



List of Figures

I.1	The A-factor regulatory cascade of <i>Streptomyces griseus</i>	07
I.2	The Birch & Donovan polyacetate hypothesis	10
I.3	Initial reactions in fatty acid and polyketide biosynthesis	11
I.4	Biosynthesis of fatty acids and polyketides	13
I.5	The structures of polyketides made by different types of polyketide synthases	15
I.6	Biosynthetic pathways; for fatty acids, (B) for aromatic polyketides, and (C) for reduced polyketides	18
I.7	Illustration of the mechanism of the type I modular PKS involved in the biosynthesis of 6dEB.	19
I.8	Digrammatic presentation of the two fungal aromatic PKS clusters	22
I.9	Digrammatic presentation of TypeIII PKS clusters	23
I.10	Biosynthetic mechanism of Chalcone and stilbene synthase	24
I.11	Digrammatic presentation of the TypeII (aromatic) PKS clusters	25
I.12	Novel polyketides produced by recombinant <i>S. coelicolor</i> CH999 strains	33
1.	Thin Layer Chromatography(TLC) and bioautogram of the crude extract of <i>S.flaviscleroticus</i>	69
1.1	Size fractionation of the partially digested genomic DNA (by <i>Sau3AI</i>) of <i>S.flaviscleroticus</i>	70
1.2	The <i>E.coli- Streptomyces</i> shuttle vector pKC505 used in construction of genomic DNA library	71
1.3	Schematic presentation of <i>in vitro</i> packaging of cosmid + genomic DNA	72
1.4	<i>PstI</i> digestion of the randomly chosen clones from the library	73

1.5	<i>Pst</i> I digestion of library pool and the <i>S.flaviscleroticus</i> genomic DNA	74
1.6	Colinearity in # MC6 with the chromosomal DNA	75
1.7	Colony hybridization	76
1.4.1	<i>Eco</i> RI digestion pattern of the cosmid clones	78
1.4.2	<i>Pst</i> I and <i>Bg</i> III digestion pattern of the cosmid clones	81
1.5.1	Southern hybridization of the <i>Eco</i> RI digested cosmid clones using <i>act</i> I DNA as the probe	82
1.5.2	Southern hybridization of the <i>Bg</i> III digested cosmid clones using <i>act</i> I DNA as the probe	83
1.5.3	Southern hybridization pattern of the <i>Pst</i> I digested cosmid clones using <i>act</i> I DNA as the probe	84
1.6	Restriction map of ~45 kb region w.r.t <i>Eco</i> RI and <i>Bg</i> III and the over lap between the cosmid clones	85
1a	Placement of five <i>Eco</i> RI fragments constituting ~45 kb region.	87
2	plasmid BlueScript vector	89
2.1.1	Restriction digestion pattern of the 5.0 kb <i>Eco</i> RI fragment with respect to four enzymes E: <i>Eco</i> RI, Bg: <i>Bg</i> III, B: <i>Bam</i> HI, P: <i>Pst</i> I.	91
2.1.1a	Restriction map for the 5.0 kb <i>Eco</i> RI fragment with respect to E: <i>Eco</i> RI, Bg: <i>Bg</i> III, P: <i>Pst</i> I and B: <i>Bam</i> HI	93
2.2.1	Restriction digestion pattern of the 11.0 kb(+) <i>Eco</i> RI fragment (derived from both the cosmid clones, 1.51 and 1.23) with respect to two enzymes E: <i>Eco</i> RI, Bg: <i>Bg</i> III.	94
2.2.1a	Restriction map for the 11.0 kb <i>Eco</i> RI fragment with respect to E: <i>Eco</i> RI, Bg: <i>Bg</i> III.	95
2.2.2	Restriction digestion pattern of the 11.0 kb(+) <i>Eco</i> RI fragment (derived from both the cosmid clones, 1.51 and 1.23) with respect to two enzymes E: <i>Eco</i> RI, P: <i>Pst</i> II.	96

2.2.2a	Restriction map for the 11.0 kb <i>EcoRI</i> fragment with respect to E: <i>EcoRI</i> , P: <i>PstI</i> .	97
2.2.3	Restriction digestion pattern of the 11.0 kb(+) <i>EcoRI</i> fragment (derived from both the cosmid clones, 1.51 and 1.23) with respect to two enzymes E: <i>EcoRI</i> , B: <i>BamHI</i> .	98
2.2.3a	Restriction map for the 11.0 kb <i>EcoRI</i> fragment with respect to E: <i>EcoRI</i> , B: <i>BamHI</i> .	99
2.2.4	Restriction digestion pattern of the 11.0 kb (+) <i>EcoRI</i> fragment (derived from the cosmid clones, 1.51 and 1.23) with respect to two enzymes Bg: <i>BgIII</i> , P: <i>PstI</i> .	100
2.2.4a	Restriction map for the 11.0 kb <i>EcoRI</i> fragment with respect to Bg: <i>BgIII</i> , P: <i>PstI</i> .	101
2.2.5	<i>BamHI</i> digestion of the 11.0 <i>EcoRI</i> fragment (in both +/- orientation)	102
2.2.5a	Diagrammatic representation of the 11kb Δ <i>PstI</i> clone in (+) and (-) orientation.	103
2.2.6	Single and double digestion of the 11.0 kb (-) orientation clone with respect to four enzymes <i>BamHI</i> , <i>BgIII</i> , <i>PstI</i> and <i>EcoRI</i> .	104
2.3.1	Single and double digestion of the 4.0kb <i>EcoRI</i> clone with respect to two enzymes B: <i>BamHI</i> , and P : <i>PstI</i> .	107
2.3.1a	Restriction map for the 4.0 kb <i>EcoRI</i> fragment with respect to B: <i>BamHI</i> , P: <i>PstI</i> .	108
2.4	Digrammatic representation of the 8.0 kb clone in (+) and (-) orientaion.	109
2.4.1	Single and double digestion of the 8.0kb <i>EcoRI</i> clone with respect to three enzymes B: <i>BamHI</i> , P: <i>PstI</i> and Bg : <i>BgIII</i>	110
2.4.1a	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to <i>BgIII</i>	111
2.4.1b	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to B: <i>BamHI</i>	112

2.4.1c	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to B: <i>BamHI</i> and Bg: <i>BglII</i>	113
2.4.1d	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to B: <i>BamHI</i> and P: <i>PstI</i>	114
2.4.2	Single and double digestion of the 8.0kb(+) <i>EcoRI</i> clone and 8.0 kb (-) <i>EcoRI</i> clone with respect to three enzymes B: <i>BamHI</i> , E : <i>EcoRI</i> and Bg : <i>BglII</i> .	115
2.4.2a	Restriction map of the 8.0 kb <i>EcoRI</i> fragment (+) and (-) orientation with respect to B: <i>BamHI</i> and Bg: <i>BglII</i>	118
2.4.3	Single and double digestion of the 8.0kb (+) <i>EcoRI</i> clone with respect to two enzymes E: <i>EcoRI</i> , and P : <i>PstI</i> .	119
2.4.3a	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to E: <i>EcoRI</i> and P: <i>PstI</i>	121
2.4.4	Single and double digestion of the 8.0kb (+) <i>EcoRI</i> clone with respect to two enzymes Bg: <i>BglII</i> , and P : <i>PstI</i> .	122
2.4.4a	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to Bg: <i>BglII</i> and P: <i>PstI</i>	124
2.4.5	Single and double digestion of the 8.0kb (+) <i>EcoRI</i> clone with respect to two enzymes B: <i>BamHI</i> , and E : <i>EcoRI</i>	124
2.4.5a	Restriction map of the 8.0 kb <i>EcoRI</i> fragment with respect to B: <i>BamHI</i>	126
2.4.6	Single and double digestion of the 4.5kb <i>PstI</i> clone with respect to three enzymes B: <i>BamHI</i> , Bg: <i>BglII</i> and P: <i>PstI</i> .	126
2.4.7	Single and double digestion of the 4.5kb <i>PstI</i> clone with respect to three enzymes B: <i>BamHI</i> , Bg: <i>BglII</i> and P: <i>PstI</i>	128
2.4.7a	Restriction map of the 4.5 kb <i>PstRI</i> fragment with respect to B: <i>BamHI</i> , P: <i>PstI</i> and Bg: <i>BglII</i>	130

2.4.8	Single digestion of the 8.0 kb $\Delta PstI$ (+) clone with respect to three enzymes B: <i>Bam</i> HI, Bg: <i>Bg</i> III and P: <i>Pst</i> I	131
2.4.9	Single digestion of the 8.0 kb $\Delta PstI$ (-) clone and 8.0 kb ΔBg III clone with respect to three enzymes B: <i>Bam</i> HI, Bg: <i>Bg</i> III and P: <i>Pst</i> I.	132
2.5.1	Single and double digestion of the 17.0 kb <i>Eco</i> RI clone with respect to three enzymes E: <i>Eco</i> RI, Bg: <i>Bg</i> III and P: <i>Pst</i> I	134
2.5.1a	Restriction map of the 17.0 kb <i>Eco</i> RI clone with respect to three enzymes E: <i>Eco</i> RI, Bg: <i>Bg</i> III and P: <i>Pst</i> I.	136
3	Partial restriction map for the ~45 kb region with respect to four enzymes, E: <i>Eco</i> RI, Bg: <i>Bg</i> III, P: <i>Pst</i> I, B: <i>Bam</i> HI	138
3.2.1	Confirmation of the clones 2.2kb <i>Bam</i> HI, 1.7kb <i>Bam</i> HI 1.9 kb <i>Bg</i> III by restriction digestion	140
3.2.2.1	Gene organization of the Various PKS clusters showing the transcriptional coupling of the KS, CLF and ACP with the exception in <i>dps/dau</i> , <i>med</i> , <i>chr</i> and <i>sfl</i> .	143
4.1.1	Schematic representation of homology dependent two-crossover recombination	156
4.2.1	Homologous recombination between the cloned internal segment 'a' of the gene a and the corresponding a sequence on the chromosome causes disruption of the gene.	157
4.3.1.1	Construction of vector for mutagenesis	160
4.3.1.2	<i>Hind</i> III digestion of the $2\Delta Bg$ III <i>Gm</i> ^r (I)	160
4.3.1.3	Construction of TDP-glucose-4,6-dehydratase deletion mutant using the homologous recombination strategy.	161
4.3.1.4	Phenotypic comparison between the Mutant <i>Gm</i> S (gentamycin sensitive) and the integrant <i>Gm</i> ^r (gentamycin resistant)	162

4.3.1.5	<i>Trans</i> complementation of the DH ⁻ genotype by the 8.0 kb <i>EcoRI</i> DNA.	163
4.3.1.6	Represents the TLC separation and bioautogram of the crude extract of WT and mutant on a flurophore containing plate	164
4.3.1.7	HPLC profile of WT crude extract	165
4.3.1.8	HPLC profile of the TDP-glucose-4,6-dehydratase deletion mutant	165
4.3.2.1	Construction of insertional inactivation vector	167
4.3.2.2	Figure <i>HindIII</i> digestion the pGMΔ <i>PstI</i> vector (II) and pGMΔ <i>PstI</i> containing the 0.2 kb <i>BamHI</i> fragment in the unique <i>BgIII</i> .	168
4.3.2.3	Strategy used to construct the insertion mutant	168
4.3.2.4	Pigment production by the WT and the DH mutant	169
4.3.2.5	Wild type-like revertant and mutant phenotypes: Repeated streaking of the mutant on R ₂ YE plate causing the reversal of DH ⁻ phenotype because of plasmid curing	170
4.3.2.6	Bioautogram and TLC of the TDP-glucose-4,6-dehydratase insertion mutant and <i>S. flaviscleroticus</i> WT crude extract developed using <i>M.luteus</i> .	171
4.3.2.7	HPLC profile of the insertion mutant # 4a	172
4.3.3.2:	<i>EcoRI</i> - <i>PstI</i> digestion the vector for mutagenesis. 0.2 kb <i>BgIII</i> DNA OMT gene cloned in pSETΔ <i>HindIII</i> vector.	173
4.3.3.3	Strategy used to construct the OMT insertion mutant	174
4.3.3.4	Phenotypic characterization of the mutant.	175
4.3.3.6	Comparison of TLC profile between O- methyltransferase mutant (M) and partially purified extract of <i>S.flaviscleroticus</i> (WT).	176

List of Tables

I.1	Cloned and Sequenced Aromatic PKS Clusters from <i>Actinomycetes</i>	16
I.2	Genetic Engineering of Polyketide Synthase for Novel Aromatic Polyketides	31
M.1	Bacterial strains, Plasmid and Lambda.	40
M.2	List of antibiotics used	49
M.3	Describing the gradient HPLC run profile	64
2.1.1	The sizes of the fragments generated by the 5.0 kb <i>EcoRI</i> fragment by different enzymes digestion	91
2.2.1	The sizes of the fragments generated by the 11.0 kb(+) <i>EcoRI</i> fragment (derived from both the cosmid clones, 1.51 and 1.23) by <i>EcoRI</i> and <i>BglIII</i> digestion as shown in figure 2.2.1	94
2.2.2	Table The sizes of the fragments generated by the 11.0 kb(+) <i>EcoRI</i> fragment (derived from both the cosmid clones, 1.51 and 1.23) by <i>EcoRI</i> and <i>PstII</i> digestion	96
2.2.3	The sizes of the fragments generated by the 11.0 kb(+) <i>EcoRI</i> fragment (derived from both the cosmid clones, 1.51 and 1.23) by <i>EcoRI</i> and <i>BamHI</i> digestion	98
2.2.4	The sizes of the fragments generated by the 11.0 kb(+) <i>EcoRI</i> fragment (derived from both the cosmid clones, 1.51 and 1.23) by <i>BglIII</i> and <i>PstI</i> digestion	100
2.2.6	The sizes of the fragments generated by the single and double digestion of 11.0 kb(-) orientation clone with respect to four enzymes <i>BamHI</i> , <i>BglIII</i> , <i>PstI</i> and <i>EcoRI</i> .	105
2.4.1	The sizes of the fragments generated by the single and double digestion of the 8.0kb <i>EcoRI</i> clone with respect to three enzymes B: <i>BamHI</i> , P : <i>PstI</i> and Bg : <i>BglIII</i> .	111

2.4.2	The sizes of the fragments generated by the single and double digestion of the 8.0kb(+) <i>EcoRI</i> clone and 8.0 kb (-) <i>EcoRI</i> clone with respect to three enzymes B: <i>BamHI</i> , E : <i>EcoRI</i> and Bg : <i>BgIII</i> .	116
2.4.3	The sizes of the fragments generated by the single and double digestion of the 8.0kb(+) <i>EcoRI</i> clone with respect to two enzymes E: <i>EcoRI</i> , and P : <i>PstI</i>	119
2.4.4	The sizes of the fragments generated by the single and double digestion of the 8.0kb(+) <i>EcoRI</i> clone with respect to two enzymes Bg: <i>BgIII</i> , and P : <i>PstI</i> .	122
2.4.5	The sizes of the fragments generated by the single and double digestion of the 8.0kb(+) <i>EcoRI</i> clone with respect to two enzymes <i>BamHI</i> , and <i>EcoRI</i>	125
2.4.6	The sizes of the fragments generated by the single and double digestion of the 4.0kb <i>EcoRI</i> clone with respect to two enzymes B: <i>BamHI</i> , and P: <i>PstI</i> .	127
2.4.7	The sizes of the fragments generated by the single and double digestion of the 4.5kb <i>PstI</i> clone with respect to three enzymes <i>BamHI</i> , <i>BgIII</i> and <i>PstI</i>	129
2.5.1	Table The sizes of the fragments generated by the single and double digestion of the 17.0 kb <i>EcoRI</i> clone with respect to three enzymes E: <i>EcoRI</i> , Bg: <i>BgIII</i> and P: <i>PstI</i>	135
3.1	BLAST result for the O-methyltransferase, showing the nearest matches in the database.	144
3.2	BLAST result for the TDP-glucose-4,6- dehydratase, showing the nearest matches in the database.	144
3.3	BLAST result for the Ketosynthase, showing the nearest matches in the database.	145
3.4	BLAST result for the Chain Length Factor, showing the nearest matches in the database	145

3.5	BLAST result for the Cyclase, showing the nearest matches in the database	146
3.6	Homology to Conserved Domain based on which putative proteins	147
3.7	BLAST result for the NRPS, showing the nearest matches in the database	149
3.8	BLAST result for the Condesation Domain, showing the nearest matches in the database	149
3.8	BLAST result for permease, showing the nearest matches in the database	150
3.9	BLAST result for the tRNA synthetase class II core domain, showing the nearest matches in the database	150
3.10	Homology to Conserved Domain based on which putative proteins were predicted	151
App.1	Bioactivity of the partially purified compound from <i>S.flaviscleroticus</i> against 5 primary cell lines.	184