SHORT COMMUNICATION

The Loss of a Large DNA Fragment is Associated with an Aerial Mycelium Negative (Amy⁻) Phenotype of *Streptomyces cattleya*

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Hybridization of various *Streptomyces cattleya* aerial mycelium negative (Amy⁻) mutants with a probe containing the gene for argininosuccinate synthetase (pTG17) has revealed the presence of two different types of mutants (stable and unstable). Stable mutants appear to have lost all or part of the region covered by the probe, while the unstable mutants demonstrate no detectable changes in this region. In one group of stable mutants (those demonstrating a partial loss of sequences hybridizing to the probe), a 4.17 kb extrachromosomal element was detected, which hybridized with the pTG17 probe. The significance of this finding is discussed with reference to the genetic instability of the genus *Streptomyces*.

INTRODUCTION

The frequent simultaneous loss of the Arg⁺ character (argininosuccinate synthetase activity) and the Amy⁺ character (ability to produce aerial mycelia) has been reported for a number of different *Streptomyces* species (Redshaw *et al.*, 1979; Nakano *et al.*, 1980). In *Streptomyces cattleya* a number of Amy⁻ variants arise spontaneously at a high frequency (0.5%) and this frequency can be enhanced by treatment with various DNA damaging agents (Coyne *et al.*, 1984). The molecular basis for this phenomenon is unknown but plasmid loss, transposition, deletion and/or gene inactivation have all been suggested as possible causes.

METHODS

Strains. *S. cattleya* NRRL 8057 was obtained from Glaxo Group Research, London, UK. Plasmid pTG17 containing the argininosuccinate synthetase gene from *S. cattleya* cloned into pBR322, and a partial map of the insert, were a kind gift from Merck, Sharpe and Dohme, Rahway, NJ, USA. The Amy⁻ Arg⁻ variants were isolated after UV irradiation (254 nm) at 100 J m⁻².

DNA extraction. Total cell DNA was isolated from the *S. cattleya* strains as described previously (Usdin *et al.*, 1984). Plasmid pTG17 was isolated by the method of Ish-Horowitz & Burke (1981).

Electrophoresis. Total cell DNA (1 µg) from each strain was digested with a threefold excess of restriction enzyme and resolved on a 0.8% Tris/borate agarose gel at 2 V cm⁻¹ for 16 h.

DNA transfer and hybridization. The DNA was transferred to nitrocellulose (Schleicher & Schüll, 0.45 µm Ba85) by the method of Southern (1975), prehybridized and hybridized in 6 × SSC (1 × SSC = 0.15 M-NaCl, 0.015 M-sodium citrate) at 68 °C. pTG17 labelled with ³²P by nick translation was used as a probe (specific activity = 5 × 10⁷ c.p.m. µg⁻¹). The filter was then washed with 0.1 × SSC four times at 68 °C for 1 h and autoradiographed at -70 °C using Cronex 4 film and a Fuji X-ray intensifying screen.

Colony hybridization. Amy⁻ clones were grown on Malt 3 agar (Kirby *et al.*, 1982) at 30 °C for 2 d in a grid pattern before transfer to nitrocellulose filters placed on a fresh Malt 3 plate. The colonies were allowed to grow up on the filters for 2 d at 30 °C. Colony hybridization was carried out using a modification of the method of Schrempf (1982). The filters were placed on filter paper saturated with 10 mM-Tris/HCl (pH 8.0), 25% sucrose and 4 mg lysozyme ml⁻¹ and incubated at 30 °C for 1 h. The filters were then transferred to filter paper saturated with 1% NaOH, 1% SDS and left for 1 h at 30 °C. They were then neutralized with 1 M-Tris/HCl (pH 7.5), 1.5 M-NaCl with three changes of buffer, briefly laid on filter paper saturated with 96% ethanol, air dried and baked under vacuum for 2 h at 80 °C. Hybridization, washing and autoradiography were done as described above.

Fig. 1. Autoradiogram of the DNA of *S. cattleya* wild-type and three Arg⁻ Amy⁻ variants hybridized with ³²P-labelled pTG17. Lane A, λ DNA digested with HindIII. Lanes B–F, *S. cattleya* DNA digested with SalGI: lane B, wild-type; lane C, stable Amy⁻ type I; lane D, stable Amy⁻ type II; lane E, unstable Amy⁻; lane F, stable Amy⁻ type II stained with ethidium bromide. Lane G, undigested DNA from a stable Amy⁻ type II mutant.

RESULTS AND DISCUSSION

In a number of *Streptomyces* species the instability of certain genes has been found to be associated with an amplification of specific DNA sequences (Fishman & Hershberger, 1983; Schrempf, 1983). We have never detected any such amplification in the *S. cattleya* Amy⁻ clones we have studied. The restriction enzyme banding patterns of chromosomal DNA also showed no apparent changes in any of the variants tested.

Two classes of Amy⁻ strains can be distinguished in *S. cattleya*. The majority (94.7%) are stable while the remainder have a high reversion frequency. The majority (48/50) of the stable Amy⁻ clones examined (type I) showed the complete absence of the sequences contained in the probe, having lost both the 6.67 kb and the 1.8 kb SalGI hybridizing bands found in the wild-type (Fig. 1, lanes B and C). Two variants (type II), however, still possessed some of these sequences hybridizing to a single SalGI fragment of 4.17 kb (Fig. 1, lane D). This DNA has an extrachromosomal location as it migrated rapidly in 0.8% agarose gels far ahead of the undigested chromosomal DNA (Fig. 1, lane G). The position of the fastest migrating band in lane G is somewhat higher than would be expected from a monomeric covalently closed circular form of the linear molecule seen in lane D, and the band could be made up of dimers. Although not visible on the gel with ethidium bromide staining (Fig. 1, lane F) or on CsCl/ethidium bromide gradients, covalently closed circular DNA could be observed under the electron microscope. This 4.17 kb extrachromosomal element has single restriction enzyme sites for SalGI, PvuI, SstII and SphI. No extrachromosomal element could be found in the DNA from the wild-type strain when hybridized with pTG17. Hybridization was limited to the high molecular weight region of the gel corresponding to the chromosomal DNA (data not shown).

All 96 unstable Amy⁻ strains retained at least part of the sequences contained in pTG17 as detected by colony hybridization. No difference from the wild-type in hybridization profile could be detected in the ten unstable strains that were tested by Southern hybridization (e.g. Fig. 1, lane E).

It is feasible that the total absence of the 5.6 kb sequence contained in pTG17 in some of these strains could be due to plasmid loss, although a plasmid has yet to be found in this species (Kirby *et al.*, 1982). Extrachromosomal DNA observed in the type II mutants could be the result of a

deletional event on such a plasmid, or be the product of the deletion and excision of hybridizing sequences from the chromosome. The results obtained with the unstable variants suggest that either a much smaller alteration is involved in these cases or, as seems more likely, the alteration that takes place is reversible. The fact that the stable Amy⁻ variants are associated with the loss of at least part of the *S. cattleya* sequences contained in pTG17, while the unstable ones retain these sequences, would suggest that at least two different molecular events are occurring.

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