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Evidence for the Wide Distribution of Repetitive DNA Sequences in the Genus *Streptomyces*

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Summary. Repeated DNA sequences were detected as rapidly reannealing sequences in the chromosomal DNA of 13 out of 14 *Streptomyces* species using either hypochromicity measurements or hydroxyapatite chromatography. These sequences made up between approximately 4% and 11% of the total DNA of these species; only in *Streptomyces rimosus* were repeated DNA sequences not detected. The repeated sequences fall into a number of distinct percentage G + C (%G + C) classes, many being of rather low %G + C. Analytical density ultracentrifugation of the DNA of these species indicated satellite bands of low %G + C, and high-resolution thermal denaturation profiles indicated the presence of blocks of DNA of low G + C content too. No such satellite band could be found in *Streptomyces coelicolor* and no low-%G + C DNA could be detected in its thermal denaturation profile. The possible relationship of this repeated DNA, an unusual occurrence in a procaryote, to genetic instability and genetic control mechanisms in *Streptomyces* is discussed.

Key words: Repeated DNA sequences — *Streptomyces* — Genetic instability

Introduction

The presence of a significant proportion of repeated sequences (rsDNA) among the DNA of eucaryotes is well documented. A small proportion of rsDNA has been detected in the Archaebacteria (Sapienza et al. 1982). Begnini et al. (1975) reported the presence of a significant proportion of rsDNA in *Streptomyces coelicolor* but not in *Streptomyces rimosus*. The present study was carried out to examine these sequences and to determine whether rsDNA occurred in other *Streptomyces* species.

Evidence has been found in a number of *Streptomyces* species that environmental stress can cause reiteration of specific DNA sequences (Robinson et al. 1981; Ono et al. 1982). It is possible that this phenomenon may be related to the presence of repeated DNA sequences in Streptomycetes.

Abbreviations used: C_ot, *the product of molar concentration of DNA nucleotides and the time (mins) of incubation (mol* l^{-1}*);* $(Et)_4NCl$, *tetraethyl ammonium chloride; Tm, the temperature at which the DNA is 50% denatured; rsDNA, repeated DNA sequences; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA %G + C, percentage G + C content of DNA*

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Experimental Procedures

Strains. Table 1 shows the strains used in this study.

Isolation and Purification of DNA. The isolation of the total cellular DNA was carried out using a modification of the procedure of Marmur (1961). The A_{260}/A_{280} ratio of the DNA used was >2.0 in all cases. The average molecular weight of the DNA obtained was >45 kilobases as assessed by gel electrophoresis, and the DNA was free of RNA.

Estimation of the rsDNA in Streptomyces Chromosomal DNA. Estimation of the amount of rsDNA was carried out by two methods; the first involved spectrophotometric measurements and the second involved hydroxypatite column chromatography. In both cases the DNA was sonicated using an MSE Soniprep to 500–600 base pairs (bp) in size as assessed by electron microscopy. Ethylene glycol was added to a final concentration of 20% to reduce the 50%-denaturation temperature (Tm) of the *Streptomyces* DNA to between 80° and 90°C (Wetmur and Davidson 1968).

The spectrophotometric method involved the denaturation and reannealing of the *Streptomyces* DNA in the presence of 1 × SSC (Marmur and Doty 1961) + 20% ethylene glycol. Measurements were carried out at the isobestic point for G + C and A + T base pairs, i.e., 272 nm. The Tm and hyperchromic shift for

Table 1. Percentage G + C and percentage rsDNA in various strains as estimated from rapidly reannealing sequences by spectrophotometric measurements and hydroxyapatite column chromatography

Species	Strain	% G + C (Buoyant density method)	% rsDNA	
			Renaturation kinetics[a]	Hydroxyapatite column[b]
Escherichia coli	C600	51	0.2 ± 0.1	0.5 ± 0.3
Streptomyces coelicolor	M130	72.1[c] 72.2	7.4 ± 0.6	8.0[d]
Streptomyces fradiae	ATCC10745	73.4[c]	9.0 ± 0.8	8.9 ± 1.8
Streptomyces olivaceus	ATCC31126	72.6	9.1 ± 0.8	8.6 ± 1.1
Streptomyces lividans	ISP5434	73.1	7.1 ± 0.5	7.1 ± 1.4
Streptomyces bikiniensis	ISP5235	71.4	5.3 ± 0.4	N/A
Streptomyces cattleya	NRRL8057	74.9	6.4 ± 0.6	N/A
Streptomyces griseus	NCIB8232	71.4[c]	4.4 ± 0.6	N/A
Streptomyces venezuelae	ISP5230	81.4[c]	5.9 ± 0.7	N/A
Streptomyces parvulus	ISP5048	71.4[c] 70.9	10.2 ± 0.5	N/A
Steptomyces alboniger	ATCC12461	N/A	3.9 ± 0.6	N/A
Streptomyces virginiae	ISP5094	N/A	7.2 ± 0.8	N/A
Streptomyces aureofaciens	ATCC10762	72.4	10.6 ± 1.1	N/A
Streptomyces acromogenes	J1054	N/A	4.8 ± 0.5	N/A
Streptomyces rimosus	NRRL24455	70.4	0.2 ± 0.2	0.0[e]

N/A, not available
[a] Renaturation kinetics results are an average of three experiments
[b] Hydroxyapatite column results are an average of two experiments
[c] From Fasman (1976)
[d] Estimate from Antonov et al. (1977)
[e] Agrees with Begnini et al. (1975)

a given DNA were determined, followed by reannealing at Tm = 25°C until linearity was well established. After correction for collapse hypochromicity, checked using *E. coli* DNA, the proportion of rapidly reannealing sequences was assessed. This was taken as a measure of the proportion of rsDNA present in the DNA (Begnini et al. 1975; Bendich and Anderson 1977). All measurements were carried out on a Beckman DU8 spectrophotometer using a Beckman Tm module and Beckman Tm Compuset.

Hydroxyapatite chromatography was carried out on *Streptomyces* DNA that had been denatured in 1 × SSC, made up in 20% ethylene glycol and renatured at Tm = 25°C to a C_0t of 0.5 [C_0t = molar concentration of DNA nucleotides × time of incubation (mol l^{-1})]. Separation of the double-stranded DNA (dsDNA) from the single-stranded DNA (ssDNA) was achieved with a thermally equilibrated hydroxyapatite column. The thermal stability of the reannealed DNA was tested to distinguish between the more stable rsDNA and the mismatched unique sequences. The assessment of the thermal stability was made either by denaturation on the DU8 spectrophotometer or by using a hydroxyapatite column and 2.5°C temperature increments followed by elution with 0.14 M phosphate buffer (Antonov et al. 1977).

Determination of the %G + C Content of Purified rsDNA. Total cellular DNA was digested to completion with Bam H1 endonuclease (Bethesda Research Laboratories) and then run on a one-dimensional formamide–urea gradient gel which separates the DNA fragments according to %G + C (Fischer and Lerman 1979). The gel was run at 12 V/cm at 60°C for 20 h. It was then soaked in Tris acetate buffer for 2 h to remove the formamide and urea, denatured and electroblotted to a nitrocellulose filter (Genescreen, New England Nuclear). Prehybridisation and hybridisation were carried out according to the method of Southern (1975). The hydroxyapatite-purified rsDNA described above was end-labelled with [^{32}P]ATP (5′ DNA Terminal Labeling System, Bethesda Research Laboratories), denatured and hybridised at 65°C for 24 h. The filters were washed twice at 65°C with 0.2× SSC for 1 h and then dried. Each gel lane was then cut from the filter and sliced into a series of 1-cm-wide by 0.5-cm-long strips. These were placed in 5 ml Packard scintillation fluid 299 and counted on a Beckman CD50 scintillation counter.

The gel system used was tested using lambda DNA digested with Eco R1 endonuclease. The results were identical to those described by Fischer and Lerman (1979). The gel system was calibrated using the chromosomal DNA isolated from three different organisms; *Clostridium acetobutylicum* (~30% G + C), *E. coli* (~50% G + C) and *S. coelicolor* (~70% G + C). This calibration was an approximation, as specific sequence effects do occur.

Studies of Streptomyces DNA in the Presence of $(Et)_4NCl$. The Tm of DNA in 2.4 M tetraethyl ammonium chloride [$(Et)_4NCl$] has been shown to be independent of base composition (Melchior and Van Hippel 1973). Therefore, the stabilities of duplexes in this solvent are influenced primarily by their degree of mismatching. Optical reassociations were monitored using the Beckman DU8 spectrophotometer with the Tm Compuset module at 272 nm.

Study of Streptomyces DNA by Analytical Cesium Chloride Density Gradient Centrifugation. Streptomyces DNA (500 bp in size) was analysed on a Beckman Model E ultracentrifuge using a Schlieren optical detection system at an initial density of 1.710. Centrifugation was carried out at 45,000 rpm for 24 h at 20°C. The DNA used was identical to that used in the denaturation-renaturation studies. Calibration was carried out using an internal *E. coli* and *S. coelicolor* DNA standard (Twefik and Bradley 1967).

High-Resolution Denaturation Profiles of Streptomyces DNA. Denaturation was carried out on DNA (average fragment size of 500 bp) at a heating rate of 0.15°C/min on a Beckman DU8 spectrophotometer at 260 nm. Only a blank and sample were used to allow calculation by the internal microprocessor of the first and second derivatives. The first-derivative data indicate groups of DNA sequences which denature in concert (Cuellar et al. 1978).

Results

Table 1 shows the %G + C of the strains studied and the percentage of rsDNA detected by both of the methods used in this study. The renaturation of the DNA to a C_0t of 0.5 under relatively stringent conditions ensures that the majority of renatured dsDNAs comprise well-matched repeated sequences. The stable (Tm ≥ 65°C) sequences are mostly repeated sequences; this thermostable fraction was used to calculate the proportion of rsDNA (Antonov et al. 1977).

The one-dimensional denaturing gradient gel system detected little difference in the hybridisation profiles of the plasmid-containing and plasmidless strains (Fig. 1a and b). This indicates that the overall rsDNA fraction is not influenced by the presence of plasmid sequences to any great extent. This would be expected, because SCP 1 and SCP 2 are low-copy-number plasmids (Kirby and Hopwood 1975; Bibb et al. 1977; J. Westpheling, personal communication). The genetic markers and genealogies of the four strains of *S. coelicolor* used here differ greatly. Our results indicate that most mutations do not affect the gross profile of the rsDNA within the genome. The other strains used here do not contain any high-copy-number plasmids (Kirby et al. 1982).

The *S. coelicolor* and *S. lividans* rsDNA profiles show few differences (Fig. 1a,b and c). These two species have distinct secondary metabolic pathways and produce different antibiotics. Thus, no direct correlation can be seen between the gross rsDNA profile and the functioning of secondary metabolism. However, where a close genetic relationship exists (these two species can exchange plasmids by conjugation), a similar rsDNA profile occurs.

S. coelicolor and *S. lividans* rsDNAs are made up of a number of distinct %G + C classes. The heterologous probe showed a single difference between these species; the *S. lividans* lacks class A of *S. coelicolor*. Three taxonomically divergent bacterial species, *E. coli*, *S. coelicolor*, and *S. olivaceus*, shared only very limited inter-specific homology when probed with labelled *S. olivaceus* rsDNA (Fig. 2a, b and c). Four classes of rsDNA were identifiable in *S. olivaceus* DNA (Fig. 2c) and three classes were identified in *S. cattleya* DNA (Fig. 2d).

Figure 3 shows the denaturation profiles in the

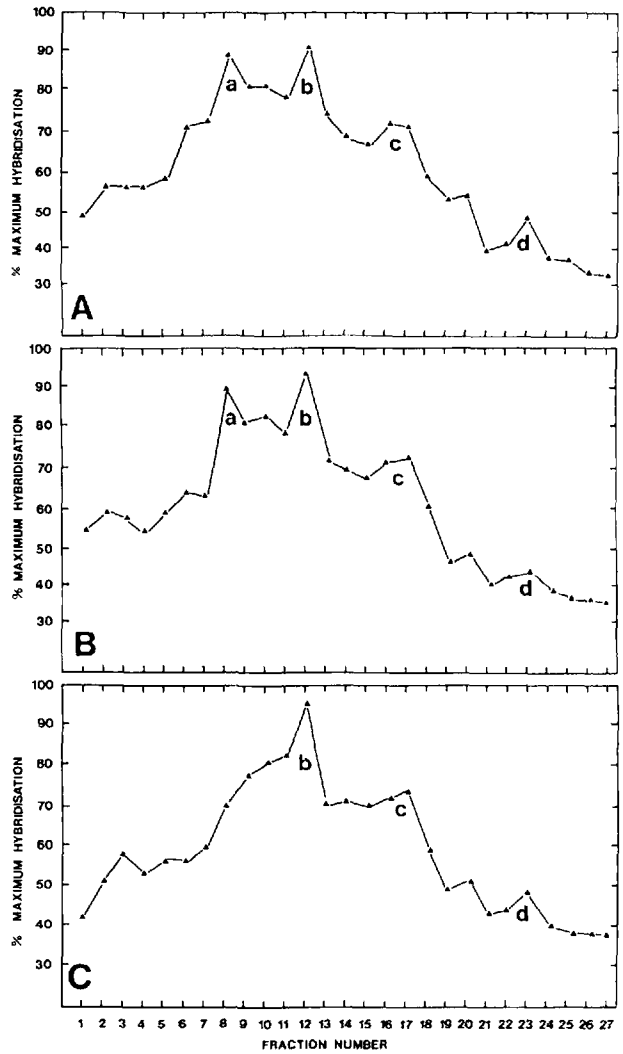

Fig. 1A-C. Hybridisation profiles of various *Streptomyces* DNAs probed with *S. coelicolor* M109 ^{32}P-labelled repeated sequences. The total cellular DNA restriction fragments were separated on a one-dimensional denaturing gel as described in the text. **A** *S. coelicolor* M109; **B** *S. coelicolor* M130; **C** *S. lividans*

presence of $(Et)_4NCl$ of the rsDNA from *S. olivaceus*, *S. cattleya* and *E. coli* after rehybridisation to a C_0t of 0.5. Heterogeneity of the rsDNA was found in both *S. olivaceus* and *S. cattleya*, confirming that the rsDNAs are made up of a number of classes.

Classes of sequences in *S. cattleya* DNA were found to denature at temperatures distinct from the main Tm in the absence of $(Et)_4NCl$. This is possibly due to blocks of DNA sequences with different %G + C contents (Fig. 4a). Similar results were obtained with *S. bikiniensis* (Fig. 4b). In both species, between 5% and 10% of the DNA was of a %G + C lower than was indicated by the main Tm.

The analytical density gradient centrifugation

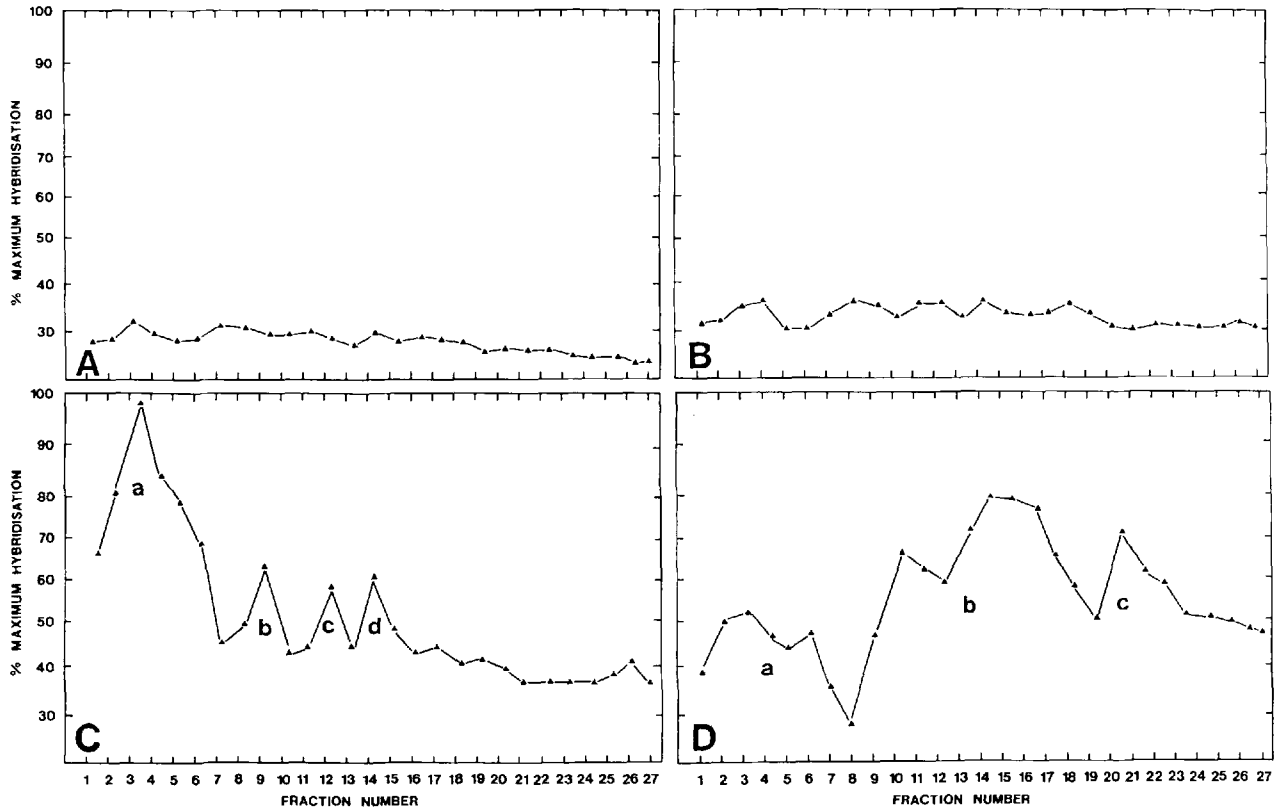

Fig. 2A-D. Hybridisation profiles of **A** *E. coli*; **B** *S. coelicolor*; and **C** *S. olivaceus* total cellular DNA probed with ^{32}P-labelled *S. olivaceus* repeated sequences. **D** *S. cattleya* total cellular DNA probed with ^{32}P-labelled *S. cattleya* repeated sequences

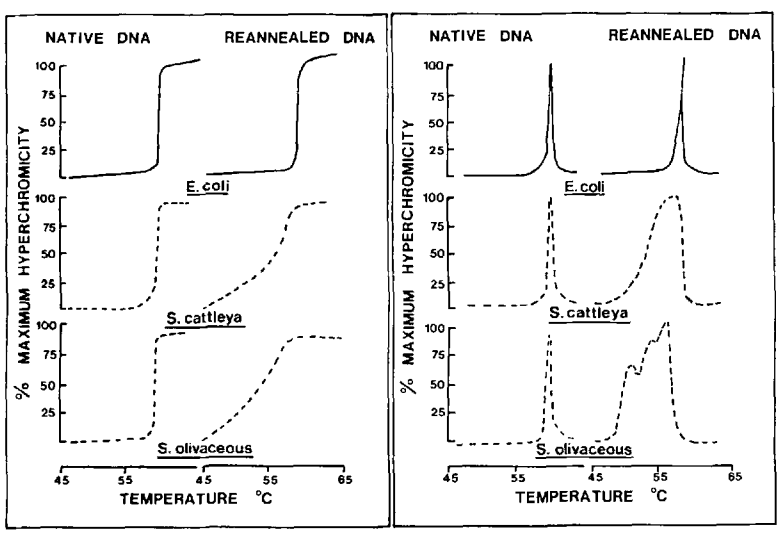

Fig. 3. Denaturation profiles of *S. olivaceus*, *S. cattleya* and *E. coli* rsDNA in the presence of $(Et)_4NCl$. *Left:* Initial profiles. *Right:* First-derivative profiles

confirmed the presence of a low-%G + C DNA band in *S. cattleya* and *S. olivaceus*. These satellite bands were sensitive to deoxyribonuclease but insensitive to ribonuclease. No such satellite band was detected in *S. coelicolor*. Neither were very low %G + C regions detected on high-resolution denaturation (Fig. 4c) or on denaturation gradient gels in this species (see previous results).

Discussion

Repeated DNA sequences are unusual phenomena in procaryotes. There is only one other case in which a significant proportion of rsDNA has been found (Archaebacteria; Sapienza et al. 1982). We have shown that rsDNA is found in a significant quantity (4%–10% of the genome) in 13 out of 14 *Strepto-*

myces species examined. The presence of rsDNA appears to be widespread in the Streptomycetales. In all cases reported here, the rsDNA sequences were detected in wild type strains. This study is unlike those of Robinson et al. (1981), who showed that after protoplast fusion between two *Streptomyces* species reiteration of a specific DNA fragment occurred to a final copy number of ~500, and Ono et al. (1982), who showed a similar amplification in a strain of *Streptomyces* after ethidium bromide treatment.

The available data (Fasman 1976; R. Kirby and K. Usdin, unpublished results) show a regular discrepancy between the %G + C values obtained by chemical analysis and buoyant density measurements and those obtained from the Tm of the DNA. The latter are consistently higher than the former. This discrepancy can be accounted for by low-%G + C sequences. Both the buoyant density method (using high-molecular-weight DNA) and the chemical method only provide information about the average %G + C of the DNA.

Detailed study of the rsDNA demonstrated a number of interesting points. The majority (±70%) of the rsDNA of *S. coelicolor* was shown by denaturation gradient electrophoresis to have a %G + C close to that of the main chromosomal DNA. This agrees with the results of Antonov et al. (1977). There does appear to be a small proportion (17.5%) of low-%G + C and high-%G + C (5.1%) rsDNA present too. The electrophoresis system used gives a distinct separation of the DNA fragments by %G + C with high reproducibility. Estimates of the %G + C are approximate, as sequence-specific effects can alter the results (Fischer and Lerman 1979). In contrast, the majority of the *S. olivaceus* rsDNA is of low %G + C (69%). A similar situation is found with *S. cattleya* rsDNA. The distinct difference between *S. olivaceus* and *S. coelicolor* rsDNA is confirmed by the limited interspecific hybridisation.

Low-%G + C sequences of a type which could be equivalent to the low-%G + C rsDNA were detected in the chromosomal DNA of *S. cattleya* and *S. bikinensis* using high-resolution thermal denaturation. They were also detected in *S. olivaceus* and *S. cattleya* using analytical density gradient centrifugation. These two methods failed to reveal a satellite band in *S. coelicolor*, thus confirming the results of Twefik and Bradley (1967); no previous experimental evidence is available for satellite bands in *S. olivaceus, S. cattleya* and *S. bikiniensis.*

As with eucaryotic rsDNA, the evolutionary importance and the molecular function (if any) of *Streptomyces* rsDNA is not clear. Streptomycetes are known to show genetic instability that in many cases does not involve plasmids (Freeman and Hopwood 1978; Kirby and Lewis 1981). The rsDNA

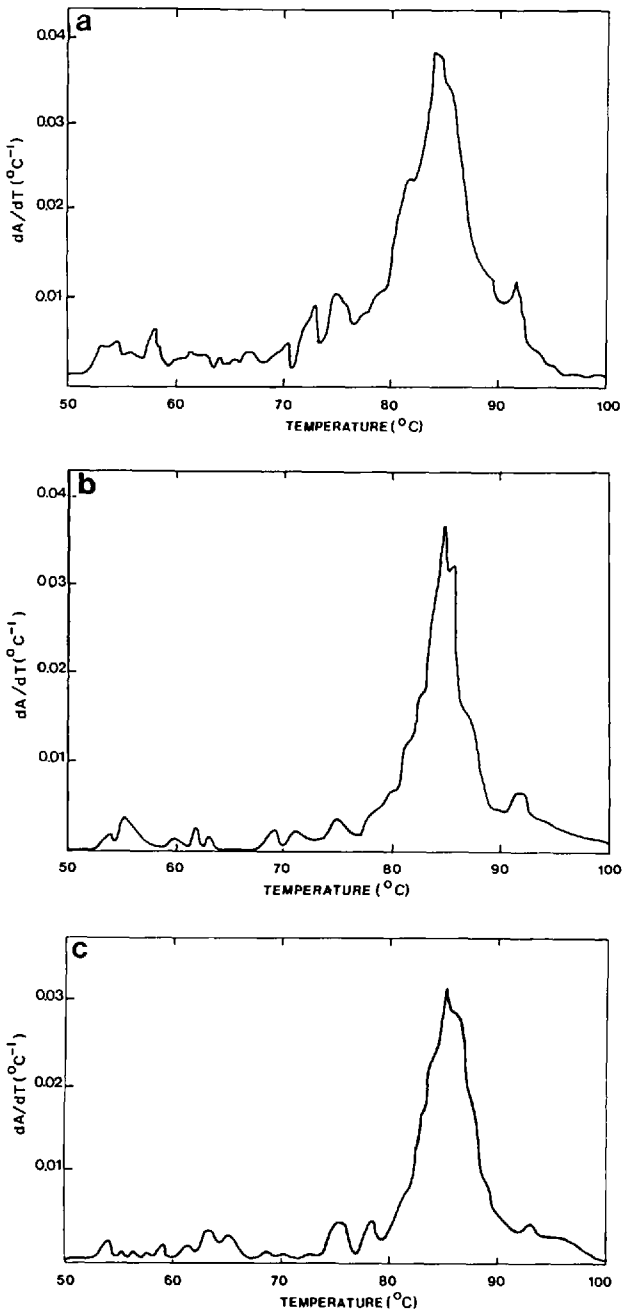

Fig. 4. High-resolution denaturation profiles of **a** *S. cattleya*; **b** *S. bikiniensis*; and **c** *S. coelicolor* total cellular DNA in 0.1 × SSC

sequences may act by virtue of their sequence homology as "hot spots" for either legitimate or illegitimate recombination events. The amplifications and deletions described by Schrempf (1983) could have resulted from such events. Another possibility is that the rsDNA is involved in the organisation and packaging of the large *Streptomyces* genome, which is three to four times the size of that of *E. coli* (Begnini et al. 1975). *S. rimosus* does not have recombinationally long but genetically non-functional silent regions in its genetic map, unlike *S.*

coelicolor (Alacèvii 1975). A role for the rsDNA as intergenic spacers is thus also possible. The Streptomycetes are amongst the most complex procaryotes and undergo a diverse series of irreversible morphological and secondary metabolic changes during their developmental cycle. Repeated DNA could be involved in the control of this process. The presence of a significant proportion of rsDNA in such a procaryote group could facilitate its use as a model system in the study of the role and evolution of rsDNA.

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