Cloning and characterization of polyketide synthase genes for jadomycin B biosynthesis in *Streptomyces venezuelae* ISP5230

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Hybridizing fragments in the genomic DNA of *Streptomyces venezuelae* ISP5230, which produces the jadomycin group of angucycline antibiotics, were detected by probing with actI DNA from *Streptomyces coelicolor* A3(2). The hybridizing regions were isolated from a 16.5 kb insert of *S. venezuelae* DNA recovered from a genomic library cloned in a λ replacement vector. Subcloning and sequencing of a 4.8 kb segment of the insert, containing regions hybridizing to actIII as well as actI, identified five open reading frames (ORFs). The deduced polypeptide products of the ORFs closely resemble in sequence the components of streptomycte type-II polyketide synthases (PKSs): the ORF1 product corresponds to the ketoacyl synthase, and the ORF2 product to a polypeptide closely related to the ketoacyl synthase and involved in determining chain length; the ORF3 product matches the acyl carrier protein; ORF4 encodes a bifunctional cyclase/dehydrase; and ORF5 encodes a ketoreductase. Integration into the chromosomal DNA of a plasmid containing a segment of the ORF2–ORF4 region severely depressed jadomycin B biosynthesis; since the integrant showed no change in growth or spore pigmentation, the cloned PKS genes are presumed to encode enzymes in the pathway for jadomycin biosynthesis.

Keywords: *Streptomyces venezuelae*, jadomycins, polyketide synthase gene cluster, gene disruption

INTRODUCTION

Compounds derived biosynthetically via polyketide intermediates represent one of the largest families of secondary metabolites, and include numerous antibiotics and pigments produced by streptomycetes (for references, see Hopwood & Sherman, 1990; O'Hagan, 1992). The polyketide synthases (PKSs) that assemble the structural skeletons of polyketides are organized either as large multifunctional enzymes (type-I PKSs) in which the biosynthetic activities are present as domains, or as multi-enzyme complexes in which the activities are distributed on individual proteins (type-II PKSs). Sequence analysis of cloned PKS genes has shown strong evolutionary relationships among systems for the biosynthesis of polyketide-derived metabolites in micro-organisms, and between these systems and the fatty acid synthases (Hopwood & Khosla, 1992). Within the type-II PKSs, the amino acid sequences deduced for proteins of equivalent function are strikingly similar (McDaniel et al., 1993; Shen & Hutchinson, 1993), and in streptomycetes the nucleotide sequences of the genes themselves have sufficient identity to hybridize under relatively stringent conditions.

Malpartida et al. (1987a, b) demonstrated that digests of genomic DNA from 14 of 18 streptomycetes known to produce polyketide-derived metabolites contained fragments hybridizing with DNA sequences from actI or actIII, the genes encoding ketoacyl synthase and ketoreductase activities, respectively, in the PKS associated with actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2). In several species, the hybridizing fragments have been confirmed as PKS genes: thus the genomic frag-
ments in *Streptomyces violaceoruber* were shown to contain genes encoding the PKS for granaticin (Sherman et al., 1989), and those in *Streptomyces halstedii* were identified with a PKS engaged in spore pigment biosynthesis (Blanco et al., 1992).

In the survey by Malpartida et al. (1987a, b), three of seven streptomycetes not then known to produce polyketide-derived metabolites, unexpectedly gave genomic DNA fragments that hybridized with the *act* probes. One of these, *Streptomyces parvulus* was later found to produce the polyketide-derived antibiotic nonactin (cited by Malpartida et al., 1987b), and *Streptomyces venezuelae* ISP5230 was subsequently reported to produce jadomycins (Ayer et al., 1991), a group of pigmented compounds believed from the pattern of isotopic labelling with [13C]acetate to be of polyketide origin (S. Ayer, personal communication).

Jadomycin B, the main component of the mixture produced during growth in an isoleucine-containing medium, is glycosylated and has an isoleucine moiety fused into the cyclic structure (Fig. 1). This and related products are formed when *S. venezuelae* ISP5230 is grown under stress conditions, and expression of the biosynthesis genes appears to be activated by heat shock (Doull et al., 1994). Although strain ISP5230 differs from *S. venezuelae* UC2374, the strain reported by Malpartida et al. (1987a, b) to hybridize with *actI* and *actIII*, both strains produce chloramphenicol, and we have verified that both hybridize similarly to the *actI* probe. We report here the cloning and sequence analysis of the hybridizing DNA in *S. venezuelae* ISP5230. A selective, severe decrease in jadomycin B production when a plasmid containing part of the cloned sequence integrated into the chromosome indicates that the cloned PKS genes are associated with jadomycin biosynthesis, and are not involved in the formation of spore pigments.

**METHODS**

**Bacteria, plasmids, phages and culture media.** The bacterial strains, plasmids and phages used are listed in Table 1. All cultures in liquid media were grown on a rotary shaker at

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<th>Table 1. Bacterial strains, plasmids and phages used</th>
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<tr>
<td><strong>Strain</strong></td>
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240 r.p.m. Escherichia coli was incubated at 37 °C in Luria-Bertani medium (Sambrook et al., 1989), supplemented for plasmid selection with ampicillin (100 μg ml⁻¹); strain TG1 was the host strain for all DNA manipulations, whereas strain ET12567 was used to generate unmethylated plasmid DNA suitable for transforming S. venezuelae ISP5230. Cultures of S. venezuelae were normally grown in MYM medium (Doull et al., 1985) at 27 °C for 24–48 h. Thiostrepton (20 μg ml⁻¹) was added for selection when required. Cultures producing mycelium from which genomic DNA was extracted were grown in MYM medium supplemented with 5 mM MgCl₂. To prepare a vegetative inoculum for jadomycin B production, S. venezuelae was grown in MYM medium as usual for 24 h. Mycelium collected from the culture by centrifugation was incubated in a galactose-isoleucine medium (Doull et al., 1994) for 6 h at 27 °C. For jadomycin B production, the culture was supplemented to 6 % (v/v) with absolute ethanol, and incubated at 27 °C for a further 48 h.

Transformation procedures. Competent cells of E. coli were prepared and transformed with plasmid DNA as described by Hopwood et al. (1985). Protoplasts of S. venezuelae were prepared and transformed by the procedure of Aido et al. (1990). They were regenerated on R5 medium, and transformants were selected by overlaying the agar surface with soft nutrient agar containing sufficient thiostrepton to give a final plate concentration of 25 μg ml⁻¹.

DNA manipulation. The general procedures described by Sambrook et al. (1989) were followed. Plasmid DNA was isolated from E. coli by the alkaline lysis method of Kieser (1984). Genomic DNA was isolated as described by Hopwood et al. (1985); plasmid DNA was isolated by the method of Meese et al. (1990). The genomic library of S. venezuelae DNA used initially consisted of BglII fragments ligated to BamHI arms of ϕGEM-11 DNA (Promega). The second library contained sized (9–23 kb) fragments from a partial Sau3AI digest ligated to XhoI half-site arms of the vector.

DNA sequencing and sequence analysis. DNA fragments were subcloned in pBluescript II SK(+) and, and overlapping deletion clones were generated. Single-stranded DNA templates were isolated from cultures of E. coli TG1 phagemid transformants as described by Sambrook et al. (1989). They were sequenced by the dideoxy chain-termination method (Sanger et al., 1977) using Sequenase version 2.0 (USB) and [α-³²P]dATP. Oligonucleotide primers were used for some regions, and to resolve anomalies where necessary, segments were resequenced with 7-deaza-dGTP in place of dGTP. Both strands of the S. venezuelae genomic DNA were sequenced, and the sequence was analysed with version 7.0 software developed by the Genetics Computer Group, Madison, WI, USA.

Hybridization. For plaque and colony hybridization, DNA was transferred to Hybond-N nylon membranes (Amersham) as described by Hopwood et al. (1985). Restriction enzyme digests were fractionated by electrophoresis in 0.7 % agarose, and transferred to nylon membranes by the Southern procedure (Southern, 1975). DNA probes were labelled with [α-³²P]dCTP or with digoxigenin (DIG)-dCTP by the random priming procedure. Hybridization was carried out at 65 °C in a solution containing 5 x SSPE (1 x SSPE is 0.18 M NaCl, 10 mM sodium phosphate and 1 mM EDTA, pH 7.7), 5 x Denhardt’s solution (Denhardt, 1966), 0.5 % SDS and sheared herring sperm DNA (100 μg ml⁻¹). Membranes were washed twice at 65 °C with the following solutions in sequence: 2 x SSPE containing 0.1 % SDS, 1 x SSPE containing 0.1 % SDS, and 0.1 x SSPE containing 0.1 % SDS. The stringency of the final wash should have removed DNA possessing less than 70 % sequence identity with the probe. ³²P-labelled DNA was detected by autoradiography; DIG-labelled DNA was detected by a chemiluminescence procedure (Boehringer-Mannheim).

Chromatographic analysis. Samples (50 ml) of S. venezuelae culture filtrate were passed through 40 μm particle size C₁₈ reverse-phase silica columns (1 g Chromosep, Chromatographic Separations); the adsorbed jadomycin B was eluted with methanol. The eluate, concentrated to 0.2 ml, was chromatographed (20 μl sample) on a 4.6 x 250 mm column of 5 μm particle size C₁₈ reverse-phase silica. The eluting solvent was a gradient of acetonitrile in 50 % aqueous acetonitrile, with both solvents containing 0.1 % trifluoroacetic acid (Ayer et al., 1991). Authentic jadomycin B eluted from the column with a retention time of 11.6 min; the concentration of jadomycin B in culture filtrates was quantified by comparing chromatographic peak areas with those given by reference solutions.

RESULTS

Detection of actI-hybridizing DNA in S. venezuelae

Samples of genomic DNA from S. venezuelae strain ISP5230 and 13s (UC2374; see Table 1) were digested with BamHI, and the fragments separated by electrophoresis were hybridized at high stringency with pJ12345, which contained an insert from the actI of S. coelicolor A3(2). The DNA from each strain showed a hybridizing fragment within the size range (2.0–2.8 kb) of that detected by Malpartida et al. (1987a, b) in S. venezuelae UC2374.

Cloning of DNA hybridizing with actI and actIII

Using pJ12345 to screen a genomic DNA library containing fragments from a BglII digest of S. venezuelae ISP5230 DNA in the replacement vector ϕGEM-11 yielded a recombinant phage containing a 1.8 kb BglII–SacI fragment that hybridized with the probe. This fragment, which was located at one end of the insert DNA, was used to probe a second S. venezuelae ISP5230 library prepared in ϕGEM-11 from a partial SacI digest. Three hybridizing λ clones (LH2, LH7 and LH24) were isolated; the DNA from each was shown by Southern analysis to contain a 2.3 kb SacI fragment that hybridized with the 1.8 kb probe. Reprobing the same Southern blots with the 1.1 kb insert excised from pJ12346 located 140 and 0.3 kb SacI fragments that hybridized at high stringency. A restriction map of the region (Fig. 2a) showed that the 2.3, 1.0 and 0.3 kb SacI fragments hybridizing to actI and actIII are adjacent. To isolate the 2.3 kb SacI fragment, a mixture of the 2.3 and 24 kb fragments from a SacI digest of DNA from λ clone LH7 was excised from an electrophoresis gels and subcloned in pHLJ400. The plasmid mixture was used to transform E. coli, and transformants containing the 2.3 kb fragment were identified by colony hybridization.

Sequence analysis

The region of S. venezuelae ISP5230 DNA containing the 2.3, 1.0 and 0.3 kb SacI fragments hybridizing with actI and actIII was subcloned from the insert in λ clone LH7.
Overlapping fragments (2.3 kb ScaI, 2.6 kb PstI and 2.4 kb SnaI; see Fig. 2a) were inserted individually into pBluescript II SK(+) and sequenced (Fig. 3). Analysis of the data with the frame program (Bibb et al., 1984) showed five ORFs, all transcribed in the same direction (see Fig. 2b). Their designations as ORFs 1–5 are based on the similarity of their deduced amino acid sequences to those of ORFs 1–5 in streptomyce gene clusters for type-II PKSs (Hopwood & Sherman, 1990). The translational start site for each ORF was predicted from the location of a potential start codon closely preceded by a plausible ribosome-binding site (RBS). The 3′ end of ORF1 overlaps by 4 bp the predicted start codon (ATG) of ORF2, which begins at nucleotide 1412 and is preceded by a likely RBS located within the ORF1 sequence. This type of organization suggests translational coupling, and occurs frequently in ORFs 1 and 2 of other PKSs. ORF2 is separated from ORF3 by 42 bp of non-coding DNA, ORF3 and ORF5 are 73 bp apart, while 39 bp of non-coding DNA separates ORF4 and ORF5.

**Deduced functions**

The deduced amino acid sequence of ORF1 is strikingly similar to sequences deduced for ORF1 in the PKS gene clusters for actinorhodin in *S. coelicolor* A3(2) (Fernández-Moreno et al., 1992), granaticin in *S. violaceoruber* (Sherman et al., 1989), tetracenomycin C in *Streptomyces glaucescens* (Bibb et al., 1989), an unidentified polyketide-derived metabolite in *Streptomyces cinnamomensis* (Arrowsmith et al., 1992) and the *wbiE* spore pigment in *S. coelicolor* A3(2) (Davis & Chater, 1990). Over a region of 420 amino acids, the polypeptide sequences exhibit more than 60% identity. The similarity is particularly strong (almost 100% identity) in the region of the presumed active site, where the cysteine and serine residues responsible for substrate attachment are located (Fig. 4a). The deduced amino acid sequence of ORF2 shows 60% identity to the sequences deduced for ORF2 in known PKS clusters. The ORF2 from *S. venezuelae* ISP5230 also resembles other ORF2s in being very similar in sequence to ORF1, but lacking the two active site motifs. McDaniel et al. (1993) have recently proposed that the protein encoded by ORF2 in the *act, gra* and *tem* PKS clusters influences the chain length of the PKS product.

The deduced product of ORF3 is a relatively small protein of 90 amino acids; its amino acid sequence shows 66% identity to the sequence deduced from ORF3 of the *S. cinnamomensis* PKS, and somewhat lower identity (40–50%) to the sequence of acyl carrier proteins (ACPs) of fatty acid and polyketide synthases. This similarity includes a conserved motif around the serine residue to which the pantetheinyl cofactor is presumably attached (Fig. 4b). The products of ORF1, ORF2 and ORF3 would be expected to form an enzyme complex able to condense acyl building units into a polyketide chain; thus the function analysis of this region indicates that the 2.3 kb ScaI fragment from *S. venezuelae* encodes part of a PKS gene cluster.

The deduced product of ORF5 showed 82% sequence identity with the deduced products of ORF5 from the *mon* PKS gene cluster of *S. cinnamomensis* (Arrowsmith et al., 1992) and 60% identity to the ORF5 products from *S. coelicolor* A3(2) (Hallam et al., 1988), and *S. violaceoruber* (Sherman et al., 1989). It also shares with these proteins the conserved sequence motif Gly-X-Gly-X-[Gly/Ala] characteristic of nucleotide binding proteins (Fig. 4c). Thus ORF5 probably encodes a ketoreductase that catalyses the reduction of a specific keto group in the polyketide to a hydroxyl group that is subsequently eliminated during chain assembly.

Strong overall similarity (59% identity) was also apparent between the amino acid sequences deduced from ORF4 of *S. venezuelae* ISP5230 and *S. cinnamomensis*, and only slightly less (45% identity) between the ORF4 products from *S. venezuelae* and the *act* and *gra* PKS gene clusters. The ORF4 product was less similar to ORFV1 in the *whiE* cluster, and resembled only the N-terminal half of ORF4 in the *tem* PKS gene cluster, but overall the results indicated that ORF4 in each cluster encodes a protein with a similar function, believed to be the correct cyclization of a region in the polyketide backbone (Zhang et al., 1990).

**Gene disruption**

A 0.6 kb *Smal* fragment of *S. venezuelae* DNA internal to ORF1 (see Fig. 2c) was subcloned in the unique *Smal* site of pHJL400, an *E. coli–Streptomyces* shuttle vector that is segregationally unstable in streptomycetes because it lacks
Fig. 3. For legend see page 3385.
Fig. 3. For legend see facing page.

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the partition function of the SCP2* parent (Larson & Hershberger, 1986). The recombinant plasmid (pJV62) isolated from E. coli TG1 transformed S. venezuelae ISP5230 to thiostrepton resistance (the marker in pHJL400 used for selection) with very low efficiency. This difficulty was exacerbated by a relatively high rate of spontaneous mutation to thiostrepton resistance. All presumptive integrants selected on thiostrepton after passage of 100 putative ThioR transformants through thiostrepton-free medium to promote plasmid loss failed to hybridize to pHJL400. However, the efficiency with which pJV62 transformed S. venezuelae ISP5230 could be markedly improved by isolating the plasmid from E. coli ET12567 (dam- dcm- hsdM-) (MacNeil et al., 1992), thereby avoiding the restriction barrier to methylated DNA apparently present in this and other streptomycetes (MacNeil, 1988). Transformation of S. venezuelae ISP5230 with pJV62 then yielded approximately 70 ThioR colonies per µg of plasmid DNA. Extraction and electrophoresis of DNA from 10 of these showed plasmid clearly present in two. However, the remaining eight harboured free plasmid in low concentration, since transformation of E. coli TG1 with extracts gave, in each case, ampicillin-resistant colonies containing pJV62. An attempt to eliminate free pJV62 from 10 ThioR transformants by two rounds of subculturing in the absence of thiostrepton selection, and then to select strains in which pJV62 had integrated into the S. venezuelae chromosome through a single cross-over between the 0.6 kb SmaI fragment and the homologous DNA region on the chromosome was not successful. Southern analysis of the subcultured colonies using pHJL400 and the 2.3 kb SacI fragment as probes detected free pJV62 in each culture and showed that the chromosomal PKS gene was still intact. Transformants containing integrated vector DNA were obtained by using a larger homologous insert. Because unfavourable secondary structure might also have contributed to the failure of pJV62 to yield stable integrants, the 2.6 kb PstI-PstI insert in the new plasmid (pJV63) was from a different region of the PKS gene cluster (see Fig. 2c). It was ligated into the PstI site of pHJL400, and
Fig. 4. Alignment by the PILEUP program (Genetics Computer Group, University of Wisconsin, Madison, WI, USA) of segments of the amino acid sequences deduced from ORFs in the S. venezuelae ISP5230 and other streptomycete PKS gene clusters (see text for references). (a) ORFl sequences around the presumed P-ketoacylsynthase active site cysteine and the presumed acyltransferase active site serine. (b) ORF3 sequences around the active site serine in putative ACPs. (c) ORF5 sequences around the presumed NAD(P)-binding domain of \(\beta\)-ketoreductases.

unmethylated plasmid DNA isolated from an E. coli ET12567 transformant was used to transform S. venezuelae ISP5230; approximately 30 ThioR colonies were obtained per \(\mu\)g of plasmid DNA. Agarose gel electrophoresis and Southern hybridization with pHJL400 as a probe failed to detect free plasmid in DNA extracted from the transformants, but only two of five samples examined gave hybridization signals. In each of these, the signal corresponded to chromosomal DNA in mobility. Integration of pJV63 into the two transformants VS651 and VS652 was confirmed by Southern analysis of restriction digests of genomic DNA using the 2.6 kb PstI-PstI fragment and pHJL400 as probes. Wild-type DNA digested with MluI or EcoRI and probed with the 2.6 kb fragment gave signals at 7.0 kb and approximately 30 kb, respectively. In contrast, VS651 and VS652 digested with EcoRI gave signals at 5.0, 8.4 and about 35 kb, and after digestion with MluI at above 30 kb (Fig. 5a). When the membrane was stripped to remove the probe and rehybridized with pHJL400, each of the signals in the wild-type DNA digests, and that at 5.0 kb in the EcoRI digests of the integrants were absent. The sizes and intensities of the signals, and the results with the different probes, were all consistent with integration into the 2.6 kb region of the chromosome of at least three copies of pJV63 (Fig. 5b).

No differences between the wild type and VS651 or VS652 were detected in growth, the abundance of spores on MYM medium, or the blue-green pigmentation of the spores. In contrast, production of jadomycin B in strains VS651 and VS652 was severely depressed. Whereas cultures of the wild type and of control strains transformed with pHJL400 or pJV62 showed strong orange pigmentation in the supernatant broth 48 h after ethanol
treatment, VS651 and VS652 cultures remained unpigmented even after 72 h. Extraction and HPLC analysis of the cultures confirmed the presence of jadomycin B at the expected concentration (30 mg l⁻¹; Doull et al., 1993) in the wild-type and control cultures. In cultures of VS651 and VS652, a small peak corresponding to jadomycin B represented 6% of that in the positive controls. A control not treated with ethanol gave no detectable peak for jadomycin B.

**DISCUSSION**

Cloning and sequence analysis of the fragment of *S. venezuelae* ISP5230 DNA that hybridizes with act1 from *S. coelicolor* A3(2) has confirmed the initial indication from hybridizations by Malpartida et al. (1987a, b) that *S. venezuelae* possesses genes for the biosynthesis of polyketide-derived metabolites. The results add yet further evidence of the value of act probes in detecting strains producing type-II polyketide metabolites. The *S. venezuelae* strain probed by Malpartida et al. (1987a, b) differs from ISP5230 in several characteristics (Ahmed & Vining, 1983; Stuttard, 1982), but both fall within the species *S. venezuelae* (Stuttard, 1982), and both are known to produce chloramphenicol (Vining & Westlake, 1984). The ISP5230 strain has only recently been reported to form the polyketide-derived jadomycins (Ayer et al., 1991), and indeed the earlier isolation of mutants blocked in chloramphenicol production (Doull et al., 1985) was consistent with this antibiotic being the sole active product; failure to detect the jadomycins is attributed in a large measure to the unusual culture conditions required for their production. Under conditions optimized for jadomycin biosynthesis, strain 13s (UC2374) produces these compounds in yields similar to ISP5230 (Singh, 1992).

The five ORFs located in the 4.8 kb of *S. venezuelae* ISP5230 DNA sequenced show marked similarity to genes in streptomycete type II PKS clusters. A tentative assignment of their functions based on comparisons of derived amino acid sequences suggests that ORF1 encodes a ketoacyl synthase, ORF2 a closely related polypeptide that influences the chain length of the PKS product, ORF3 an ACP, ORF4 a bifunctional cyclase/dehydrase, and ORF5 a ketoreductase. The presence of ORF5 among the jad (jadomycin biosynthesis) genes is consistent with the absence from the jadomycin B structure of a hydroxyl group at C-10 (see Fig. 1). In containing the ORF5 ketoreductase function, the jad PKS differs from those of *tcm* (Bibb et al., 1989), *whiE* (Davis & Chater, 1990) and *sch* (*S. halstedii* spore pigment; Blanco et al., 1993; whereas the *tcm* metabolite (tetracenomycin C) retains an oxygen substituent at C-3, jadomycin B at the corresponding position (nine carbons from the carboxyl end of the polyketide chain; Bartel et al., 1990) is unsubstituted. The arrangement of ORFs 1, 2 and 3 in the group of jad PKS genes has features in common with most type-II streptomycete PKS genes (Fig. 6), but the location of ORF5 places jad in the subset represented by *otc* (Kim et al., 1994), ‘fren’ (Bibb et al., 1994), *gris* (Yu et al., 1994) and the mon cluster of *S. cinnamonensis* (Arrowsmith et al., 1992). In these groups, ORF5 is located between ORF3 and ORF4, although with various spacings; the jad arrangement most closely resembles that of *gris*, the PKS gene cluster for grisescin biosynthesis in *Streptomyces griseus*. Grisescin (Tsuij et al., 1976) and jadomycin are probably each derived from C₉₀ polyketide intermediates that fold in a similar pattern. The arrangement of the mon gene cluster differs from those of jad and gris only in the closer spacing of ORFs 3 and 5. Although the mon metabolite has not yet been chemically characterized, the polyether antibiotic monensin and spor pigments are excluded as candidates (Arrowsmith et al., 1992) and a structural relationship to jadomycin is possible.

No attempt was made to determine whether the size or DNA structure of the insert accounted for the failure of pJV62, containing the 0.6 kb *SmaI* fragment of ORF1, to integrate into the *S. venezuelae* ISP5230 chromosome, whereas integration occurred when the vector carried the 2.6 kb *PstI* fragment containing the C-terminal portion of ORF2, the complete sequence of ORF3 and ORF5, and the N-terminal portion of ORF4. Information on expression of the jad PKS genes is lacking, but the corresponding act PKS genes all lie within a single transcriptional unit (Fernández-Moreno et al., 1992). The isolation of *S. venezuelae* transformants disrupted in jadomycin B production not only indicates that the PKS gene cluster is involved in jadomycin B biosynthesis, but also supports the assumption that ORFs 2–4 are co-transcribed. Production of a small amount of jadomycin B by the disruptants suggests that during growth of the culture, some of the mycelium had excised pJV63, thereby

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**Fig. 6.** A diagrammatic comparison of gene organization in the PKS clusters cloned from *S. coelicolor* A3(2) (act and whiE), *S. violaceoruber* (gra), *S. glaucescens* (tcm), *S. rimosus* (otc), *S. roseoflavus* (fren), *S. griseus* (gris), *S. cinnamonensis* (mon), and *S. venezuelae* ISP5230 (jad); see text for references.
restoring jadomycin B synthesis. Jadomycin production increased as expected when disruptant cultures were supplemented with 50 µg thioestrepton ml⁻¹, which would select for strains with high-level thioestrepton resistance resulting from excision of the multi-copy vector. Excision of integrated plasmids is not unexpected (MacNeil et al., 1992).

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