Genome size of *Streptomyces*

(Deoxyribonucleic acid; *Streptomyces; Streptoverticillium*; genome size; renaturation rate constant; base ratio)

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1. SUMMARY

The genome sizes of 13 *Streptomyces* and 2 *Streptoverticillium* strains were determined by the initial renaturation rate method described by De Ley and Gillis. The estimated genome sizes are within the range of $3.69 \times 10^9$ Da to $5.15 \times 10^9$ Da.

2. INTRODUCTION

Little is known about the genome size of organisms belonging to the genus *Streptomyces*. These actinomycetes can be expected to have large genomes because of their morphological differentiation and physiological variation, and this has in fact been shown to be true for the *S. rimosus* [1] and *S. coelicolor* [2] strains examined by the methods of Wetmur and Davidson [3] and Britten and Kohne [4]. More recently, however, these two methods have been criticized by Gillis and De Ley [5] as giving too high values.

In the present study 13 *Streptomyces* and 2 *Streptoverticillium* strains were examined by the initial renaturation rate method described by Gillis and De Ley [6]. All the test strains were representatives of discrete clusters defined in an extensive numerical taxonomic survey on *Streptomyces* [7].

3. MATERIALS AND METHODS

3.1. Test strains and growth conditions

The test strains are listed in Table 1. The *Micrococcus* and *Pseudomonas* reference strains were grown in shake flasks (160 strokes per minute for 11 h) containing complex nutrient broth at 37°C, and the *Streptomyces* and *Streptoverticillium* strains in modified Sauton’s broth [8] at 30°C for 18 h. Biomass was collected at the late log phase by centrifugation, washed with 0.15 M NaCl + 0.1 M EDTA at pH 8.0, and stored at $-20$°C.

3.2. DNA preparation

DNA was isolated using the modification of the method of Saito and Miura [9] described by Mordarski et al [10]. The purity of DNA preparations was assessed on the basis of the absorbance at $E_{260/280}$ and $E_{230/255}$, and the reaction for protein with Folin reagent. The concentration of DNAs was determined by Burton’s chemical method [11,12] using deoxyribose standard solutions as reference.

3.3. Analysis of base compositions

The mol% of the guanine plus cytosine (G + C) was estimated on the basis of the thermal denaturation temperature ($T_m$) [13]. The mol% G + C was calculated from the equation of Mandel and Marmur [14]: mol% G + C = $(T_m - 53.9) \times 2.44$. 

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3.4. Shearing of DNA, analytical centrifugation

Sheared DNA was prepared by passing liquid DNA (0.1 SSC, \(E_{260} = 2.0\)) through a French pressure cell at 21000 lb/in\(^2\) as described by De Ley [6]. To control the fragment size of sheared DNA samples the sedimentation coefficients were determined by analytical ultracentrifugation (Beckman model E analytical ultracentrifuge fitted with an optical system [15,16]).

3.5. Measurement of DNA reassociation

The initial optical renaturation rate method of Gillis and De Ley was chosen [6,17]. The DNA (\(E_{260} = 2.0\), 0.1 SSC) was denatured by boiling at 100°C for 10 min. After adjusting the salt concentration to 2 SSC, the DNA samples were quickly transferred to thermostatically controlled cuvettes in the holder of a spectrophotometer (Beckman). The reassociation reaction was carried out at optimal renaturation temperature (\(T_{OR}\)) calculated from the equation:

\[
k = \frac{v}{c^2}
\]

where: \(v\) = renaturation rate, expressed as decrease of extinction/min, and \(c\) = concentration of DNA (as mM nucleotide pairs). (The DNA concentrations were based on estimates determined chemically.)

3.6. Estimation of the genome size

Genomic \(M_r\) values were calculated by reference to the "Micrococcus lysodeikticus" control of known genome size, \(1.86 \times 10^9\) [5], using the equation [6]:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>G + C (mol%)</th>
<th>Average renaturation rate constant (K \cdot 10^{-2})</th>
<th>S.D. ± %</th>
<th>Genome size (M_r) ((\cdot 10^9))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus lysodeikticus</td>
<td>ATCC4696</td>
<td>72.0</td>
<td>23.8</td>
<td>2.5</td>
<td>1.88 (1.86)*</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC27853</td>
<td>66.4</td>
<td>11.0</td>
<td>3.6</td>
<td>4.25 (4.20)</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>NCTC3756</td>
<td>62.0</td>
<td>13.9</td>
<td>2.9</td>
<td>3.48 (3.48)</td>
</tr>
<tr>
<td>/DNA E. coli B SIGMA/</td>
<td>ISP326</td>
<td>72.7</td>
<td>10.9</td>
<td>3.7</td>
<td>4.06</td>
</tr>
<tr>
<td>Streptomyces alboviridis</td>
<td>ISP313</td>
<td>74.9</td>
<td>10.1</td>
<td>3.0</td>
<td>4.38</td>
</tr>
<tr>
<td>S. albus</td>
<td>ISP447</td>
<td>73.8</td>
<td>10.1</td>
<td>2.0</td>
<td>4.38</td>
</tr>
<tr>
<td>S. almiridii</td>
<td>ISP186</td>
<td>75.9</td>
<td>10.4</td>
<td>2.9</td>
<td>4.26</td>
</tr>
<tr>
<td>S. coelicolor</td>
<td>ISP233</td>
<td>74.7</td>
<td>11.8</td>
<td>6.7</td>
<td>3.75</td>
</tr>
<tr>
<td>S. felleus</td>
<td>ISP130</td>
<td>74.9</td>
<td>8.6</td>
<td>3.5</td>
<td>5.15</td>
</tr>
<tr>
<td>S. flavoviricini</td>
<td>ISP152</td>
<td>74.1</td>
<td>11.7</td>
<td>7.7</td>
<td>3.78</td>
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<tr>
<td>S. floccus</td>
<td>ISP327</td>
<td>74.4</td>
<td>10.3</td>
<td>2.9</td>
<td>4.30</td>
</tr>
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<td>S. goshikiensis</td>
<td>ISP190</td>
<td>74.7</td>
<td>11.3</td>
<td>3.5</td>
<td>3.92</td>
</tr>
<tr>
<td>S. griseus</td>
<td>ISP236</td>
<td>75.4</td>
<td>11.3</td>
<td>9.7</td>
<td>3.92</td>
</tr>
<tr>
<td>S. lavendulae</td>
<td>ISP069</td>
<td>72.5</td>
<td>8.9</td>
<td>3.4</td>
<td>4.97</td>
</tr>
<tr>
<td>S. oitaceus</td>
<td>ISP072</td>
<td>73.4</td>
<td>11.0</td>
<td>8.2</td>
<td>4.02</td>
</tr>
<tr>
<td>S. polychomogenes</td>
<td>ISP316</td>
<td>74.3</td>
<td>9.2</td>
<td>2.1</td>
<td>4.81</td>
</tr>
<tr>
<td>Streptozetricollum cinnamoneum</td>
<td>ISP005</td>
<td>73.0</td>
<td>11.6</td>
<td>5.2</td>
<td>3.82</td>
</tr>
<tr>
<td>Streptozetricollum griseocarneum</td>
<td>ISP004</td>
<td>71.7</td>
<td>12.0</td>
<td>5.8</td>
<td>3.69</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>DNA base composition, renaturation rate constant and genome size of the control and tested strains</th>
</tr>
</thead>
</table>

* In parentheses data from literature [5,19].
where $K_A$, $K_B$, $M_A$ and $M_B$ represent the second-order renaturation rate constants and genome $M_r$ values of the control (A) and test organisms (B), respectively. The genome sizes of "M. lysodeikticus" and the other control organisms were calculated according to the equation [5]:

$$M = \frac{70.03 - 0.35 \cdot GC}{K} \cdot 10^7$$

4. RESULTS

4.1. Base composition

The DNA base composition of the Streptomyces strains ranged from 71 to 76% GC (Table 1). The base composition of the control strains were in line with previously published data [18].

4.2. Degradation of the DNA

Sedimentation coefficients were estimated for the DNA of 2 of the control strains Escherichia coli and "M. lysodeikticus" and for Streptoverticillium griseocarneum. The average $M_r$ of the sheared DNA was within the range given by De Ley [6,17].

4.3. Genome size

The genome size and the average renaturation rate constant of the test strains are shown in Table 1. The corresponding results for the control DNA from E. coli, "M. lysodeikticus" and the two Pseudomonas strains were compared with data from the literature [5,11,19] based on the same method. The genome size of the reference "M. lysodeikticus" strain was 1.88 x 10^9 Da which is in agreement with the result of a previous study [5]. The estimated genome sizes of the 13 Streptomyces and 2 Streptoverticillium strains ranged from 3.69 x 10^9 Da to 5.15 x 10^9 Da. The average standard deviation of the data was ±10%.

4. DISCUSSION

The genome sizes of the Streptomyces and Streptoverticillium strains were calculated by comparison with the M. lysodeikticus reference strain of known genome size and with a similar DNA base composition. The genome sizes of the "M. lysodeikticus" strain (1.88 x 10^9 Da) is in excellent agreement with the value recorded (1.86 x 10^9 Da) by Gillis and De Ley using the same method [5]. Similarly, values for the E. coli, Pseudomonas aeruginosa and P. fluorescens strains are in good agreement with the corresponding results of Gillis and De Ley [5,19].

The morphologically complex streptomyces and streptoverticillia have genome sizes about 1.5 times larger than that of E. coli. Genome sizes of this value are comparable with that of P. aeruginosa, which has one of the largest bacterial genomes described.

The recently published values [1,2] for the genome sizes of S. coelicolor and S. rimosus obtained using the method of Wetmur and Davidson were 7.09 x 10^9 Da and 6.77 x 10^9 Da, respectively, that is nearly 50% higher than the values revealed in the present experiments based on the method of Gillis and De Ley. Similarly, the corresponding values for the control DNAs used in the present experiments were lower than those based on the method of Wetmur and Davidson [20–23]. These differences are a reflection of the reproducibility and reliability of the methods used. According to Gillis and De Ley the method of Wetmur and Davidson is neither as reproducible nor reliable as the initial renaturation rate technique and this explains the higher values recorded for the former [5,6,19,20] and Mordarsky et al., unpubl.). The inaccuracies of the method of Wetmur and Davidson have already been discussed in detail [5].

Information on genome sizes may influence the results of nucleic acid hybridization experiments and may account for the relatively high degree of binding found between ribosomal RNA and DNA in preliminary homology experiments carried out on representative Streptomyces strains (Mordarsky et al., unpubl.). It is possible that in these organisms a repetition of a short genome region and a small fraction of repeating base sequences (empty regions) exist [2].

REFERENCES