SUMMARY

The phage PIS136 was isolated at IMTECH, Chandigarh. The phage was known to have a DNA genome with a G+C content of 69 mol percent. The genome of the phage PIS136 was further characterized, to show that the genome is made up of double stranded DNA of ~90-kb is linear, circularly permuted and terminally redundant. The phage genome does not have any cohesive termini (*cos*-site). A probable *pac*-site was clearly visible. The presence of *pac*-site suggests a headful mechanism of DNA packaging. The phage genome can be digested extensively by several restriction enzymes therefore the restriction map was drawn by using the restriction enzymes: *Nhe*I, *Not*I, *Sca*I and *Sna*BI because all the four enzymes cut the PIS136 genome 2-4 times.

The phage PIS136 has a very wide host range within actinomycetes. The host range of the phage PIS136 is variable. The cause of variable host range was investigated in detail. The phage PIS136 was isolated from its natural lysogen a species of *Saccharomonospora* PA136 which produced dark green pigmented colonies. Dilution plating of the strain PA136 formed two types of colonies (a) green pigmented (b) non-pigmented. However, upon subculturing some of these non-pigmented colonies reverted back to mostly pigmented colonies and some non-pigmented colonies. Each of these colonies either pigmented in non-pigmented underwent lysis and produced phage PIS136. Therefore phage was isolated separately from the pigmented colonies PIS136(G), and from non-pigmented colonies PIS136(W).

The very first barrier to a phage infection is the adsorption. Phage adsorption is receptor mediated. Phages anchor through their tail fibers to the receptor. It was known that the PIS136 has a narrow host range. However, comparison of the total protein profile of the phages PIS136(G) and PIS136(W) did not show any difference. It is not known, as yet, if there was any change in the nature of the proteins. It appears that the phage PIS136 can cross the extracellular barrier with ease.

The genomic digests of the phage PIS136(G) and PIS136(W) were more revealing. The *Cla*I digest of the PIS136(W) had a fragment of 2.33-kb [C(i)] which was missing in the *Cla*I digest of the PIS136(G). However, the *Cla*I digest of the PIS136(G) had two fragments of 1.98-kb(C6) and 0.642-kb (C10) which were missing

in the PIS136(W). But the Southern hybridization showed that none of the three fragments, *i.e.* C(i), C6 and C10 were actually totally absent from both PIS136(G) and PIS136(W). Therefore, all the three fragments of the *Cla*I digests:C(i), C6 and C10 are integral part of the genome of the phage PIS136. It was found that the phage PIS136 codes for a DNA polymerase α-subunit gene. The present study demonstrated that the inversion of the sequences in the fragment C6 and in C10 due to recombination between 9 bp inverted repeat at the positions 54 to 60 and 2207 to 2215 disrupts the ORF encoding DNA polymerase α-subunit. The inversion leads to the appearance of the fragment C(i) in place of C6 and C10. However, why does the colonies which produce the phage PIS136(W) fail to produce green pigment is not yet clear. It is assumed that the disruption of the DNA polymerase α-subunit may be the cause of narrow host range of the phage PIS136(W). Downstream to the DNA polymerase α-subunit, ORFs coding for putative helicase and DnaJ were also found.

The phage PIS136 lysogenized a strain of *Amycolatopsis mediterranei*, which produces rifamycin. The phage PIS136 integrated randomly and at multiple sites on the *A. mediterranei* genome. The four lysogens studied in detail, produced metabolites which were different from the non-lysogenic parent. The lysogens produced metabolites which were different from each other.

All the metabolites produced by the lysogens: AR4, AR14, AR17 and AR35 metabolites were purified and analyzed for structure determination. Most of the compounds produced by these lysogens were long chain aliphatic acids. The lysogen AR17 produced two benzene derivatives: Phenylacetic acid, 4-hydroxybenzoic acid. The lysogen AR17 also produced a compound AR17-7 which has a polyketide chain grown on a loader molecule which is not the natural (3-amino, 5-hydroxybenzoic acid) molecule. Similarly, the lysogen AR14 also produced a compound AR14-7-3 which also has a polyketide chain grown on a molecule which is not the natural (3-amino-5-hydroxybenzoic acid) loader molecule. These results suggest that the phage PIS136 is a mutator phage which alters various biosynthetic/ degradative pathways. Products of the altered pathways may be shunted to other biosynthetic pathways which may lead to the production of novel/hybrid derivatives. The work is continuing in the lab.