CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE
1.1 INTRODUCTION

In recent years it has become clear that phages have played a pivotal role in understanding the prokaryote biology. Phages have been used as an important genetic tool for fine-structure mapping, site-directed mutagenesis and transposon-related genetic manipulation, even in highly developed model system such as *E. coli*, *Salmonella typhimurium* and *Bacillus subtilis* etc. In organisms such as *Streptomyces*, *Mycobacterium*, *Amycolatopsis* and certain other actinomycetes the need for such a genetic tool is even greater as these organisms are commercially and medically very important.

Despite their interesting biology, commercial importance and availability of the genome sequences of *S. coelicolor* and *M. tuberculosis* relatively little is known about the gene expression pathways that regulate morphological development; antibiotic biosynthesis, pathogenesis etc. A major limitation in the study of morphogenesis in these organisms has been the inability to clone genes identified by morphological mutations. For example, despite intensive efforts to clone and study the *bld* loci, sites of mutations that cause pleiotropic defects in morphological development, antibiotic production and extracellular signaling, only five *bld* genes have been characterized at the molecular level since the first description of these mutation in 1976 (Merrick, 1976, Nodwell *et al.*, 1996; Elliot *et al.*, 1998; Pope *et al.*, 1998; Bignell *et al.*, 2000).

The reasons for such a slow progress are that that the obvious genetic approaches for recovering genes, identified by chemically induced mutations, have been difficult to implement in these genera. In actinomycetes relatively few genetic markers exist has making the fine structure mapping difficult. Cloning by complementation is slow and tedious. Transformation of plasmid libraries constructed either in *E. coli* or in these genera is inefficient and the libraries are often incomplete. Transposons indigenous to actinomycetes (Baltz *et al.*, 1992; Olsen *et al.*, 1998) or derived from other bacteria (Volff *et al.*, 1997; Herron *et al.*, 1999) have been identified, but they have proven ineffective for insertion mutagenesis in actinomycetes. The ineffective insertion mutagenesis is in partly due to the nature of transposon delivery systems currently available that are typically dependent on
temperature-sensitive plasmid vectors. Curing is not effective, and exposure to high temperature is mutagenic, which result in a high background of mutations not caused by transposition. Mutations resulting from transposition are not easily distinguished from the background because genetic tools have not been available to establish causal relationship between transposition insertions and mutant phenotypes. Gehring and group have (Gehring et al., 2000) recently developed a method for efficient in vitro transposition of Tn5 in S. coelicolor that takes advantage of chromosomal transformation (Oh et al., 1997) to show linkage between phenotypes of interest and transposition insertions. With this method, co-transformations, however, may not be efficient enough to allow genome scale screening of transposon related mutations.

Bacteriophages are usually very specific about their host. Very often it has also been found that the actinophages are not a good tool for generalized transduction, e.g. φC31, VP5 and R (Stuttard, 1979; Lomovskaya et al., 1980) were not able to transduce markers. Recently five more phages of S. coelicolor have been reported (Burke et al., 2001). These phages although have a wide host range and can infect several species of Streptomyces but are not yet fully characterized. Further, their host range does not cross the generic barrier. Several phages for mycobacteria are known e.g.: Bxb1 (Mediavilla et al., 2000); D29 (Ford et al., 1998a); L5 (Hatfull et al., 1993); TM4 (Ford et al., 1998b). However, phages are not known for a large number of important genera of actinomycetes. Therefore to fill the lacunae, by serendipity a new phage 'PIS136' was isolated from a active compost heap at IMTECH, Chandigarh. The phage PIS136 has a hexagonal head with a long contractile tail (Fig. A). The phage has a very wide host range amongst various genera of actinomycetes (Table A). The phage has been deposited as a lysogen in the strain Saccharomonospora PA136 at the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh and carries an accession number 'A0001'.

1.2 REVIEW

1.2.1 General biology of the actinophages

Actinomycetes represent an extremely diverse group of Gram positive bacteria which can be either bacilli or filamentous. The group includes organisms which has
The phage PIS136 was negatively stained with phosphotungstic acid and observed under a transmission electron microscope. Three hook-like structures can be clearly seen (shown with an arrow) which may help the phage in anchoring.
### Table A  HOST RANGE OF THE PHAGE PIS 136

- *Streptomyces albus G* (D.A. Hopwood)
- *Streptomyces albus* (*SalI* defective mutant, D.A. Hopwood)
- *S. albunaceus*
- *S. achromogenes subsp. achromogenes* (DSM 40028)
- *S. coelicolor A3(2)* (D.A. Hopwood)
- *S. coelicolor ΔC31 sensitive mutant* (D.A. Hopwood)
- *S. clavuligerus* (NRRL-B 3585)
- *S. canescens* (ISP 5001)
- *S. galileus* (K. Dharmalingam)
- *S. hygroscopicus* (ISP 5578)
- *S. lividans* (D.A. Hopwood)
- *S. varsoviensis* (DSM 40346)
- *S. vastus* (DSM 40309)
- *S. vinaceus* (DSM 40257)
- *Saccharopolyspora erythreus* (NRRL)-B 5616)
- *Saccharothrix aerocolonigenes* (DSM 40034)
- *Streptoverticillium albireticuli* (DSM 40051)
- *Microtetraspora inositola* (DSM 43189)
- *Micromonospora pusilla* (IFO 14684)
- *Actinomadura madurae* (IFO 14623)
- *Amycolatopsis mediterranei* (ATCC 27643)
- *A. mediterranei* (ATCC 21789, S699)
- *Nocardia amarae* (Gordona amarae)
- *Mycobacterium fortuitum*
- *Mycobacterium smegmatis*
both commercial and medical important genera such as *Streptomycetes*, *Mycobacterium*, *Amycolatopsis*, *Nocardia*, *Arthrobacter* etc. of all the actinomycetes, streptomycetes and mycobacteria are the most extensively studied genera. Bacteriophages, have played an important role in understanding the biology of these genera those bacteriophage which infect actinomycetes are known as actinophages.

1.2.2 Morphology

All the actinophages studies so far belong to group 2 morphology (Bradley, 1967a) which have a polyhedral head and long tails with little, if any, more complex structure (Smirnova et al., 1976; Suarez et al., 1984). However, three phages with elongated heads *i.e.* MSP2 (Bradley et al., 1967b), MSP8 (Inman et al., 1971), R2 (Coyette et al., 1967) and one with a very short tail *i.e.* 17 (Mattern et al., 1963) have also been reported.

1.2.3 Host range

1.2.3.1 Adsorption specificity

Host range of both virulent and temperate actinophages are usually narrow. Some of the phages such as φC31 (Smith et al., 1999), FP43 (Hahn et al., 1991) have wide host range but it rarely crosses the generic barrier. Adsorption specificity of the phages, wherein a phage is unable to get absorbed on the host because of the lack of the phage specific receptors, is the most common factor which limits the host range (Okanishi et al., 1968; Lomovskaya et al., 1977). However, some of the important intracellular barriers such as host specific restriction and modification system (R-M system) also limits the host range.

1.2.3.2 Restriction-modification systems

Of more than 24 bacterial and archaeal genomes which have been completely sequenced revealed a fact that >80% of the genome appears to have at least one DNA restriction modification (DNA R-M) system and 75% of these genomes appear to contain multiple R-M systems (Kong et al., 2000). Restriction modification systems are also known in several other bacteria whose complete genome sequence is not yet available *e.g.* *Streptomyces fradiae* (Matsushima et al., 1989b), *Streptomyces lipmanii*
(Matsushima et al., 1989a), Streptomyces avermitilis (MacNeil, 1988), Streptomyces albus G (Rodicio et al., 1988; Cox et al., 1984). Most of these R-M systems traditionally fall in Type II (Wilson et al., 1991) category and have two independent polypeptides: a restriction endonuclease which cleaves DNA and a corresponding DNA methyltransferase (methylase), which protects endogenous DNA from endonuclease digestion by methylating the N^6 of adenine to N^6-methyladenine or N^4 of cytosine to N^4-methylcytosine or cytosine to 5-methylcytosine (Roberts, 1990).

1.2.3.3 Evasion of host R-M systems by bacteriophages

1.2.3.3.1 Modification of DNA by methyl transferase

Some bacteriophages can evade the R-M system of the host. Usually either of the following means are followed: The T-phages overcome the host restriction barrier of E. coli by encoding a methyltransferase of their own (Schroeder et al., 1986; Scherzer et al., 1987; Kossykh et al., 1995; Kossykh et al., 1997) that is [N^6-adenine] methyltransferase. The Mu phage has a gene *mom* which encodes a DNA modification function. The *mom* modifies ~15% of the adenines of the phage Mu which ultimately confers protection against a wide variety of restriction enzymes (Allet et al., 1975; Kahmann, 1984).

1.2.3.3.2 Specific modification systems

Bacteriophages φC31 and R4 specify a 'g' system that interferes with modification by the *SalGI* R-M system in *Streptomyces albus G* (Chater, 1978, 1979; Lomovskaya et al., 1980), and *S. albus P*. Very rarely it was found that some of the phages which were grown on restriction- (r-) host could form plaques on *S. albus G* (r^+ m^+) host these phages were found to be 'g' mutants and were able to form plaques because their DNA was modified by the host machinery (in r background) and therefore provided protection against host restriction machinery.

1.2.3.3.3 Absence of target sites

It has also been observed that some phages lack recognition sites for large number of restriction enzymes. The *Bacillus* phages (Kruger et al., 1983) are known to follow this strategy. A *Streptomyces* phage, FP22 (Cox et al., 1984) also follows
the above strategy. This phage does not have any target site for many restriction enzymes, such as SalI, SalPI, SacI, SalI, SphI and Stul, produced by Streptomyces species and therefore is able to survive within the hosts having R-M systems for these enzymes.

1.2.4 Genome of actinophages

All the actinophage studied so far are known to contain double-stranded DNA genome with a G+C mol percentage in the range of 59-73% and a genome size in the range of ~39 kb to more than 100 kb (Table B). The genome organization can be linear with cohesive termini R4 (Chater et al., 1979), SH10 (Klaus et al., 1979), TG1 (Chater, 1986), φC31 (Smith et al., 1999), D29 (Ford et al., 1998a), TM4 (Ford et al., 1998b), L5 (Hatfull et al., 1993) or circularly permuted and terminally redundant e.g.: Pg2 (Sladkova, 1982), φSF1, (Chung et al., 1983); FP43 (Hahn et al., 1991).

1.2.4 Replication in double stranded DNA phages

The replication strategy of phages depends on the nature of its genetic material. The double stranded DNA (dsDNA) phages with a very compact genome coding for a few proteins rely altogether on host cell for their replication. Alternatively, phages with large genomes encode most of the information necessary for replication and the phage is only reliant on the cell for the provision of energy and some of the accessory factors (Cann, 1997) e.g. phages φC31 (Smith et al., 1999), Bxb1 (Mediavilla et al., 2000) D29 (Ford et al., 1998), L5 (Hatfull et al., 1993), M2 (Matsumata et al., 1989) PRD1 (Jung et al., 1987), T5 (Laevitt et al., 1989), T3 and T7 (Patel and Loeb, 2001) are known to encode their own DNA polymerase I. Similarly phage RB69 (Wang et al., 1987), φ29 (Escarmis and Salas, 1982), T4 and T6 (Patel and Loeb, 2001) are known to encode their own DNA polymerase II. There is one report of a phage SPBc2 (Lazarevic et al. unpublished with GenBank accession number NP_046685) which codes for a putative DNA polymerase III.

Type I and Type II DNA polymerases do not have enough processivity to replicate phage genomes (Lewin, 1997) but still these phages are reported to use these
### Table B  
A table showing general characteristics of the genome of various actinophages

<table>
<thead>
<tr>
<th>Phage</th>
<th>G+C in DNA (mol %)</th>
<th>Size of DNA (kb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperate phages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ϕC31</td>
<td>63.6</td>
<td>41.5</td>
<td>Smith et al., 1999</td>
</tr>
<tr>
<td>VP45</td>
<td>59</td>
<td>40.5</td>
<td>Chater, 1980</td>
</tr>
<tr>
<td>S14</td>
<td>59</td>
<td>53.85</td>
<td>- do -</td>
</tr>
<tr>
<td>ϕ448</td>
<td>59</td>
<td>51</td>
<td>- do -</td>
</tr>
<tr>
<td>R4</td>
<td>67</td>
<td>45</td>
<td>Chater et al., 1979</td>
</tr>
<tr>
<td>SH3</td>
<td>73</td>
<td>51</td>
<td>Klaus et al., 1979</td>
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<td>- do -</td>
</tr>
<tr>
<td>SH12</td>
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<td>- do -</td>
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<td>DAH1</td>
<td>NR</td>
<td>117</td>
<td>Burke et al., 2001</td>
</tr>
<tr>
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<td>NR</td>
<td>121</td>
<td>- do -</td>
</tr>
<tr>
<td>DAH4</td>
<td>NR</td>
<td>118</td>
<td>- do -</td>
</tr>
<tr>
<td>DAH5</td>
<td>NR</td>
<td>120</td>
<td>- do -</td>
</tr>
<tr>
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<td>- do -</td>
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<tr>
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<td>65</td>
<td>56</td>
<td>Hahn et al., 1991</td>
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<tr>
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<td>NR</td>
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<td>Chater, 1986</td>
</tr>
<tr>
<td>L5</td>
<td>63.2</td>
<td>50</td>
<td>Hatfull et al., 1993</td>
</tr>
<tr>
<td>Bxb1</td>
<td>63.6</td>
<td>50.55</td>
<td>Mediavilla et al., 2000</td>
</tr>
<tr>
<td><strong>Virulent phages</strong></td>
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</tr>
<tr>
<td>VP12</td>
<td>67</td>
<td>39.45</td>
<td>Chater et al., 1979</td>
</tr>
<tr>
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<td>55</td>
<td>99</td>
<td>- do -</td>
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<td>67.5</td>
<td>Lomovskaya et al., 1980</td>
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<td>- do -</td>
</tr>
<tr>
<td>D29</td>
<td>63.6</td>
<td>49.14</td>
<td>Ford et al., 1998a</td>
</tr>
<tr>
<td>TM4</td>
<td>67.1</td>
<td>52.8</td>
<td>Ford et al., 1998b</td>
</tr>
</tbody>
</table>

NR = Not reported
polymerases for replication of their DNA. The mechanism by which these polymerases work has been very well studied in the phages T7 and T4.

1.2.5.1 Replication in the phage T7

The bacteriophage T7 DNA polymerase I has been shown to be a 1:1 complex of DNA pol and the host thioredoxin (a 12 kDa protein encoded by \textit{trxA}). The DNA \textit{Pol}I has a limited processivity of only 1-50 nucleotides (Lewin, 1997) but the DNA pol-TrxA complex has been shown to have a processivity in the range of thousands (Tabor et al., 1987). This increase in processivity is accompanied by a 20-50 fold increase in half-life of DNA Pol-TrxA complex on primer-template and 200 fold increase in dsDNA exonuclease activity of the complex compared to DNA pol alone (Huber et al., 1987). \textit{TrxA} does not alter the affinity of DNA pol for ssDNA. Thus TrxA acts to clamp DNA pol to the primer template. The reducing activity of TrxA is not required for this effect (Russel and Model, 1986; Huber et al., 1986).

1.2.5.2 Replication in the phage T4

The bacteriophage T4 DNA polymerase II is also a relatively non-processive DNA pol (Goulin et al., 1968; Nossal et al., 1974; Morris et al., 1975; Alberts et al., 1975; Lewin, 1997) and is encoded by the gene 43. The gene 43 protein was shown to carry out replication on primer-template when the products of gene 45 (27 kDa protein), gene 44 (34 kDa protein) and gene 62 (20 kDa protein) were added to the reaction (Morris et al., 1975; Alberts et al., 1975). This stimulation required ATP hydrolysis (Piperno and Alberts, 1978; Piperno et al., 1978). The g44P and g62P form a tight complex (g44/62P) (Barry and Alberts, 1972; Huang et al., 1981). This complex along with g45P acts as a sliding clamp and eliminated pausing of g43P (Huang et al., 1981) while it is replicating long single stranded region (Roth et al., 1982).

1.2.5.3 Replication in eubacteria

Replication in bacteria is carried out by DNA polymerase III holoenzyme (Pol III holoenzyme). Pol III holoenzyme is a multisubunit replicase with a very high processivity. Pol III holoenzyme of \textit{Escherichia coli} is one of the best studied
holoenzyme (Kornberg and Baker, 1992; Kelman and O'Donnell, 1995). Pol III holoenzyme of E. coli consists of 10 different subunits, some of which are present in multiple copies for a total of 18 polypeptide chains (Onrust et al., 1995). The subunits of the holoenzyme can be grouped functionally into three components: (i) the DNA polymerase III core is the catalytic unit and consists of the α (DNA polymerase), ξ (3'-5'-exonuclease) and θ subunits (McHenry and Cros, 1979) (ii) the β-sliding clamp is the ring-shaped protein that secures the core polymerase to DNA for processivity (Kong et al., 1992) (iii) the five protein γ complex (γ δ ε χ) is the 'clamp loader' that couples ATP hydrolysis to assembly of β clamps around DNA (O'Donnell, 1987; Maki and Kornberg, 1988). A dimer of ζ subunit acts a 'macromolecular organizer' holding together two molecules of core and one molecule of γ complex forming the Pol III* subassembly (Onrust et al., 1995). Two β-dimers associate with the two cores within Pol III* to form the holoenzyme capable of replicating both strands of duplex DNA simultaneously.

1.2.6 DNA rearrangement

DNA rearrangement play an important role in both prokaryotic and eukaryotic regulation particularly in the generation of programmed genetic diversity. Some of the very good examples of DNA rearrangements in eukaryotes include the generation of immunoglobulin diversity by multiple deletion events which join immunoglobulin coding sequences (Tonegawa, 1983; Lee et al., 2000), mating type switching in yeast (Egel, 1977; Arcangioli and Lahondes, 2000), sequential variation of the variant surface glycoprotein in Trypanosomes by duplicative transposition of the silent copy of an expression region (Borst et al., 1982; Rudenko et al., 1998).

1.2.6.1 Rearrangement in prokaryotes

In prokaryotes DNA rearrangement is involved in the switching of pilus antigenic types in Neisseria gonorrhoeae (Meyer et al., 1982; Mehr and Seifert, 1998), phase variation of fimbriae in Escherichia coli (Abraham et al., 1985; Henderson et al., 1999), phase variation of H-segment in Salmonella typhimurium (Zieg et al., 1978; Nanassy and Hughes, 1998) host range specificity through C-segments of phages P1 and P7, invertible P-segment on the E. coli chromosome (Iida et al., 1982; Rozsa et al.,
1995) and alteration of host range through inversion of G-region in bacteriophage Mu (van de Putte et al., 1980; Kamp et al., 1981). The reaction is catalysed by a group of very closely related enzymes, invertases that are Hin, Pin, Cin and Gin for inversion of H-segment in *S. typhimurium* P-segment in *E. coli*, C-segment in phages PI and P7 and G-region in phage Mu respectively. These enzymes share more than 60% homology on the amino-acid level and can complement each other (Plasterk et al., 1984).

1.2.6.2 Inversion in the phage Mu

The phage Mu has a wide host range but the host range is variable. The variation in host range is controlled by the G-region. The G-region is 3-kb long and located in the right end of the genome between 'a' and '13' regions. The G-region has the genes S and U (Howe et al., 1979) which encode for tail fiber. It has been reported (Howe et al., 1979) that the 'G' region inverts its orientation. When the G-region is in '+ orientation, S and U genes are expressed but in '- orientation, S' and U' genes are expressed. The S' and U' genes are actually S and U genes but in opposite orientation. The change in orientation of the S and U genes changes the specificity of the tail fiber proteins which then recognize different receptors on host cell surface (Kamp et al., 1983; Grundy et al., 1984).

The inversion of G-region is mediated by the site-specific recombination function, Gin, which is encoded in the neighboring β-region. The boundaries of G-region have a 34-bp inverted repeat (IR) sequences (Hsu et al., 1974) which are substrate for Gin-mediated inversion (Plasterk et al., 1981). For Gin-mediated inversion to occur presence of an *E. coli* host factor, FIS (Factor for Inversion Stimulation) (Kahmann et al., 1985), Mg⁺² and supercoiled DNA are the other requirements (Plasterk et al., 1984; Mertens et al., 1984).

1.2.6.3 Inversion of fimS in *E. coli*

*Escherichia coli* colonizes various hosts *e.g.* Human gut and urinary tract by virtue of interaction between its type 1 fimbriae and receptors on the host cell surface (Bloch et al., 1992). A chromosomally located fim gene cluster is (Klemm et al., 1985) of 9.5-kb which encodes the components of fimbrial organelle. The expression of
fimbriae is phase variable, with individual cells switching between fimbriated and non-fimbriated states. This characteristic is due to the inversion of a 314-bp DNA segment, \textit{fimS}. A promoter residing in \textit{fimS} phase switch, drives the expression of other \textit{fim} genes (Olsen and Klemm, 1994) when the switch is in the 'on' orientation but not when the switch is in the 'off' orientation.

The inversion in \textit{fim} switch, \textit{fimS}, is mediated by two site-specific recombinases: FimB and FimE (Kawala \textit{et al.}, 1991; Guo \textit{et al.}, 1997). Inversion of the 314-bp switch is the basis of phase-variable expression of type 1 fimbriae (Abraham \textit{et al.}, 1985; Henderson \textit{et al.}, 1999). FimB can invert \textit{fimS} in either direction with approximately equal facility however FimE can invert the switch predominantly from 'on' to 'off' direction (Gally \textit{et al.}, 1996; Kulasekara \textit{et al.}, 1999). Both FimB and FimE require the accessory proteins: integration host factor and the leucine-responsive regulatory protein (Lrp) for efficient inversion of the switch (Blomfield \textit{et al.}, 1993; Dorman and Higgins, 1987; Eisenstein \textit{et al.}, 1987; Roesch and Blomfield, 1993). Inversion of \textit{fimS} involves site-specific recombination between two 9-bp inverted repeats that border the switch and orientation of the switch controls the expression of type 1 fimbriae.

1.2.7 Antibiotic biosynthetic pathways

In the ever evolving microbial world, the number of antibiotic resistant pathogenic microbes are increasing everyday which is further increasing the risk to human life at an alarmingly high rate. Antibiotics are the first line of defense to combat the pathogenic organisms. One of the most important group of antibiotics which come to the rescue of human life is polyketide antibiotics. The polyketide antibiotics are characterized by a long aliphatic carbon chain, which may be saturated or unsaturated, attached to a naphthalenic/benzenic chromophore via an amide linkage. Some of the important polyketide antibiotics used for the management of human health are rifamycin, erythromycin, oxytetracycline, Rapamycin, FK506, Candididin, Avermectin \textit{etc.}
1.2.7.1 Synthesis of polyketide antibiotics by polyketide synthesis (PKS)

The polyketide chain is synthesized by an enzyme polyketide synthase (PKS). The PKS is a multifunctional enzyme and shares a very high homology to fatty acid synthases (FAS) (Birch and Donovan, 1953; Lynen, 1980; O'Hagan, 1991). The biosynthesis of polyketide antibiotics proceeds by an iterated cycle of reaction which involves repeated decarboxylative condensations of acylthioesters (usually acetyl, propionyl, butyrate malonyl or methyl malonyl). Following each condensation, the β-keto group of the growing side chain undergoes complete reduction by following a cycle of ketoreduction, dehydration and enoyl reduction. This cycle of reduction-dehydration-reduction is omitted or curtailed at some or all points in the polyketide chains. After the carbon-chain has grown to a specific length, which is characteristic of a specific PKS and is controlled by Chain Length Factor (CLF). The polyketide back bone is released from the synthase by thiolysis, the reaction is catalysed by an thioesterase, which hydrolyzes the bond between completed polyketide and the 4-phosphopantetheine prosthetic group on the acyl carrier protein (ACP) of the last chain-extending module (Cortis et al., 1990; Donadio et al., 1991; Bevitt et al., 1992).

1.2.7.2 Types of PKSs

Polyketide synthases (PKS) like FAS are of two main types depending upon the architecture of these enzymes. In type I PKS the catalytic site for the various steps in the biosynthesis of polyketide are carried as domains along the length of multifunctional proteins. Type I PKS are responsible for the biosynthesis of the polyketide chain of erythromycin, rapamycin, rifamycin etc. (Hopwood, 1998). In type II PKS each catalytic site for a particular step in biosynthesis of polyketide is carried on a separate protein subunit (McCarthy et al., 1984). Type II PKSs are responsible for the biosynthesis of polyketide chain of actinorhodin, tetracenomycin and doxorubicin (Hopwood, 1998).

The type I PKSs are organized into group of active sites known as modules. Each module is responsible for one cycle of polyketide chain extension and functional group modification. Each module has catalytic domains where each domain is made up of 100-400 amino acids that are analogous in both function and sequence to the
individual enzyme of fatty acid biosynthesis. All the modules possess at a minimum ketosynthase (KS), acyltransferase (AT), or chain length factor (CLF), and acyl carrier protein (ACP) domains, termed as minimal PKS. In addition specific combinations of ketoreductase (KR), dehydratase (DH), enoyl reductase (ER) and thioesterase (TE) domains may also be present depending upon the degree of functional group modification taking place after each elongation step (McDaniel et al., 1993; Fu et al., 1994; Hopwood, 1997; Khosla et al., 1999).

1.2.7.3 Clustering of genes in antibiotic biosynthetic pathways

Cloning and sequencing of the various polyketide antibiotic biosynthetic pathways have shown that all the genes needed for biosynthesis of an antibiotic are clustered and each cycle of reaction is completed by a module (Sherman et al., 1989; Yu et al., 1994; Gaisser et al., 1997; Alexander et al., 1998; McHenney et al., 1998; Xue et al., 1998; Aparicio et al., 1999; Fouces et al., 1999; Mao et al., 1999; Chen et al., 2000; Du et al., 2000; Tillet et al., 2000; Trygve et al., 2000; Waldron et al., 2001).

1.2.7.4 Modularity of PKS and hybrid antibiotics

The modularity of the biosynthetic pathways suggests that splicing together of the structural genes for different PKS should be possible, which might result into production of hybrid multienzymes. These hybrid multienzymes might synthesize novel/hybrid molecules by incorporating elements of different polyketide natural products (Katz and Donadio, 1993; Hutchinson and Fujii, 1995). Hopwood et al. (1985) reported, for the first time the production of mederrhodin A which was a hybrid of actinorhodin and medermycin and proved that hybrid biosynthesis is possible and novel/hybrid compounds can be produced. Various strategies to produce novel/hybrid antibiotics include:

- Precursor directed approach or Mutasynthesis
- Domain swapping or 'Mixing and Matching'

In the 'precursor directed approach' or 'mutasynthesis' the organism is mutated so that the endogenous synthesis of one of the antibiotic precursors is blocked. The missing component is then replaced by analogs which are added to the culture. Once the analogue of the missing component is added the formation of analogous structures
or novel/hybrid antibiotics occurs (Rinehart, 1977; Omura et al., 1980; Denoya et al., 1995; Jacobsen et al., 1997).

In the 'domain swapping' or 'mixing and matching' approach (Hopwood, 1997; Marsden et al., 1998) a particular domain of PKS, which may not be functional, absent or selective for a particular starter/extender unit is replaced with another domain from a different PKS with different specificities. These additions generally, lead to the production of a novel/hybrid antibiotics (Hopwood et al., 1985; Epp et al., 1989; Weber et al., 1991; Donadio et al., 1993; McDaniel et al., 1993; Neimi et al., 1994; Ruan et al., 1997; Sezonov et al., 1997; Bohm et al., 1998; Ritsema et al., 1998; Tropf et al., 1998; McDaniel et al., 1999; Tang et al., 1999; Tang et al., 2000; Wilkinson et al., 2000; Xue et al., 2000).

1.2.8 Amycolatopsis

The genus Amycolatopsis is a member of the family pseudonocardiaceae and produces several antibiotics such as rifamycin, vancomycin, dethimycin, balhimycin and 31-Homorifamycin W (Sensi and Thiemann, 1967; Barna and Williams, 1984; Uemo et al., 1992; Wang et al., 1994; Pelzer et al., 1999).

Of all the antibiotic biosynthetic pathway in Amycolatopsis, rifamycin biosynthetic pathway is well characterized (Tang et al., 1998; Schupp et al., 1998; Hu et al., 1999; Stratmann et al., 1999; Yu et al., 2001). Balhimycin biosynthetic gene cluster in Amycolatopsis mediterranei has also been identified and characterized (Pelzer et al., 1999).

1.2.9 Rifamycins

Rifamycins (Fig. B) are clinically important ansamycin antibiotics, composed of a naphthalenic chromophore bridged by a long aliphatic ansa chain that terminates at the chromophore in an amide linkage. The rifamycins and their semisynthetic drugs exert antibiotic activity by specific inhibition of bacterial DNA-dependent RNA polymerase (Wehrli, 1977). At higher concentrations these antibiotics also inhibit the RNA-dependent DNA polymerase of retroviruses (Szabo et al., 1976). Rifamycin derivatives are primarily used against Mycobacterium tuberculosis and M. leprae,
Fig. B  Structure of Rifamycin B
causative agents of tuberculosis and leprosy respectively, but they are also active against variety of other bacteria and viruses (Szabo et al., 1976; Oppenheim et al., 1986; Bachs et al., 1992; Barakett et al., 1993; Zimmerli et al., 1998).

1.2.10 Rifamycin biosynthetic pathway

The complete rifamycin biosynthetic pathway has been sequenced and analyzed by August et al., (1998). The starter unit for the polyketide assembly, the mC7N unit, is a part of chromophore and is derived from 3-amino-5-hydroxybenzoic acid (AHBA) (Ghisalba and Nuesch, 1981). AHBA is synthesized via a modified shikimate pathway termed as aminoshikimate pathway (Kim et al., 1996; Kim et al., 1998) (Fig. C).

The polyketide chain of rifamycin is synthesized from propionate and acetate units (Chiao et al., 1995; Lancini and Cavalli, 1997) via a multifunctional type I polyketide synthase (Katz and Donadio, 1993) (Fig. C).

The gene rifF, which is located between the modular polyketide synthase and AHBA biosynthetic gene cluster has been shown to catalyze the release of the completed polyketide from the PKS by intramolecular amide formation to generate macrocyclic lactam structure (Ohsako et al., 1988; Stratmann et al., 1999).

1.2.11 Modulation of rifamycin biosynthesis

AHBA is the natural starter unit for various rifamycins however various other starter units such as 3-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid have also been reported (Hunziker et al., 1998). However, 3-hydroxybenzoic acid and 3,5-dihydroxybenzoic acid are processed only up to a tetraketide Desamino P8/1-OG (Fig. D), an analogue of P8/1-OG formed in rifamycin biosynthetic pathway (Hunziker et al., 1998; Hu et al., 1999).
**Fig. C** Schematic diagram of the rifamycin biosynthetic pathway

The rifamycin biosynthetic pathway as shown by August *et al.* 1998. The panel (a) shows the comparison of early shikimate pathway with the alternate amino-shikimate pathway for the biosynthesis of the 3-amino-5-hydroxybenzoic acid (AHBA), the loader molecule of rifamycin biosynthetic pathway. DAHP is 3-deoxy-D-arabino-heptulosonate 7-phosphate, DHQ is dehydroquinate, DHS is dehydroshikimic acid, PEP is phosphoenolpyruvate, E4P is erythrose-4-phosphate. The panel (b) shows the rifamycin PKS gene cluster. Also shown are the genes encoding the rifamycin PKS pathway, together with their inferred biosynthetic intermediates. KS is ketosynthase, AT is acyltransferase, DH is dehydratase, KR is ketoreductase, ACP is acyl carrier protein.
Fig. D  Various loading molecules of the rifamycin biosynthetic pathway

Various loading molecules of the rifamycin biosynthetic pathway are (a) 3-amino-5-hydroxybenzoic acid (AHBA, the natural loading molecule), (b) 3-hydroxybenzoic acid and (c) 3,5-dihydroxybenzoic acid. Only AHBA is capable of synthesizing rifamycin, the other two stop polyketide extension after tetraketide stage (Hunziker et al., 1998). P8/1-OG and are the tetraketide products when AHBA is the loading molecule. However desamino P8/1-OG and 5-hydroxydesamino P8/1-OG are the tetraketide products when 3-hydroxybenzoic acid and 3,5-hydroxybenzoic acid are the loading molecules respectively.
AIMS AND OBJECTIVES

The phage PIS136 was isolated at IMTECH from a strain of *Saccharomonospora*. The phage has a very wide host range within actinomycetes. Commercially and medically important genera such as *Streptomyces*, *Mycobacterium*, *Amycolatopsis*, *Arthrobacter*, and *Rhodococcus* are the part of the group actinomycetes. Cell wall composition varies a lot among the genera of actinomycetes. Therefore, it is intriguing as to how the phage PIS136 infect several phylogenetically diverse genera. It was also known that (1) the host range of the phage PIS136 is variable (2) upon lysogenization the phage PIS136 generates random mutations. This study was started with two main objectives:

i). Study of general biology of the phage PIS136
ii). Can the phage PIS136 modulate the antibiotic biosynthetic pathway to generate novel antibiotics.

1. Study of general biology of the phage PIS136

Under this heading the aim was to study:

a) general organization of the phage PIS136
b) properties of the phage PIS136
c) reason for the variation in host range

2. Can the phage PIS136 modulate the antibiotic biosynthetic pathway

The phage PIS136 was shown to be a mutator phage which generates mutations by random integration into the host genome. The phage PIS136 infects a strain of *Amycolatopsis mediterranei* which produces rifamycin. Rifamycin derivatives *e.g.* rifampicin, rifabutin and rifapentene are widely used in tuberculosis therapy. However, with the increase in the number of rifampicin resistant cases, new rifamycin derivatives are required. Therefore, another objective of the present study is to test the ability of the phage PIS136 to modulate the rifamycin biosynthetic pathway.

Rifamycins are synthesized by a modular type I polyketide synthase. During the course of present study, the rifamycin biosynthetic pathway was sequenced
(August et al., 1998). The study showed that the organization of rifamycin biosynthetic pathway is similar to rapamycin, erythromycin FK506, avermectin etc. Further, rifamycin biosynthetic pathway is not thoroughly explored. Therefore, in the present study, the rifamycin biosynthetic pathway was used as a model to study the capability of the phage PIS136 to modulate type I polyketide synthases.