NOTES

Estimate of the Genome Size by Renaturation Studies in Streptomyces

ROMUALDO BENIGNI, PETER ANTONOV PETROV,¹ AND ANGELO CARERE*

Istituto Superiore di Sanita, Rome, Italy

Received for publication 1 April 1975

The genome sizes of Streptomyces coelicolor and Streptomyces rimosus as calculated by deoxyribonucleic acid reassociation kinetics are approximately 10.5 × 10⁴ 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 (Kₛ) 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

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

where \( k = 8.83 \times 10^4 \), \( s \) is the sedimentation coefficient of single-stranded DNA measured at pH 7 (14), and \( Kₛ \) is calculated in \( l \cdot mol^{-1} \cdot s^{-1} \). 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.

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 for 30 min in 0.1× 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 \( Kₛ \) values were calculated in 1 M Na⁺ according to Wetmur and Davidson (16). For samples which do not give simple reaction kinetics, the \( Kₛ \) 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 × 10⁴ and 6.77 × 10⁴ daltons, respectively, for the DNA samples extracted from mycelia, and 7.23 × 10⁴ and 6.33 × 10⁴ daltons for the DNA samples extracted from spores. No significant difference has been found between the results obtained for spores

¹Present address: Higher Medical Institute, Department of Physics and Biophysics, Sofia, Bulgaria.
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%) renatures steeply, while the bulk renatures 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.

We wish to thank G. Sermonti and C. Frontali for their continuous interest in the study, V. Angeli for measurements of the sedimentation coefficients, and E. Maroccia and G. Di Giuseppe for technical assistance. This investigation was partially supported by the Italian National Research Council.

**LITERATURE CITED**

1. Alacevic, M., M. Strasek-Vesilaj, and G. Sermonti. 1973. The circular linkage map of *Streptomyces rimosus*. J. Gen. Microbiol. 77:173-185.
2. Bak, A. L., C. Christiansen, and A. Stenderup. 1970. Bacterial genome sizes determined by DNA renaturation studies. J. Gen. Microbiol. 6A:377-380.
3. Cairns, J. 1963. The chromosome of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 28:43-46.
4. Cooper, S., and C. E. Helmstetter. 1968. Chromosome replication and the division cycle of *Escherichia coli*. Br. J. Mol. Biol. 31:519-540.
5. Enquist, L. W., and S. G. Bradley. 1971. Characterization of deoxyribonucleic acid from *Streptomyces venezuelae* spores. Dev. Ind. Microbiol. 12:225-236.
6. Friend, E. J., and D. A. Hopwood. 1971. The linkage of *Streptomyces rimosus*. J. Gen. Microbiol. 68:187-197.
7. Gillis, M., J. de Ley, and M. de Cleene. 1970. The determination of molecular weight of bacterial genome
DNA from renaturation rates. Eur. J. Biochem. 12:143-153.
8. Hopwood, D. A., K. F. Chater, J. E. Dowding, and A. Vivial. 1973. Advances in Streptomyces coelicolor genetics. Bacteriol. Rev. 37:371-405.
9. Hsiang, J. L. 1974. Isolation of a short cytosine-rich repeating unit from the DNA of Escherichia coli. Biochim. Biophys. Acta 349:13-22.
10. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208-218.
11. Monson, A. M., S. G. Bradley, L. W. Enquist, and G. Cruces. 1969. Genetic homologies among Streptomyces violaceoruber strains. J. Bacteriol. 99:702-706.
12. Shapiro, H. S. 1968. In H. A. Sober (ed.), Handbook of biochemistry, 2nd ed., p. H-80. Chemical Rubber Co., Cleveland, Ohio.
13. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base compositions of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. 4:430-443.
14. Studier, F. W. 1965. Sedimentation studies of the size and shape of DNA. J. Mol. Biol. 11:373-390.
15. Tewfik, E. M., and S. G. Bradley. 1967. Characterization of deoxyribonucleic acids from Streptomycetes and Nocardiae. J. Bacteriol. 94:1994-2000.
16. Wetmur, J. C., and N. Davidson. 1968. Kinetics of renaturation of DNA. J. Mol. Biol. 31:349-370.