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Abstract of the Thesis

The thesis entitled “Studies on Cytochrome P-450 Monooxygenase System from alkaliphilic Bacteria” deals with study the reactions of alkaliphilic bacterial cytochrome P-450 \( \text{cam} \) monooxygenase system, its involvement in the degradation or biotransformation of camphor and alterations in the activities of these enzymes system by exposure to camphor. Similarly the effect of various parameters like different initial camphor concentration pH, salinity, temperature, carbon sources, nitrogen sources and on the biotransformation ability of cytochrome P-450 monooxygenase system. Metabolites generated during biotransformation of camphor were indentified spectroscopic technique and on the basis of the structural metabolites, probable degradation pathways for all four bacterial strains were elucidated.

Chapter I: Introduction, Literature review, aim and objective.

The imbalance of a nature due to modern civilization and progress of human being through more complicated activities seems to be a great challenge. Extreme industrialization, modern scientific development, modern cultivation of farms has led the more guanine problem of concern. The environmental pollution, which involves direct or indirect threat to every living organisms. During the ages of development the living organisms has acquired the ability to adapt themselves to the changes in the ecosystem. The adaptation process has helped the living organism to acquire the capacity to modulate their biological phenomenon and biological system so as to make their survival continue. In the defiance against adverse changes in the ecosystem and environment, living system has adapted the biological mechanisms, the defense mechanism of environmental pollution, medication and other xenobiotics takes place mainly in the liver of animals. The cytochrome P-450 monooxygenase system or mixed function oxidase system is major enzymatic defense mechanism, well established in various species of animals and organisms like bacteria, fungi and plants. In bacteria cytochrome P-450 containing monooxygenase system is present in soluble fraction of the cell. Microbial cytochrome P-450 containing monooxygenase system involved and efficiently performs the removal of organic waste from environment. Cytochrome P-450 is a terminal oxidase
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plays an important role in this case. The microorganisms which possess significant amount of these enzymes system are useful to develop the technology to clean up the contaminated environment. In the context of topic more stress is on the mechanism and etiology of cytochrome P450 enzyme versatility to alleviate the problem of organic pollutant. There is very less literature available on the microbial cytochrome P-450 monooxygenase system and its role in degradation of waste organics particularly in alkaliphilic bacterium. Therefore the present studies are planned to examine the cytochrome P-450 monooxygenase system from alkaliphilic bacteria. The studies are further extended to purified and characterized cytochrome P-450 from various strains of alkaliphiles and compared these with data of neutrophilic bacteria and their degradation potential towards organic compound like camphor.

Chapter II: Identification of alkaliphilic and Neutrophilic bacteria.

Authentic information of bacterial species is very essential. In past two decades, it was a difficult task to identify and characterize bacteria by conventional phenotype methodology because of their difference in phenotypic characteristics. Amplification of smaller subunit of ribosomal 16S rRNA genes and their specific differentiated regions made help in identification and phylogenetic tree of the strain. By using such 16S rRNA sequencing classification, phylogenetic relation and novelty has been determined. In present work a three alkaliphilic bacterial strains were isolated from sediment soil sample from Lonar lack and neutrophilic bacteria Lysinibacillus sphaericus DL 8 from textile soil sample of dye finishing industry disposal site, Ichalkaranji, India. To screen the degradation potential of the strain, total four isolates were collected from soil and supplemented with organic compound camphor. Purification, morphology and molecular identification by 16S rRNA of strains were studied thoroughly. According to phylogenetic study, morphology examination the isolates are identified as non-pathogenic aerobic Bacillus badius D1, Kocuria sp DL, Aquiflexum sp. DL 6 alkaliphilic bacteria and neutrophilic bacteria Lysinibacillus sphaericus.
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Chapter III: Expression of microbial proteins by camphor

It is well known that exposure of organisms with a certain chemical or drug results into expression of genes, which code for a particular protein. In present studies one alkaliphilic strain *Bacillus badius D1* and one neutrophilic strain *L.sphaericus sp DL8* were grown in presence of camphor. Incubation with camphor has resulted in expression of new genes, which encoded into respective proteins. The gene expression in camphor treated microorganisms compared with their respective controls with the help of 2-D gel electrophoresis.

Chapter IV: Purification and characterization of cytochrome P-450\(_{\text{cam}}\) from Alkaliphilic and Neutrophilic bacteria.

Cytochrome P450 is a terminal oxidase and plays an important role in the biotransformation of various endogenous and exogenous chemicals. It is present in wide animal species, bacteria, fungi and plants. Cytochrome P-450 has been purified from various animal species including human being, many bacterial species and plant.

In present studies attempt has been made to purify and characterize cytochrome P-450\(_{\text{cam}}\) from the cytosol of both alkaliphilic and neutrophilic bacterial strains. Purification of cytochrome P-450\(_{\text{cam}}\) was carried out by using different analytical methods including sonication, ammonium sulfate precipitation, dialysis, ion-exchange chromatography, molecular exclusion chromatography and electrophoresis. The molecular weight of cytochrome P-450\(_{\text{cam}}\) from all microbial strain was determined by SDS-PAGE with molecular mass standards. The study was further extended to observe the effect of pH and temperature on the catalytic activity of cytochrome P-450\(_{\text{cam}}\). Determination of \(K_m\) and \(V_{\text{max}}\) was done by standard procedure and comparison was made between alkaliphilic and neutrophilic bacteria. Similarly, spectral properties like absorption spectra in various oxidized states and type-I and type-II or substrate binding spectra have been reported.
Chapter V: Degradation of camphor by Alkaliphilic and Neutrophilic bacteria.

Camphor is a universal inducer for microbial proteins and cytochrome P-450. Camphor induced microbial cytochrome P-450 is known as cytochrome P-450_{cam}. Involvement of cytochrome P-450 containing mixed function oxidase system in biotransformation of wide range of chemicals is well known. In present work attempt has been made to examine the degradation potential of both alkaliphilic and neutrophilic bacterial strain. Studies are further extended to determine the structure of intermediates formed during the degradation of camphor by these bacteria using advance technique like FTIR, GC-mass and H^{1}NMR. The tentative degradation pathway of camphor by these bacteria has been proposed.