ammonium chloride served as a best nitrogen sources.*

*It was observed that uptake of TBTC was more in presence of both succinate and glycerol in MSM.*

*The spectrophotometric analysis of chloroform extract of cells grown in TBTC containing medium showed a sharp absorption peak at 241nm.*

*Preliminary TBTC degradation studies (TLC analysis) have revealed that TBTC is definitely transformed to different organotin derivatives, but very slowly. Time course study of TBTC degradation has revealed that the bacterial isolate can degrade TBTC within 75 days of incubation.*

*It has been observed that both succinate and glycerol together significantly enhance the TBTC degradation process, possibly due to enhanced bioavailability of TBTC as well as cell population.*

*The test organism significantly degrades (transforms) the TBTC as 280mg of degradation product is produced out of 1gm of TBTC (28% aprox).*

*The IR and NMR analysis of pure TBTC degradation compound has revealed that the molecular formula of the degradation product is Monobutyltin dichloro hydride (BuSnHCl$_2$).*

*Mono-thiol (Mercaptoethanol) and chelating agent (EDTA) significantly reduced the toxicity of TBTC to \textit{Pseudomonas aeruginosa} strain USS25, since this isolate could tolerate 4mM and 5mM TBTC in presence of thiol and EDTA respectively.*

*\textit{Pseudomonas aeruginosa} strain USS25 showed a significant effect of TBTC on EPS production, as the yield of EPS was recorded 4.9mg/gm of dry cell mass.*

*TBTC induced EPS showed enhanced surfactant activity as compared to control, which*
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is produced only in presence of glucose.

* The surfactant activity of TBTC induced EPS was higher in benzene-water as well as chloroform–water systems.

* The test organism *Pseudomonas aeruginosa* strain USS25 also produces green fluorescent pigment in the growth medium, which has been identified as phenazine and it is interesting to note that TBTC causes significant increase in pigment synthesis.

* SDS-PAGE analysis of protein sample of *Pseudomonas aeruginosa* strain USS25 has revealed expression of an additional a novel protein of 45 KDa. Further characterization of this polypeptide will be done to explore its involvement in degradation mechanisms.

* TBTC resistant *Pseudomonas aeruginosa* strain USS25 possesses a plasmid of 41Kbps. We have also confirmed that it has no role in TBTC degradation since plasmid cured bacterial cells still show TBTC resistance and degradation capability.

* Therefore we can infer that gene governing TBTC degradation in *Pseudomonas aeruginosa* strain USS25 is located in genomic DNA.

* NTG-mutagenesis studies using *Pseudomonas aeruginosa* strain USS25 revealed that NTG-induced mutants can grow upto 10mM TBTC in MSM broth and also shows significant degradation of TBTC as compared to the wild type strain.
Future prospects of the present study

Microorganisms indigenous to polluted sites often have a limited ability to degrade xenobiotics and toxic pollutants which are highly substituted or which have especially novel chemical structure (Pipke et al. 1992). Though microorganisms have been shown to bioremediate heavy metal and aromatic hydrocarbon of polluted sites, but bioremediation of organotin contaminated sites mediated by microbes is far away from real large scale commercial process, since very little work has been done to explore the exact biochemical mechanism of organotin biodegradation and genes involved in the process. We have isolated five *Pseudomonas* strains and studied extensively *Pseudomonas aeruginosa* strain USS25 as a potent strain for organotin degradation in marine and estuarine environment of west coast of India. It has novel characteristics to produce EPS which shows significant surfactant activity to emulsify TBTC in the medium. The structural analysis of the EPS may give rise to the exact molecular basis of the TBTC solubilization in the medium and subsequent degradation of the biocides which is a serious threat to marine biota.

As this pigmented isolate can bioaccumulate TBTC on the cell surface, therefore this culture can be used for immediate removal of TBTC from the contaminated marine and estuarine site. Role of EPS in emulsification and biosorption of TBTC needs to be explored in detail in order to answer various questions related to TBTC biodegradation and bioremediation.

The molecular biological and genetic studies have confirmed that the TBT degradation gene(s) is located on chromosomal genome. We have planned to find out the gene(s) responsible for degradation. Further characterization of the gene(s) may certainly reveal exact molecular mechanism of TBTC degradation and resistance. We can explore and examine these TBTC inducible genes by cloning these genes in *lux* reporter plasmid pUCD615 (Gift from Dr. Kado) using standard techniques (Sambrook et al. 1989).
Therefore, we suggest that much focussed research is required to elucidate the mechanism of TBTC bioaccumulation, biodegradation and bioremediation in TBTC resistant marine bacteria employing molecular biological and genetic tools (Sambrook et al. 1989). This study would ultimately enable us to check the potential of these natural as well as genetically engineered bacterial strains for bioremediation of TBTC contaminated marine and estuarine sites of west coast of India.