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 x 10⁶ nucleotide pairs.

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 E₂₆₀/E₃₂₈ (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 to 30 min in 0.1 x SSC and then immediately transferred in a thermostatically controlled chamber (Tₚ, 25 C) of a Beckman-Acta III spectrophotometer. The Tₚ 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 K₂ values were calculated in 1 M Na⁺ according to Wetmur and Davidson (16). For samples which do not give simple reaction kinetics, the K₂ 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 x 10⁶ and 6.77 x 10⁶ daltons, respectively, for the DNA samples extracted from mycelia, and 7.23 x 10⁶ and 6.33 x 10⁶ daltons for the DNA samples extracted from spores. No significant difference has been found between the results obtained for spores

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 (K₂) and the molecular weight of the renaturing single-stranded DNA, as deduced from the sedimentation coefficient (s). The formula for calculation of genome size (Gₛ) was derived by Bak et al. (2) as

\[ Gₛ = \frac{k \cdot s^{0.811}}{K₂} \]

where \( k = 8.83 \times 10^8 \) s is the sedimentation coefficient of single-stranded DNA measured at pH 7 (14), and \( K₂ \) is calculated in l·mol⁻¹·s⁻¹. Lyophilized logarithmic 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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Table 1. Some physicochemical parameters of DNA in Streptomyces*

| Species          | No. of determinations | Mol% G+C* | $K_v$ value (l·mol⁻¹·s⁻¹) | Genome size*  |
|------------------|-----------------------|-----------|--------------------------|---------------|
|                  |                       |           |                          | Dalton (× 10⁶) | Pairs of nucleotides (× 10⁶) | Extended DNA (nm) |
| 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        |

*In all calculations the $s_{DNA}^t$ value used was 13.2 ± 0.5 corresponding to a molecular weight of $4.4 \times 10^7$.

*These values were calculated from buoyant density in CsCl as reported by Schildkraut et al. (13).

*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).

*Pairs of nucleotides × 0.34 nm.

![Graph](image.png)

**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⁺; Δ, 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). $A_2$ and $A_4$ 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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