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Cloning and analysis of DNA sequences from Streptomyces hygroscopicus encoding geldanamycin biosynthesis

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Abstract A gene library constructed from large (~20 kb) fragments of total DNA from the geldanamycin-producing strain *Streptomyces hygroscopicus* 3602 cloned in the plasmid vector pIJ61 were used to transform *S. lividans* TK24. Three transformants of about 800 tested were found to have acquired the ability to produce an antibiotic lethal to a geldanamycin-sensitive strain of *Bacillus subtilis*. The plasmids isolated from these transformants, pIA101, pIA102 and pIA103, each contained an insert of ~15 kb. A 4.5 kb DNA fragment from the insert in pIA102 hybridised to DNA from *S. hygroscopicus* 3602 and to DNA encoding part of the erythromycin polyketide synthase but not to *S. lividans* TK24 DNA. The integration-defective phage vector ϕC31 KC515 containing this 4.5 kb fragment was able to lysogenise *S. hygroscopicus* 3602 to produce lysogens defective in geldanamycin production. Loss of the prophage restored the ability to produce geldanamycin. Extracts of fermentation broth cultures of *S. lividans* containing pIA101, pIA102 and pIA102 and pIA103 analysed by thin-layer chromatography (TLC) contained compounds identical or very similar to purified geldanamycin, which were not present in *S. lividans*. These compounds showed a mass spectrum indistinguishable from geldanamycin. The evidence suggests that the clones contain DNA sequences encoding functions required for geldanamycin biosynthesis including components of the polyketide synthase.

Key words *Streptomyces hygroscopicus*
Geldanamycin biosynthesis · Ansamycins
Polyketide antibiotics · Insertional mutagenesis

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Introduction

Geldanamycin (Fig. 1) is an ansamycin antibiotic produced by the gram-positive bacterium *Streptomyces hygroscopicus* var. *geldanus* NRRL 3602 with antiprotozoal and antitumour activity and, to a lesser extent activity against gram-positive bacteria (Sasaki et al. 1970; DeBoer et al. 1970). The ansamycin antibiotics are produced by a variety of Actinomycete bacteria and also by some plants (Wehrli 1977; Lancini 1983). As a class their structure is characterised by an aliphatic bridge (the ansa chain) linked to two non-adjacent positions of an aromatic nucleus (Rinehart and Shield 1976); Wehrli 1977; Lancini 1983). The aromatic nucleus can be either a naphthalenic ring system, as in the rifamycins, tolypomycins, naphthomycin and streptovaricins, or a benzenic ring system as in geldanamycin, herbimycins, macbecins, ansamytocins and maytansinoids. Generally the naphthalenic ansamycins are antibacterial agents although some show antifungal activity, whereas the benzenic compounds are predominantly active on eukaryotic cells.

The structural similarities of the ansamycins suggest closely related biosynthetic pathways and for the three ansamycins that have been studied, rifamycin, geldanamycin and ansamytocin, this is confirmed by bio-

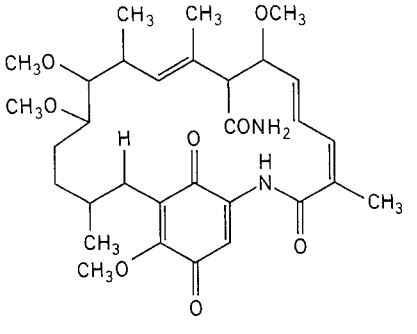

Fig. 1 Structure of geldanamycin (mol. wt 560)

genetic evidence (Ghisalba et al. 1974; Rinehart and Shield 1976; Lancini 1983). Biosynthesis of all ansamycins is initiated by a seven-carbon unit, 3-amino-5-hydroxybenzoyl-CoA (C_7N), from which the aromatic nucleus is constructed. The precursor of C_7N is an intermediate in the shikimate pathway, probably 3-dehydroxyquinic acid or 3-dehydroxyshikimic acid (Lancini 1983; Ghisalba et al. 1984). The ansa chain, which is a polyketide structure, is formed on this aromatic nucleus by methylmalonate-malonate condensation with propionate (P) and acetate (A) units. Biosynthetic branch points for the different ansamycins can be identified as C_7N-PA, leading to the ansamytocins and maytansinoids, and C_7N-PAP leading to geldanamycin, herbimycins, streptovaricins and rifamycins (Ghisalba et al. 1984). The structural variety and functional specificity is therefore determined partly by the sequence and subsequent modification of the remaining P and A units in the ansa chain. This route for the formation of the polyketide chain is reminiscent of that occurring in macrolide synthesis, furthermore the stereochemistry of the aliphatic chains of rifamycins and streptovaricins is very similar to that of erythromycin, suggesting that synthesis occurs by a similar polyketide synthase (PKS) enzyme system. However, the ansamycins do not contain lactone bonds and therefore are clearly distinct from the macrolide antibiotics.

Recent studies of the erythromycin biosynthetic pathway may provide some clues to understanding the mechanism of ansamycin biosynthesis (Cortes et al. 1990; Weber et al. 1990; Donadio et al. 1991; Revill and Leadlay 1991). Within a DNA sequence of at least 60 kb required for erythromycin A biosynthesis in *Saccharopolyspora erythraea* a contiguous region of about 30 kb codes for the PKS functions, which catalyse the successive condensations of the six propionate units required to form the erythromycin polyketide chain. This region is subdivided into three genes, each coding for the PKS functions necessary for two cycles of polyketide chain extension. The erythromycin PKS therefore has a modular structure with complete sets of functions organised as a multifunctional complex and has been referred to as a Type I system because of its similarity to Type I fatty acid synthases. By contrast, the PKS functions of the antibiotics actinorhodin, tetracenomycin, granaticin and oxytetracycline have a Type II PKS. In Type II PKS the individual enzyme components are organised as a linked cluster of separate genes, each coding for a specific function in polyketide chain extension and modification (Hopwood and Sheerman 1990). In the case of the ansamycins the organisation and functions of the biosynthetic genes are largely unknown and while it can be presumed that PKS functions are involved they remain to be characterised. As an initial stage in the analysis of the ansamycin biosynthetic pathway genes we report the isolation of cloned DNA sequences from *S. hygroscopicus* 3602, which control the synthesis of geldanamycin when expressed in *S. lividans* TK24.

Materials and methods

Bacterial strains, plasmids and phages

The wild-type geldanamycin-producing strain *S. hygroscopicus* var *geldanus* NRRL 3602 (DeBoer et al. 1970) was used as the source of chromosomal DNA for the construction of the DNA library. *S. lividans* TK24 (thiostrepton sensitive) was used as the recipient strain for plasmid transformation and for the propagation of plasmid vectors pIJ61 (low copy number, thiostrepton resistant, viomycin resistant) and pIJ699 (a *Streptomyces* - *Escherichia coli* high-copy-number shuttle vector expressing thiostrepton resistance and viomycin resistance in *S. lividans*) and their constructs (Hopwood et al. 1985; Kieser and Melton 1988). *S. hygroscopicus* NRRL 3672 and *Amycolatopsis mediterranea* are producers of the ansamycin antibiotics herbimycin and rifamycin, respectively (Sensi et al. 1960; Iwai et al. 1980). *S. coelicolor* M145 and *S. lividans* TK24 both carry the biosynthetic genes for the polyketide antibiotic actinorhodin but neither is known to produce any ansamycin antibiotics. *Bacillus subtilis* (geldanamycin sensitive, thiostrepton resistant) was used as the indicator strain for antibiotic activity. Plasmids pIJ2345 and pIJ2346 contain the *actI* and *actIII* DNA sequences, which respectively, encode components of the actinorhodin PKS in *S. coelicolor*. Plasmid pBROC397 contains the 10 kb open reading frame-A (ORFA) sequence encoding erythromycin polyketide synthase functions cloned from *Saccharopolyspora erythraea* (Cortes et al. 1990). The phage cloning vector ϕC31 KC515 (*attP* site deleted, thiostrepton resistant, viomycin resistant) was propagated in *S. lividans* 1326 (thiostrepton sensitive) and used for the homologous recombination studies with cloned *S. hygroscopicus* DNA (Hopwood et al. 1985).

Media, chemicals and enzymes

R2YE agar medium (Hopwood et al. 1985) was used for the regeneration of *S. lividans* protoplasts. Yeast extract agar medium (YEM) and yeast extract-malt extract liquid medium (YEME) were used for the propagation of all *Streptomyces* strains where antibiotic expression was studied (Hopwood et al. 1985). Liquid cultures were grown at 30° C in baffled shake flasks shaken at 240 rpm and supplemented with thiostrepton at 5 μg/ml to maintain selection where necessary. *B. subtilis* stocks were grown overnight at 37° C in Luria broth and maintained on Luria agar plates. Phage stocks were prepared with Difco soft nutrient agar (SNA) overlays on Difco nutrient agar plates supplemented with 2% glucose, 2 mM $CaNO_3$ and 2 mM $Mg(SO_4)_2$. Spore overlays were made with SNA for bioassays and with soft R2YE agar containing 50% v/v P buffer for phage infections and transfections (Hopwood et al. 1985). Thiostrepton was a gift from E.R. Squibb and Sons and the geldanamycin samples were gifts from E.M.H. Wellington (Warwick University, UK) and K.L. Rinehart (University of Illinois at Urbana-Champaign). DNA restriction endonucleases, alkaline phosphatase and T4 DNA ligase were all purchased from Boehringer Mannheim and used as recommended by the manufacturer.

Isolation of DNA

DNA was isolated from *Streptomyces* cultures grown in YEME medium, with antibiotic selection where appropriate, for 4-6 days with shaking at 30° C. Total DNA was isolated by Procedure 1 of Hopwood et al. (1985) modified by including the RNase step with the lysozyme treatment, omitting the polyethyleneglycol (PEG3000) step and using isopropyl alcohol to precipitate the DNA. Plasmid DNA was extracted by the method of Kieser (1984) modified by including the RNase step with the lysozyme treatment and purified by caesium chloride-ethidium bromide gradient centrifugation (Hopwood et al. 1985; Maniatis et al.

1982). Phage DNA was isolated from SNA overlays by the procedure of Hopwood et al. (1985).

Cloning *S. hygroscopicus* total DNA

A DNA library was constructed from *S. hygroscopicus* 3602 total DNA partially digested with *Sau*3A and size-fractionated on a sucrose density gradient (Maniatis et al. 1982). Restriction endonuclease cleavage and fractionation conditions were selected to produce DNA fragments of about 20 kb. DNA bands were extracted from agarose gels by the Geneclean (NEN DuPont) method and ligated into pIJ61 previously linearized by cleavage of the single *Bam*HI restriction site and treated with alkaline phosphatase. The ligation mixture was used to transform protoplasts of *S. lividans* TK24 prepared and regenerated at 30° C on R2YE plates as described by Hopwood et al. (1985). After 24 h incubation the regeneration plates were overlaid with thiostrepton (500 μg/ml) in P buffer or distilled water to select for thiostrepton-resistant plasmid transformants and incubated a further 5-7 days at 30° C. Transformed colonies were either replicated or transferred as an agar plug to YEM plates supplemented with thiostrepton and overlaid with SNA containing the *B. subtilis* indicator strain. After overnight incubation at 30° C the plates were scored for zones of antibiosis surrounding the transformed colonies.

Mutational cloning

Two *Bam*HI restriction fragments of about 3.5 kb and 4.5 kb in size were cut from the *S. hygroscopicus* 3602 DNA insert in pIA102 (see the Results and discussion). The fragments were isolated from a 0.6% agarose gel by the Geneclean (NEN DuPont) procedure and after treatment with alkaline phosphatase were ligated separately into *Bam*HI-cleaved ϕC31 KC515. The ligated DNA was used to transfect fresh protoplasts of *S. lividans* 1326 which were overlaid on R2YE plates with spores of the same strain suspended in soft R2YE agar and the plates incubated for 24 h at 30° C (Hopwood et al. 1985). The resulting plaques were individually isolated, titred, and the phage used to infect a lawn of spores of *S. hygroscopicus* 3602. The plates were incubated at 30° C for 4-7 days and the resulting mycelial lawns replicated to YEM plates containing thiostrepton (50 μg/ml) to select for lysogenic colonies, which were then isolated for further analysis.

DNA hybridizations

DNA probes were labelled with [α-^{32}P]dCTP (Amersham International) using a random priming DNA labelling kit (Boehringer-Mannheim) and purified by chromatography through Sephadex G50 columns. Aliquots of target DNA species (5 μg) were denatured by heat in 1 M NaCl, 0.1 M NaOH, 10 mM EDTA and applied to Hybond hybridisation membranes (Amersham International) and allowed to dry. The DNA was crosslinked to the membrane by ultraviolet light with Stratalinker (Stratagene) and hybridised to probe DNA at 70° C in an oven with Quickhybe hybridisation buffer (Amersham International) according to the manufacturers' instructions. Membranes were subsequently washed at high stringency as described by Hopwood et al. (1985).

Thin-layer chromatography (TLC) bioautography

S. lividans TK24 containing the clones pIA101, pIA102 and pIA103 and the thiostrepton-resistant ϕC31 KC515 lysogens of *S. hygroscopicus* 3602 were tested for the production of antibiotic activity by TLC bioautography. All strains were grown for about 5 days at 30° C in shake flasks containing YEME supplemented with thiostrepton (5 μg/ml), the mycelium removed from the broth by centrifugation (4000 rpm; 10 min) and the broth extracted with an equal volume of ethyl acetate. The extracts were reduced to dryness *in vacuo*, the residue resuspended in 10-50 μl ethyl acetate and an aliquot applied to the base-line of a pre-coated thin-layer chromatoplate (Sigma Chemical Co.) alongside a geldanamycin standard. The chromatoplates were developed in ethyl acetate/dichloromethane/*n*-hexane/methanol (9:6:6:1) until the solvent front had migrated to near the top of the plate and excess solvent was allowed to evaporate from the plates. For bioautography assays the chromatoplates were then overlaid with molten SNA seeded with *B. subtilis*, incubated for 24-48 h at 30° C and scored for areas of antibiosis in the *B. subtilis* lawn.

Mass spectrometry

Broth extracts of *S. lividans* containing the clones pIA101, pIA102 and pIA103 were prepared and chromatographed by TLC alongside geldanamycin standards as described above. A single spot ($R_f \sim 0.69$) was isolated for each clone and for geldanamycin from the developed chromatoplate, extracted in ethyl acetate and applied to a second chromatoplate, which was developed in chloroform/methanol (9:1). A single spot was again isolated for each clone and the geldanamycin standard and these purified products analysed by mass spectrometry. Mass spectrometry analyses were determined with a single focusing magnetic sector instrument with an electron impact ion source (VG Micromass 7070F). Solid samples were individually admitted to the ion source using a direct inlet probe via a vacuum rod, and volatilised at a temperature of about 150° C. A potential equivalent to 70 eV was applied to the ion source.

Results and discussion

Isolation and screening of clones of *S. hygroscopicus* total DNA

Attempts were made to identify DNA sequences specifying PKS functions associated with geldanamycin biosynthesis, by hybridising *S. hygroscopicus* 3602 total DNA with the *actI* and *actIII* sequence probes. These probes encode components of the actinorhodin PKS (Malpartida et al. 1987) and were unsuccessful in identifying DNA sequences of interest (data not shown). This result suggests that the PKS involved in geldanamycin biosynthesis is not a Type II PKS. As an alternative approach, a *S. hygroscopicus* 3602 gene library was constructed by transforming protoplasts of *S. livicans* TK24 with *Sau*3A-cleaved and size-fractionated total DNA (average fragment size, 20 kb) ligated into pIJ61. Plasmid transformed colonies, selected on plates containing thiostrepton, arose at a frequency of $1.5 \cdot 10^4$/μg DNA. The transformants were either replicated or individually transferred to thiostrepton-supplemented YEM plates and tested for antibiotic activity by overlaying with lawns of the geldanamycin-sensitive *B. subtilis* indicator strain. Of the 800 transformants tested, 3 produced zones of antibiosis. These clones, designated pIA101, pIA102 and pIA103, were isolated and stored on thiostrepton-supplemented medium to maintain selection for the plasmid.

Plasmid DNAs isolated from *S. lividans* TK24 containing pIA101, pIA102 and pIA103 where used to retransform *S. lividans* TK24 protoplasts. Again

thiostrepton-resistant transformants were produced, which formed zones of antibiosis on overlays of *B. subtilis* thus confirming that antibiotic production was inherited with the plasmid. Restriction analysis of the plasmid DNA showed the presence of some bands in submolar amounts, suggesting the presence of a mixed population of the original clones and deleted products (data not shown) which may have arisen by intraplasmid recombination (Tsai and Chen 1987). Nevertheless, digests of the cloned DNA with *Bam*HI identified several bands, confirming the presence of insert DNA since pIJ61 contains only a single *Bam*HI site. This plasmid instability prevented precise mapping of the cloned inserts. However, the restriction patterns of DNA from the three clones showed some similarities, suggesting that the clones contained common sequences, and it was possible to conclude that each clone contained an insert of approximately 15 kb.

Two *Bam*HI bands from pIA102, of approximately 3.5 and 4.5 kb in size, were individually subcloned into the single *Bam*HI site of the *Streptomyces* region of the *E. coli/Streptomyces* shuttle vector pIJ699. The 4.5 kb fragment was radiolabelled and used as a probe in dot blot hybridisations at high stringency with total genomic DNA from a series of Actinomycetes. Strong hybridisation was detected with *S. hygroscopicus* 3602 DNA, weak hybridisation with DNA from the herbimycin producer *S. hygroscopicus* 3672 and no hybridisation with DNA from *S. lividans* TK24, *S. coelicolor* M145 or the rifamycin producer *A. mediterranea*. This result confirms the presence of *S. hygroscopicus* DNA in clone pIA102. Similar results were obtained for pIA101 and pIA103. In addition, we have shown that a probe consisting of the 10 kb ORFA PKS region from *Saccharopolyspora erythraea* isolated from pBROC397 also hybridizes to the pIA101, pIA102 and pIA103 clones and to the 4.5 kb sub-cloned fragment of pIA102 (data not shown).

Bioautography of culture extracts

Ethyl acetate extracts of YEME broth cultures of *S. lividans* TK24 containing either pIA101, pIA102 or pIA103 were fractionated by TLC and analysed for antibiosis by overlaying the separations with *B. subtilis*. Purified geldanamycin and broth extracts from cultures of wild-type *S. hygroscopicus* 3602 and untransformed *S. lividans* TK24 were run as standards on the same chromatoplates. The results (Table 1) allow several conclusions. Purified geldanamycin is a mixture of compounds with antibiotic activity against *B. subtilis*, of which those with R_f values of ~ 0.33 and ~ 0.68 are specific to geldanamycin since they are absent from culture extracts of *S. lividans* TK24. The relationships of these two compounds are not known; however, both were present in two samples of geldanamycin from independent sources and they may represent the two unresolved compounds reported by DeBoer and Peterson (1971). It is highly significant that zones of antibiosis at the same R_f values were present in culture extracts of *S. lividans* TK24 containing clones pIA101, pIA102 and pIA103. This finding strongly suggests that they are related structurally and functionally to geldanamycin. The bioactive zone at $R_f \sim 0.15$ is probably also related to geldanamycin but the presence of a killing zone of similar R_f in the *S. lividans* TK24 extracts makes this conclusion tentative. Antibiotic zones at R_f values of ~ 0.04 and ~ 0.08 were also present in all samples but as these compounds had barely migrated from the baseline of the chromatoplate it is not possible to draw any conclusions about their relationships. From the TLC analysis we conclude that the cloned inserts in pIA101, pIA102 and pIA103 express functions when present in *S. lividans* TK24, which control the synthesis of compounds closely related, if not identical, to geldanamycin.

Table 1 (TLC) bioautography of fermentation broth extracts[a]

Geld[b]	TK24 (pIA101)	TK24 (pIA102)	TK24 (pIA103)	TK24[c]
0.15±0.01	0.15±0.01	0.13±0.00	0.15±0.02	0.15±0.03
0.33±0.02	0.31±0.01	0.35±0.02	0.31±0.02	
0.68±0.01	0.69±0.01	0.70±0.02	0.69±0.02	

[a] Numbers refer to R_f values of zones of antibiosis detected on TLC chromatoplates overlaid with the geldanamycin-sensitive *Bacillus subtilis* indicator strain. Average values with standard errors were calculated for 3 to 7 independent determinations
[b] Purified geldanamycin
[c] *Streptomyces lividans* TK24. Where present, the plasmids containing cloned inserts of *S. hygroscopicus* 3602 DNA are shown in brackets

Table 2 TLC bioautography of *S. hygroscopicus* KC515 lysogens[a]

Geld[b]	*S. hygroscopicus*[c]		
	Wild type	Class A lysogens	Class B lysogens
0.15	0.19		
	0.24	0.23	0.23
0.33	0.34		
	0.52		
0.68	0.65	0.69	

[a] Numbers refer to R_f values of zones of antibiosis detected on TLC chromatoplates overlaid with the geldanamycin-sensitive *B. subtilis* indicator strain
[b] Purified geldanamycin
[c] Extracts of fermentation broth cultures of *S. hygroscopicus* 3602 wild type and lysogens containing ΦC31 KC515 prophage with the 4.5 kb insert subcloned from pIA102. Of the 15 lysogens tested 6 were of Class A and 9 of Class B

Mutational cloning

The 3.5 and 4.5 kb *Bam*HI fragments of *S. hygroscopicus* 3602 DNA sub-cloned from pIA102 into pIJ699

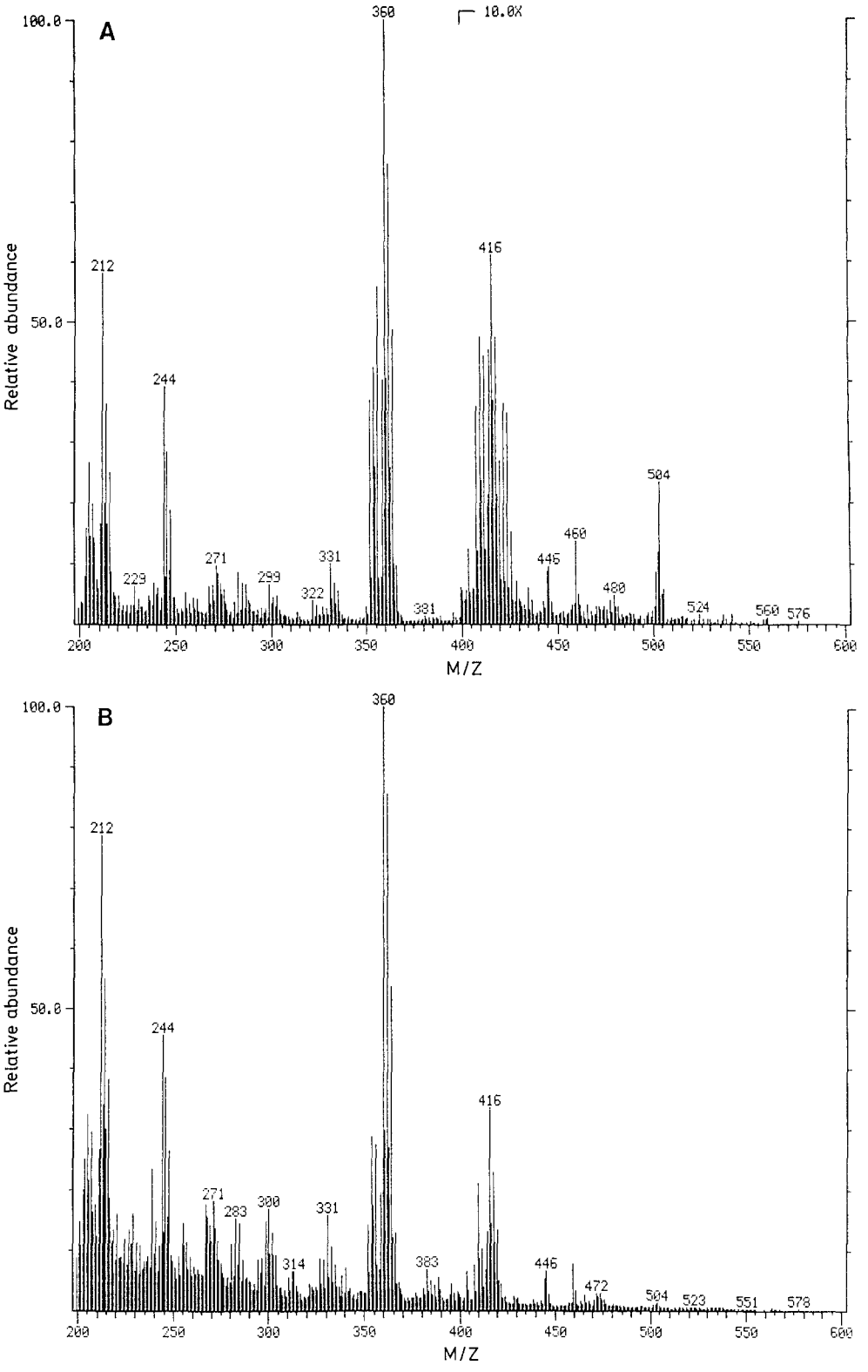

Fig. 2 Mass spectra of geldanamycin **A** and an extract of *Streptomyces lividans* TK24 (pIA102) fermentation broth **B**. Samples were purified as described in the Materials and methods The *bracket* in **A** indicates a tenfold increase in detection sensitivity

were re-cloned into the *Bam*HI site of the phage vector ϕC31 KC515 (see the Materials and methods). This vector is an *attP*-deleted derivative of the temperate phage ϕC31 and is able to enter the lysogenic state only by recombination between a DNA sequence cloned into the phage and a homologous sequence in the host cell. Lysogens were isolated for both fragments upon infection of *S. hygroscopicus* 3602, indicating that homology-driven integration had taken place in both cases. Lysogens derived from both classes of fragment were assayed for geldanamycin biosynthesis by TLC bioautography. Extracts of those lysogens produced with the 3.5 kb

fragment showed patterns of antibiotic zones identical to *S. hygroscopicus* 3602 wild-type extracts and were therefore unaltered with respect to geldanamycin production. This suggests that either the cloned DNA is not involved in geldanamycin biosynthesis or it contains a transcriptional junction within the geldanamycin biosynthetic cluster such that phage integration does not disrupt expression of the biosynthetic pathway (Chater and Bruton 1983).

Lysogens containing the 4.5 kb fragment of pIA102 produced TLC bioautography phenotypes of two classes, both of which were different from the *S. hygroscopicus* 3602 wildtype and both of which had lost the ability to synthesise active geldanamycin (Table 2). The class A lysogens produced the antibiotic zone of $R_f \sim 0.69$ detected in purified geldanamycin and extracts of *S. hygroscopicus* 3602 wild type and indicating partial expression of the geldanamycin biosynthetic genes. Class B lysogens produced none of the antibiotic zones associated with geldanamycin. The antibiotic zones at $R_f \sim 0.23$ was identified as the antibiotic nigericin, which is also produced by *S. hygroscopicus* 3602 but is unrelated to geldanamycin. The absence of the antibiotic zone of $R_f \sim 0.15$ from both class A and class B lysogens suggests that this may also be a geldanamycin biosynthetic compound.

These results indicate that at least part of pIA102 is involved in geldanamycin biosynthesis since phage integration has caused pathway disruption and we conclude that the 4.5 kb fragment must therefore be internal to a transcription unit within the geldanamycin biosynthetic gene cluster. The two classes of lysogen probably arose by insertion of the 4.5 kb fragment into ΦC515 in two orientations (Chater 1986). On this basis the phage producing class A lysogens would contain fragments cloned in the sense orientation with respect to the vector and with partial expression of geldanamycin biosynthetic genes occurring by readthrough from phage promoters. Class B lysogens would therefore contain prophage with the inserted DNA in the opposite orientation such that readthrough from a phage promoter would produce nonsense transcripts and hence a geldanamycin non-producing phenotype.

S. hygroscopicus 3602 (KC515) lysogens carrying the 4.5 kb insert of both groups A and B were grown on R2YE plates without selection for thiostrepton and viomycin resistance and the colonies replicated to lawns of *S. lividans* 1326 to test for the loss of prophage. Plaques were produced on the replica plates from several colonies indicating phage excision and replication. Loss of the prophage from these colonies was confirmed by their loss of thiostrepton resistance. When overlaid with *B. subtilis* the non-lysogenic colonies had regained the capacity to produce antibiosis. The loss of the integrated phage is therefore linked to the restoration of antibiotic production, providing further proof that the 4.5 kb insert in the phage contains the sequences from the geldanamycin biosynthetic pathway and that insertion resulted in the disruption in geldanamycin production.

Mass spectrometry of clone broth extracts

Broth extracts from the clones pIA101, pIA102 and pIA103 were fractionated on TLC plates together with geldanamycin and in each case the material in the band at $R_f \sim 0.69$ was extracted, purified by two further TLC fractionations and the mass spectrum determined. The mass spectra for geldanamycin and the clone broth extracts showed almost identical sets of peaks (Fig. 2). While there is considerable fragmentation a small peak of molecular weight 560 corresponding to the molecular weight of geldanamycin (DeBoer et al. 1970) was present in all samples. From the correspondence of the mass spectral "fingerprints" it can be assumed that the same sets of molecular fragments were present in all cases. The fragments differing by only a few mass units from the corresponding geldanamycin fragments sometimes detected in the clone broth extracts might reflect the presence of modified compounds, such as a dihydroquinone substituent, formed in the aqueous broth.

From the biological and physical data presented above we conclude that the *S. hygroscopicus* 3602 DNA sequences cloned in plasmids pIA101, pIA102 and pIA103 control the expression of functional geldanamycin activity in *S. lividans* TK24. The size of the cloned inserts (~15 kb), when compared to the sizes of the gene clusters known to encode the biosynthetic enzymes for other antibiotics, suggest that these clones may not contain all the genes required to encode geldanamycin biosynthesis. This may indicate that the *S. lividans* T24 host is providing some ansamycin pathway intermediates, which are converted to geldanamycin in the presence of the *cloned S.* hygroscopicus 3602 sequences. Hybridisation of the cloned geldanamycin DNA to the erythromycin polyketide synthase sequences but not to those for actinorhodin (data not shown) leads us to speculate that geldanamycin biosynthesis is catalysed by a Type I PKS of the kind associated with macrolide biosynthesis.

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