

SUMMARY

Gaseous alkanes are widespread in nature and numerous types of microorganisms have the capability to utilize these hydrocarbons as sole source of carbon and energy. Attempts were made earlier by some workers to correlate the abundance of such microorganisms with the hydrocarbon content of the region being prospected. Methane, the largest component of natural gas is also produced biogenically and thus may not be diagnostic of oil deposits. On the other hand, abundance of organisms able to utilize ethane, propane and butane in subsoil indicate the presence of oil reservoirs. Although some basic study was done on the physiological aspects of propane and butane metabolism, absolutely nothing is known about genetics and molecular biology of such organisms. In this study attempts have been made to understand the uniqueness of these organisms at DNA level and to find out the feasibility of using specific nucleic acid sequences as probes for detection of hydrocarbon utilizing organisms in the environment.

Twenty three microorganisms capable of utilizing gaseous alkanes were isolated by enrichment technique from oil fields of Gujarat, India. These microorganisms were characterized in terms of gram staining and ability to utilize different gaseous alkanes. There was predominance of gram negative bacteria amongst the isolates but gram positive and gram refractory bacteria were also found. These bacteria were aerobic in nature and could grow on a wide range of carbon sources including putative intermediates of propane and butane metabolic pathways. Two isolates were identified and belong to the genera (*Pseudomonas* and *Rhodococcus*) whose members have been reported earlier to oxidize propane and butane. Most of the microorganisms isolated in the course of this study could utilize both propane and

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butane but three utilized only butane. Some of the isolates were found to grow faster on these hydrocarbons than the ones reported in the literature.

Attempts were made to check by various methods the presence of extrachromosomal elements in five propane and butane utilizing bacteria. However, no plasmids were found in them. Exposure of one gaseous alkane utilizing isolate *Pseudomonas* sp. IMT40 to mitomycin C and ethidium bromide did not produce any propane and butane negative segregants. These findings indirectly suggested that enzymes of propane or butane metabolic pathway are not plasmid encoded.

It was shown earlier that two novel polypeptides (42 and 58kDa) were present in the membrane of *Rhodococcus* sp. IMT35 when grown on propane or butane. Antibodies against these two polypeptides reacted with polypeptides of same mass in the membrane fraction of *Pseudomonas* sp. IMT40 grown under similar conditions. In fact, these antibodies showed the presence of these polypeptides in almost all propane and butane utilizing bacteria (V. Nair, unpublished data). In light of this observation, antibodies raised against these two polypeptides were used to screen a genomic library of *Pseudomonas* sp. IMT40 constructed in an expression vector, λ gt11. The strategy used here for cloning was expected to ensure the expression of DNA sequences (encoding the 58kDa protein in particular) involved in propane and butane metabolism. Four positive clones were obtained with anti 58 kDa antibody. These clones were characterized in terms of insert size and homology among themselves. It was found that only two clones had insert of 4.9 kb and both gave similar restriction patterns. Lysogens of these recombinant phage clones were generated. A fusion protein (170 kDa, of which 112 kDa is β -galactosidase part) was detected by immunoblotting technique only in lysogen lysates of positive recombinant clones. The positive clones showed strong and reproducible reaction with the antibody raised against the 58 kDa polypeptide. The expression was high in both lytic as well as lysogenic forms of the recombinant phages when grown on appropriate hosts. The size (4.9 kb) of the cloned fragment was more than enough to encode a polypeptide of 58 kDa. The 4.9 kb DNA fragment from the λ TCI was cloned in the EcoRI site of pUC18 and a restriction map of the cloned fragment has been drawn. The orientation of the 4.9 kb insert DNA in the original clone was resolved by subcloning Hind III fragments of this insert in λ gt11. It was found that epitopes of 58 kDa were encoded within a 2 kb HindIII-EcoRI fragment. Northern analysis revealed that the cloned 4.9 kb DNA

fragment encodes a 1.6 kb transcript which gets induced only when cells were grown on propane. The southern analysis has shown that cloned DNA fragment is present as a single copy in its immediate neighborhood on the genome.

In the present work, a 4.9 kb DNA fragment from *Pseudomonas* sp. IMT40 was found to encode at least one polypeptide (58 kDa) involved in propane and butane metabolism. This DNA fragment and its subfragments were tested for their specificity in detecting propane and butane utilizing microorganisms belonging to different physiological and taxonomic groups as well as other bacteria unable to utilize these gases. The 4.9 kb DNA fragment as a probe showed specificity in detecting only gaseous alkane utilizing bacteria at high stringency (in presence of 35–40% formamide at 45°C). Hybridization studies showed that this fragment was fairly conserved amongst the gaseous alkane utilizing isolates of Indian oil fields and also in two species from other far away country. This showed the universal application of this probe in discriminating microbes which can utilize these two hydrocarbons from the ones which can not. This probe showed nonspecific signal with *Micrococcus* sp. 45R even at high stringency and this can be attributed either to the presence of homologous sequences which were earlier involved in propane and butane metabolism but got functionally redundant due to accumulation of mutations or these sequences encode different functions. It was further observed that 2.0 and 2.9 kb EcoRI-HindIII fragments (of 4.9 kb DNA) as probes showed similar specificity. This indirectly suggested that 2.9 kb EcoRI-HindIII may have some role in gaseous alkane metabolism. Two DNA fragments (0.8 kb SalI-EcoRI and 1.4 kb SalI-SalI) of the 4.9 kb cloned DNA were found to be most useful as probes since they reacted positively with almost all propane and butane utilizing microorganisms tested and showed no nonspecific hybridization with *Micrococcus* sp. 45R. It is concluded here that a 4.9 kb DNA fragment from *Pseudomonas* sp. IMT40 was fairly conserved amongst propane and butane utilizing microorganisms. Moreover, the DNA sequences from cloned DNA fragment, especially 0.8 kb SalI-EcoRI and 1.4 kb SalI-SalI fragments have the potential to be used in geomicrobiological prospecting of petroleum and natural gas.