Summary

It is well known that many bacteria are able to utilize gaseous hydrocarbons as sole source of carbon and energy. Metabolism of long chain hydrocarbons have been studied extensively in various laboratories, whereas, very little is known about the metabolism and uptake of gaseous *n*-alkanes (C_2–C_4). Knowledge pertaining to genetics and molecular biology of hydrocarbon utilization, specially propane and butane is virtually non existent. Very little is known about propane/butane induced membrane changes. The present study was carried out to understand the cellular changes associated with propane/butane utilization. It is any uniqueness in the protein profile of hydrocarbon utilizing bacteria, it was assumed that antibody could be developed against such induced polypeptides for detection of specific microflora in the soil. Since hydrocarbon utilizing bacteria are reported to be the indicators of underground petroleum /natural gas deposits, these antibodies may provide an easy and reliable method for microbiological petroleum prospecting.

Many hydrocarbon utilizing bacteria were isolated from known oil fields and some of them were studied in detail. Three bacteria could grow on butane and not on propane, the others could utilize these gaseous alkanes for their growth. Antibodies raised against whole cells of two of these organisms showed more specificity towards hydrocarbon grown cells compared to the cells grown on other carbon sources. This observation suggested that there was antigenic variation among cells grown on different substrates. Although antibodies showed some specificity but they were not satisfactory enough for diagnostic purpose. Membrane preparations of the bacteria on SDS-PAGE revealed that three unique proteins were

induced in presence of propane/butane. The approximate molecular weight of the unique proteins are 58 kDa, 42 kDa and 27 kDa. Two of the unique proteins (58 kDa and 42 kDa) were purified and used for developing antibodies. The specificity of the antibodies was confirmed by western immunoblot, solid phase immunoassay and dot-ELISA. These antibodies were highly specific to the cells grown on propane/butane. They did not show any reaction towards the same bacteria grown on other carbon sources or with other non hydrocarbon utilizing bacteria. Antigenically similar proteins were found to be present in all propane/butane utilizing bacteria tested so far.

Western immunoblotting of the soluble fractions using these two antibodies as probes revealed the presence of similar (in terms of molecular weight and antigenicity) proteins in the cytoplasm. FPLC fractionation and dot-ELISA of the fractions showed that both these antibodies reacted with a protein of molecular weight of more than 300 kDa. This observation suggested that these two proteins were subunits of a larger molecule. This idea was further strengthened by analysis of the immunoprecipitates of these two antibodies. Immunoprecipitate of anti 58 kDa antibody on SDS-PAGE revealed four distinct bands (58 kDa, 42 kDa, 37 kDa, and 32 kDa). When immunoprecipitate of anti 42 kDa antibody was analyzed on SDS-PAGE the protein bands were identical to the ones observed with the anti 58 kDa antibody immunoprecipitate . Attempts were made to estimate the molecular weight of the partially purified native protein by chromatography on Sepharyl S-300 and Sephacryl S-400. In both the cases the induced protein was found to be in void volume, indicating that the molecular weight of the induced protein is more than 670 kDa. The possibility that the high molecular weight could be due to aggregation of the molecules can not be ruled out at present.

Propanol and butanol, the first intermediates of propane and butane metabolism respectively, could not induce these 58 kDa and 42 kDa proteins. Presence of these two proteins in propane/butane grown cells and their absence in cells grown on propanol and butanol strongly suggest that these proteins are part of a monooxygenase, most likely propane monooxygenase, the first enzyme of the pathway. Inhibition of growth on propane of the cell in presence of anti 58 kDa and 42 kDa antibodies, separately or in combination, clearly indicates the involvement of these two proteins in propane/butane metabolism. The presence of these two proteins in propane/butane utilizing bacteria belonging to different taxa, indicated

that a common mechanism exists for their metabolism.

Peptide mapping and epitope analysis of 58 kDa protein suggested that the 34 kDa and 32 kDa fragments (generated by partial digestion with V_8 and trypsin respectively) to be the immunogenic component of the protein. First twelve N-terminal amino acids of the protein were found to be The-Gln-Ser-Val-Tyr-Val-Leu-Asn-* * *-Gln-Met-Gln.

Based on the findings of this study, a simple and reliable method was developed for the rapid detection of propane and butane utilizing bacteria in the soil. The dot-ELISA method is very simple, sensitive and requires only 10^3 cells per spot. In principle the method consists of spotting 5μl of soil suspension onto nitrocellulose membrane, washing of the soil particles, reaction with the specific antibody and detection of the reaction using secondary antibody-enzyme conjugate. These steps do not require special laboratory facility and can be easily performed under field conditions.