NOTE

Genetic Relatedness, Among Streptomycetes Producing Tetracycline Antibiotics, Studied by Means of Deoxyribonucleic Acid Association

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The genetic relatedness among streptomycetes capable of producing tetracycline, chlortetracycline, and oxytetracycline was studied by using deoxyribonucleic acid (DNA) association techniques. Reciprocal DNA association experiments yielded genetic similarity values ranging from 75 to 97% among *Streptomyces aureofaciens* ATCC 10762, *S. aureofaciens* ATCC 13911, *S. lusitanus* CBS 101A, and *S. lusitanus* var. *tetracyclini* 106-T ATCC 15843. These four strains should thus be considered members of the same species on the basis of genetic relatedness. On the bases of DNA association experiments, the degree of nucleotide similarity of morphologically heterogeneous strains with *S. rimosus* ATCC-10970 was 28 to 33%.

The deoxyribonucleic acid (DNA)-DNA association technique has proved to be extremely useful for taxonomic studies. The genetic homology among actinomycetes has been extensively studied by Tewfik and Bradley (9), Monson et al. (5), Farina and Bradley (3), and Bradley (1). A method for the determination of DNA homologies of streptomycetes has been described by Okanishi and Gregory (7), whereas Okanishi et al. (6) evaluated taxonomic criteria in streptomycetes on the basis of DNA homologies. Furthermore, Toyama et al. (10) investigated genetic relatedness of 26 species of whorl-forming streptomycetes.

The present note deals with the nucleotide similarity of *Streptomyces aureofaciens* ATCC 10762 and some related strains, and further among wild-type and variant strains.

The strains listed in Table 1 are capable of producing antibiotics with Cl⁻ present in the medium.

Escherichia coli was used as a control organism. The streptomycetes were cultured with shaking at 27°C for 72 h in a medium consisting of 1.0% glucose, 0.5% peptone, 0.5% meat extract, and 0.5% NaCl. The final pH was adjusted before autoclaving to 7.2 to 7.4. *E. coli* was cultured with shaking at 37°C for 48 h in a medium consisting of 0.5% peptone, 0.5% meat extract, and 0.5% NaCl, pH 7.2 to 7.4. For

radioactive labeling of DNA, the strains were grown in the minimal medium described by Okanishi and Gregory (7). The DNA samples were prepared by the phenol procedure (8). Such DNA preparations were found to contain less than 2% ribonucleic acid (RNA) (11) and 8% protein (4). The specific activity of the labeled DNA preparations was 3,000 to 5,000 cpm per μg as determined by liquid scintillation counting. The DNA-DNA association method was that described by Denhardt (2), carried out on a small scale. About 5 μg of unlabeled, denatured DNA was immobilized on a small, membrane filter (Sartorius, 5.5-mm diameter, 0.45- μm pore size). The immobilized DNA on the membrane filter (IM-DNA) was preincubated for 6 h at 75°C in 0.2 ml of 6 $\times$ SSC (SSC, 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and twice-diluted PM as described by Denhardt (2). After the preincubation, the DNA-DNA association reaction was done for 20 h at 75°C in small test tubes containing the IM-DNA and 0.25 μg of sheared-denatured DNA (SD-DNA) in 0.2 ml of 6 $\times$ SSC and 0.003 M MgCl₂. The SD-DNA was prepared from ³H-labeled DNA by sonic extraction and Sephadex G-200 gel filtration. For the present study, the adequacy of the association method was established by studies on the effects of temperature, of chain length on the association reaction, and the thermal stability of the associated DNA. The results demonstrated that SD-DNA, having about 300 nucleotide units and incubated at 75°C, gave the most reliable and reproducible data.

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TABLE 1. *Cultures investigated and their origin*

Organism	Antibiotic produced	Source ^a
<i>Streptomyces aureofaciens</i> ATCC 10762	Chlortetracycline (with chlorine added); tetracycline (without chlorine)	Pridham
<i>S. aureofaciens</i> ATCC 13911	Tetracycline (with chlorine added)	ATCC
<i>S. lusitanus</i> CBS 101A	Chlortetracycline (with chlorine added); tetracycline (without chlorine)	ACC
<i>S. lusitanus</i> var. <i>tetracyclini</i> 106-T ATCC 15843	Tetracycline (with chlorine added)	ATCC
<i>S. rimosus</i> ATCC 10970	Oxytetracycline	ATCC
<i>Escherichia coli</i>		IFM

^a Pridham, Thomas G. Pridham, N. Reg. Lab., U. S. Department of Agriculture, Peoria, Ill.; ATCC, American Type Culture Collection, Rockville, Md.; ACC, American Cyanamid Co., New York; IFM, Institute of Food Microbiology, Chiba University, Japan.

TABLE 2. *Reciprocal DNA association assays of DNA samples from selected strains of antibiotic-producing streptomycetes and Escherichia coli*

Source of IM-DNA ^a	Nucleotide similarity value (%)			
	<i>Streptomyces aureofaciens</i> ATCC 10762 ^b	<i>S. aureofaciens</i> ATCC 13911	<i>S. lusitanus</i> CBS 101A	<i>S. lusitanus</i> var. <i>tetracyclini</i> 106-T ATCC 15843
<i>Streptomyces aureofaciens</i> ATCC 10762	100	75	89	77
<i>S. aureofaciens</i> ATCC 13911	75	100	82	88
<i>S. lusitanus</i> CBS 101A	87	85	100	89
<i>S. lusitanus</i> var. <i>tetracyclini</i> 106-T ATCC 15843	80	80	97	100
<i>S. rimosus</i> ATCC 10970	33	30	32	28
<i>Escherichia coli</i>	2	2	1	3

^a IM-DNA, Immobilized DNA on membrane filter.

^b Source of reference DNA (sheared-denatured DNA).

Table 2 represents the nucleotide similarity among DNA samples isolated from selected strains of antibiotic-producing streptomycetes. The reciprocal DNA association among morphologically homogeneous strains (*S. aureofaciens* ATCC 10762, *S. aureofaciens* ATCC 13911, *S. lusitanus* CBS 101A, and *S. lusitanus* var. *tetracyclini* 106-T ATCC 15843) gave from 75 to 97% nucleotide similarity values. The morphologically different strain, *S. rimosus* ATCC 10970, associated with the above strains from 28 to 33%. These results provide evidence that the former four strains indeed are members of the same species and that *S. rimosus* ATCC 10970 is appreciably different on the basis of genetic relatedness. Nucleotide dissimilarity of *S. aureofaciens* ATCC 10762 and *S. rimosus* ATCC 10970 was reported earlier by Tewfik and Bradley (9).

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