Communications to the editor

POSSIBLE PLASMID CONTROL OF SPORULATION, SOLUBLE PIGMENT PRODUCTION AND TETRACYCLINE RESISTANCE IN A WILD *STREPTOMYCES* SP. STRAIN

Sir:

Since OKANISHI *et al.*[1] stated that events such as sporulation, melanin and antibiotic production in actinomycetes might be controlled by plasmids, an increasing amount of experimental data supporting this hypothesis has accumulated.[2,3] Besides a better understanding of the regulation of metabolism[3], plasmids have gained increasing interest in view of the possibility of applying recombinant DNA techniques to industrial strain improvement[4]. In this respect, actinomycete plasmids carrying antibiotic resistance genes would be of particular interest, but only little is known about the natural occurring resistance of actinomycete species against different antibiotics and whether this resistance is due to the presence of R-factors, as in other bacteria[5]. Plasmid determined resistance of a producing organism to its own antibiotic is well documented in *Streptomyces coelicolor* A3(2)[6]. In *Streptomyces rimosus* LST-118, a plasmid seems to be involved in oxytetracycline resistance, since this strain gives rise spontaneously and, at an increased frequency after acridine dye treatment, to oxytetracycline-sensitive mutants[7].

We report here on the isolation of plasmid DNA from a strain derived from a soil isolate of *Streptomyces* sp. strain and its possible relationship to soluble pigment production, sporulation and tetracycline resistance.

From the original soil isolate, which produces an as yet unidentified antibiotic active against Gram-positive bacteria, a series of strains were derived, differing in sporulation ability, soluble pigment production and antibiotic production. Strains were routinely grown on medium S[8]. One of these strains, strain A16, characterized by white spores (Sp^w), soluble pigment production ($S.P.^+$), tetracycline resistance (Tc^r) and lack of antibiotic production (A^-), gives rise spontaneously, at high frequency (2.5%), to sporulation deficient colonies or sectors, visible as poorly or nonsporulated sectors on white sporulated colonies (Fig. 1). The frequency of sporulation deficient colonies is increased approximately twofold when strain A16 spores are grown on medium S containing 17 μg acridine orange/ml. Upon repeated subculturing, these colonies remain Sp^-, $S.P.^-$ and no revertants to Sp^w, $S.P.^+$ have been detected. Tetracycline resistance was tested on lawns derived from dense suspensions of A16 spores and from vibrated mycelium of unsporulated colonies. Three hours after plating, agar plugs from a tetracycline producing *Streptomyces aureofaciens* strain were transferred on to these lawns. The inhibition zones were determined after a 48 hours incubation period at 28°C. Lawns of strain A16 exhibit no inhibition zones. Lawns derived from Sp^-, $S.P.^-$ colonies, however are tetracycline sensitive, exhibiting inhibition zones with diameters between 12~16 mm. If suspensions of Sp^w, $S.P.^+$, Tc^r spores and vibrated mycelium of Sp^-, $S.P.^-$, Tc^s colonies are mixed in a 1:3 ratio respectively and plated, the resulting colonies are of the A16 phenotype, showing on subculture the same segregation pattern as strain A16. Since no growth inhibition between strain A16 and the non-sporulating variant was detected when plugs from one strain were tested on lawns

Fig. 1. Colonies of white sporulating strain A16, producing nonsporulated colonies and nonsporulating sectors.

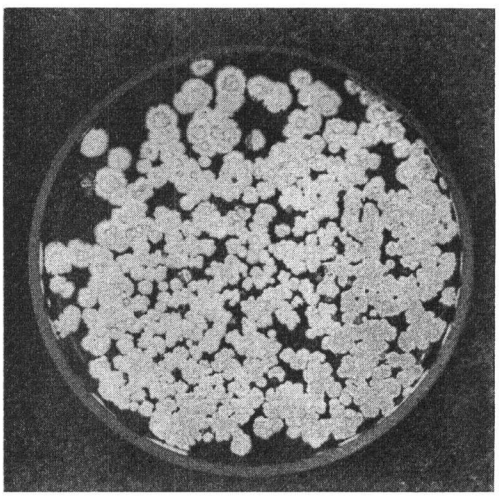
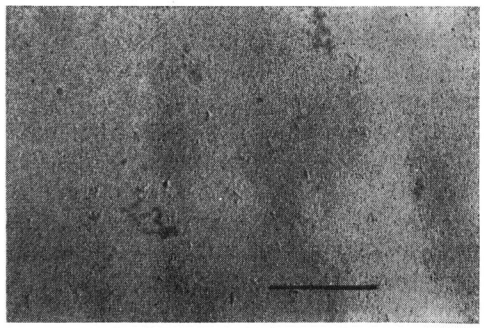
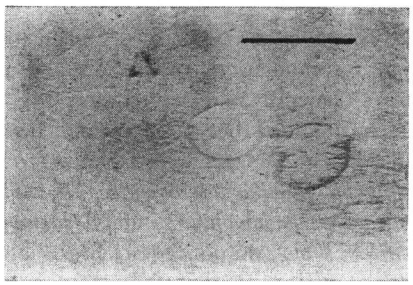


Fig. 2. Electron micrographs of DNA molecules isolated from cleared lysates of strain A16 on CsCl gradients. (Bar represents 1 μm.)

(a) Covalently closed circular form.



(b) Open circular form.



derived from the other, this finding suggests an infectious transfer of sporulation ability, soluble pigment production and tetracycline resistance.

From cleared lysates of A16 mycelium, a dense DNA band could be isolated on CsCl-ethidium bromide gradients, essentially by the methods of SCHREMPF *et al.*[9] and BIBB *et al*[10]. Electron microscopy[11] of this DNA revealed the presence of plasmid DNA molecules in the covalent closed circular and open circular forms (Fig. 2). Contour length measurements indicate a molecular weight of approximately 5.2×10^6 daltons. This plasmid DNA is missing in cleared lysates of the Sp^-, S.P.$^-$, Tc^S, A^- colonies derived from A16.

Our data strongly suggest the implication of a self-transmissible plasmid in the control of sporulation, soluble pigment production and tetracycline resistance in this particular strain. Whether one or all of these features are coded for by the isolated plasmid DNA is not clear yet.

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