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NOTES

Estimate of the Genome Size by Renaturation Studies in *Streptomyces*

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The genome sizes of *Streptomyces coelicolor* and *Streptomyces rimosus* as calculated by deoxyribonucleic acid reassociation kinetics are approximately 10.5×10^6 nucleotide pairs.

Genome sizes have been estimated for numerous bacterial species (2, 3, 12) but not for representative species of the genus *Streptomyces*. Certain species of *Streptomyces* are genetically the best explored of prokaryotes after the *Enterobacteriaceae* (8). We have considered in the present study two genetically well-studied species, *Streptomyces coelicolor* A3 (2) and *Streptomyces rimosus* ATCC 10970 R7 (6, 8). Their genetic maps are known to be quite comparable except for the occurrence of two "empty" regions in *S. coelicolor* that are apparently missing from *S. rimosus* (1).

The genome size was calculated by the application of the technique of renaturation of single-stranded deoxyribonucleic acid (DNA) (16). This process follows second-order reaction kinetics. Genome size corresponding to a simple nonrepetitive DNA can be calculated from the second-order reaction rate constant (K_2) and the molecular weight of the renaturing single-stranded DNA, as deduced from the sedimentation coefficient (s). The formula for calculation of genome size (G_s) was derived by Bak et al. (2) as

$$G_s = \frac{k \cdot s^{0.911}}{K_2}$$

where $k = 8.83 \times 10^8$, s is the sedimentation coefficient of single-stranded DNA measured at pH 7 (14), and K_2 is calculated in $l \cdot mol^{-1} \cdot s^{-1}$. Lyophilized logarithimic phase mycelium from submerged cultures was lysed enzymatically with glusulase (Endo Laboratories, Inc.). Lyophilized spores were broken mechanically in a mortar by using silica powder.

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The DNA was purified according to Marmur's method (10). *Escherichia coli* K-12 DNA was obtained by the same procedure with the exception of the use of lysozyme to lyse the cells.

The DNA preparations were checked by the extinction ratio E_{260}/E_{280} (1.8 to 2.0) and thermal denaturation in SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7) (2).

The DNA (10 to 20 μg/ml) was denatured and degraded by boiling at 100 C fo 30 min in $0.1 \times$ SSC and then immediately transferred in a thermostatically controlled chamber (T_m, 25 C) of a Beckman-Acta III spectrophotometer. The T_m values in 1 M Na^+ were calculated for all the DNA samples from the formula of Bak et al. (2). The sedimentation coefficient values were obtained in a Spinco-Beckman E ultracentrifuge in 1 M NaCl at pH 7.0.

The K_2 values were calculated in 1 M Na^+ according to Wetmur and Davidson (16). For samples which do not give simple reaction kinetics, the K_2 values were calculated from the slope of the linear part of the renaturation curve relative to the bulk of DNA.

The mole percent guanine plus cytosine content (mol% G+C) of the DNA samples was estimated according to the equation $d = 1.660 + 0.098$ (mol% G+C), where d = buoyant density (13) (Table 1). These values were in fairly good agreement with similar estimations of G+C content in other *Streptomyces* species (5, 11, 15).

The genome sizes of *S. coelicolor* and *S. rimosus* turned out to be 7.09×10^9 and 6.77×10^9 daltons, respectively, for the DNA samples extracted from mycelia, and 7.23×10^9 and 6.33×10^9 daltons for the DNA samples extracted from spores. No significant difference has been found between the results obtained for spores

TABLE 1. *Some physicochemical parameters of DNA in Streptomyces*[a]

Species	No. of determinations	Mol% G+C[b]	K_2 value ($l \cdot mol^{-1} \cdot s^{-1}$)	Genome size[c]		
				Dalton ($\times 10^9$)	Pairs of nucleotides ($\times 10^6$)	Extended DNA[d] (mm)
S. coelicolor						
Spore	6	72.5	1.72 ± 0.15	7.23 ± 0.51	11.0 ± 0.7	3.7 ± 0.2
Mycelium	5	73.0	1.75 ± 0.19	7.09 ± 0.73	10.8 ± 1.0	3.6 ± 0.3
S. rimosus						
Spore	5	71.4	1.90 ± 0.17	6.33 ± 0.30	9.7 ± 0.4	3.2 ± 0.1
Mycelium	9	71.4	1.86 ± 0.16	6.77 ± 0.26	10.4 ± 0.4	3.5 ± 0.1
E. coli K-12	5	51.0	4.18 ± 0.35	2.28 ± 0.19	3.5 ± 0.3	1.2 ± 0.1

[a] In all calculations the $s_{20,w}^{pH\ 7}$ value used was 13.2 ± 0.5 corresponding to a molecular weight of 4.4×10^5.
[b] These values were calculated from buoyant density in CsCl as reported by Schildkraut et al. (13).
[c] The values of genome sizes of *Streptomyces* are corrected to account for the G+C effect according to J. C. Wetmur (Ph.D. thesis, California Institute of Technology, Pasadena, 1967).
[d] Pairs of nucleotides × 0.34 nm.

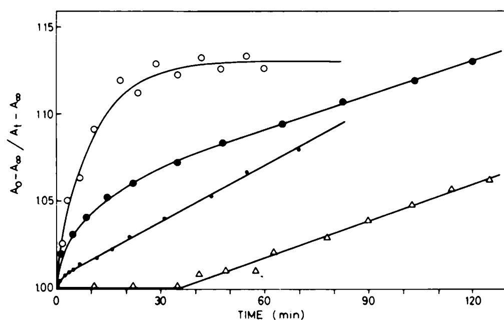

FIG. 1. *Typical curves of S. coelicolor and S. rimosus DNA. Symbols: O, S. coelicolor DNA in 60 mM Na⁺; ●, S. coelicolor DNA in 150 mM Na⁺; △, S. rimosus DNA in 150 mM Na⁺; and ●, E. coli K-12 DNA in 150 mM Na⁺. The data are plotted according to Wetmur and Davidson (16). A_0 and A_t are absorbance at 260 nm at time zero and time t; A is the absorbance of native DNA. The DNA concentrations used were 25 to 35 μg/ml. The curves of Streptomyces refer to DNA extracted from mycelia.*

and mycelia; moreover, the genome sizes calculated for the two *Streptomyces* species are superimposable within the standard error and are about three times larger than that of *E. coli* K-12 calculated in similar conditions (Table 1). Similar values for *E. coli* K-12 genome size have been obtained previously by other authors (2-4, 7, 16).

The renaturation curve of *S. coelicolor* DNA as compared with that of *S. rimosus* showed a biphasic appearance at Na^+ concentrations lower than 1 M. Best estimates were made between 60 to 150 mM Na^+ (Fig. 1). Here the renaturation reaction of *S. rimosus* DNA begins 35 min later than that of *S. coelicolor* DNA. In fact a small fraction of *S. coelicolor* DNA (about 3 to 5%) renaturates steeply, while the bulk renaturates at a slower rate. Such apparent heterogeneity was not observed in *S. rimosus* and in *E. coli*, whereas it was observed in *S. coelicolor* irrespective of the presence in the strain of the SCP 1 plasmid (sex factor) (8). It could indicate the occurrence in *S. coelicolor* of a small fraction of repeating base sequences, possibly corresponding to the so-called empty regions, i.e., map segments devoid of detectable genes. Repetition of short regions of genome is also possible, as Hsiang (9) has found for *E. coli*.

The lack of renaturation of *S. rimosus* DNA for 35 min is unexpected, and we cannot yet provide a good explanation for this phenomenon.

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