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Reexamination of the Genome Size of Myxobacteria, Including the Use of a New Method for Genome Size Analysis

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The genome sizes of two myxobacteria, *Myxococcus xanthus* and *Stigmatella aurantiaca*, were measured by renaturation analysis and also by a new method involving the quantitation of individual restriction fragments. In contrast to several previous reports, which indicate that *M. xanthus* has a genome size which is three to four times that of *Escherichia coli*, the present measurements indicated that the *M. xanthus* genome is only about 24 to 53% larger than that of *E. coli*. *S. aurantiaca* had a genome size nearly identical to that of *M. xanthus*. Of possible significance is the fact that the renaturation curves of *M. xanthus* and *S. aurantiaca* deoxyribonucleic acid both exhibited significant fractions which renatured with rapid, unimolecular kinetics. However, we were unable to establish that these fractions represented inverted repeats or repetitive sequences.

Myxobacteria are unique among bacteria in having an ability for multicellular development. Their developmental cycle consists of the aggregation of many cells into spore-filled fruiting bodies, as much as several tenths of a millimeter in diameter, which in some species may attain a remarkable degree of morphological complexity (23).

Myxobacteria have a number of other peculiar properties. Various species have guanine-plus-cytosine (G+C) contents approaching 70%. They are motile by gliding rather than by swimming. Also, they secrete lytic enzymes to digest solid extracellular materials, including other bacteria, and have evolved cooperative patterns of behavior which, by allowing increased local concentrations of extracellular lytic and hydrolytic enzymes, increase the rate of release of nutrient materials, allowing for faster growth. Indeed, swarms of these bacteria acting in concert have been termed "wolf packs" (8).

A number of previous reports have indicated that *Myxococcus xanthus* has a genome size three to four times that of the *Escherichia coli* genome (9, 24), and this has led to speculations that an extremely large number of genes may be required for cellular differentiation and, in particular, the formation of fruiting bodies.

In this paper we present data showing that, contrary to previous reports, the genome sizes of two tested species of myxobacteria, *M. xanthus* and *Stigmatella aurantiaca*, appear to be only marginally greater than those of the more typical bacteria *E. coli* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Cultures of *M. xanthus* DZF1 and *S. aurantiaca* DW4 were grown in Casitone-yeast extract broth (5) at 30°C. *E. coli* W1485 and *P. aeruginosa* were grown in tryptone-yeast extract broth (L broth) (11) at 37°C. Cultures were grown on rotary shakers with vigorous aeration.

Preparation of DNA. DNA was extracted from stationary-phase cultures according to the following procedure, scaled up as necessary according to the actual volume of culture used. Cells harvested from 50 ml of stationary-phase culture were suspended in 1 ml of 25% sucrose in 50 mM Tris-hydrochloride (pH 8.0). Lysozyme (Worthington Biochemicals Corp.) was added to a final concentration of 3.3 mg/ml; after incubation at 37°C for 30 min, 0.4 ml of 0.25 M EDTA and 0.16 ml of 10% sodium dodecyl sulfate was added, and the mixture was swirled until clearing was observed. Pronase (Sigma Chemical Co.; type VI; 20 μl of a 10-mg/ml solution in 25 mM Tris-hydrochloride, pH 8.0, autodigested at 37°C for 1 h before use) was added, and the mixture was incubated at 37°C for 30 min. The lysate was diluted with 2 ml of 50 mM Tris-hydrochloride (pH 8.0) and then extracted twice with phenol and twice with chloroform-isoamyl alcohol (24:1, vol/vol). The DNA was precipitated with ethanol and redissolved in 2 ml of SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). To this was added 50 μl of RNase mix (2 mg of pancreatic RNase per ml [Worthington] plus 1,000 U of RNase T_1 per ml [Worthington] in 0.15 M NaCl; heat treated at 80°C for 15 min), and the mixture was incubated at 30°C for 1 h. To the mixture was then added 5 μl of Pronase solution; after a 1-h incubation, the DNA was again twice extracted with phenol and chloroform-isoamyl alcohol. After ethanol precipitation, the DNA pellet was dissolved in 0.01 M Tris-hydrochloride, pH 8.0,

plus 0.001 M EDTA.

Preparation of DNA fragments and determination of size. DNA fragments for C_0t analysis were prepared by dissolving the DNA in SSC and shearing by sonication (Branson Sonifier model W185 and microtip), using 10-s bursts and allowing 30 s of cooling in ice water between bursts to minimize thermal denaturation. The sheared fragments were precipitated from solution with 2 volumes of cold ethanol and pelleted at 10,000 rpm for 15 min in a Sorvall SS-34 rotor.

The sizes of the fragments were measured on 0.7 and 2.0% agarose gels, using the series of fragments generated from ϕX174 DNA by *Hae*III as a size standard.

Optical reassociation kinetics. Sheared DNA (500 to 1,100 base pairs [bp]) in 0.2 M sodium phosphate buffer (pH 6.8) containing 8 M urea was denatured for 10 min. The solution was then transferred into cuvettes (2-mm light path) equilibrated to the incubation temperature. A Gilford 240 spectrophotometer with a chart recorder was used to monitor the reassociation by following hypochromicity changes at 260 nm. After 10 to 15 min of monitoring the initial reassociation, silicone oil (Dow Corning 200) was overlaid to prevent evaporation. Use of urea in the reassociation buffer was necessitated by the high G+C content of *M. xanthus* DNA (68%) (13), *S. aurantiaca* DNA, and *P. aeruginosa* DNA (67%) (12).

By using the temperature-dependent absorbance at 260 nm of a standardized EDTA solution, we established that, under the conditions of the experiment, the temperature of solutions pipetted into the 2-mm-path-length cuvettes dropped from 100°C to within 10°C of the final incubation temperature during the first 10 s of incubation and to within 5°C of the final incubation temperature during the first 30 s of incubation. In all DNA solutions tested, a large hypochromic shift was observed during the first 30 s of incubation, which was presumably due to nonspecific base stacking effects as the DNA cooled to the incubation temperature (3). This collapse hypochromicity appeared to amount to as much as 3% of the total hypochromic shift. The remaining nonspecific hypochromic shift, during the final 5°C drop to the incubation temperature, appeared to be relatively negligible. Hence, for computation purposes, time zero was arbitrarily chosen to be 30 s after the initial pipetting of DNA solution into the cuvettes, and the absorbance corresponding to fully denatured DNA was assumed to be the absorbance measured at this time.

Hydroxylapatite reassociation kinetics. Sheared *M. xanthus* DNA and *E. coli* DNA (500 to 1,000 bp) in 0.1X SSC were denatured at 100°C for 10 min and then equilibrated to 68°C. Phosphate buffer (PB) (2 M, pH 6.8) was then added to the DNA solution to a final concentration of 0.12 M (time zero). Samples were taken at intervals and frozen in a dry ice-ethanol bath.

DNA samples were diluted with 0.12 M PB to 1 ml and applied to disposable Pasteur pipette columns containing 0.6 ml of hydroxylapatite (Bio-Rad HTP) equilibrated with 0.12 M PB and maintained at 68°C. Columns were washed with 3 ml of preheated 0.12 M PB and eluted with 3 ml of preheated 0.4 M PB, using mild air pressure to speed the collection of fractions. DNA content in the collected fractions was measured by absorbance at 260 nm.

Quantitation of restriction fragments. Agarose gels were stained at least 4 h with 0.5 μg of ethidium bromide per ml. Short-wave UV-illuminated gels were photographed through a Tiffen 23A filter on Kodak Royal Pan film. Negatives were scanned with a Joyce-Loebl microdensitometer. Measurements of areas under peaks were performed with a Numonics electronic graphics calculator.

RESULTS

Renaturation analysis. Optical reassociation analysis was performed on DNA from *E. coli*, *S. aurantiaca*, *M. xanthus*, and *P. aeruginosa*. In the 0.2 M PB–8 M urea solution used for these experiments, the T_m values for *E. coli*, *S. aurantiaca*, and *M. xanthus* DNAs were, respectively, 75.4, 81.7, and 83.1°C. Since it has been reported that the optimal temperature for reassociation is generally about 25°C below the T_m (3), optical reassociation experiments were conducted at either of two temperatures, 50 to 51°C and 58 to 59°C. The optical reassociation runs were usually terminated after about 50% renaturation, since optically monitored reassociation deviates strongly from second-order kinetics after this point (2).

Figures 1 and 2 present the data for optical reassociation for *E. coli*, *S. aurantiaca*, *M. xanthus*, and *P. aeruginosa*. A small fraction of rapidly renaturing material was repeatedly seen in the renaturation curves of *M. xanthus* and *S. aurantiaca*, whereas it was not seen in the renaturation curves of *E. coli* and *P. aeruginosa*.

The apparent genome size of *M. xanthus* DNA as determined by renaturation at 50°C appeared to be 2.04 times that of the *E. coli* standard (Fig. 1a). Likewise, the apparent genome size of *S. aurantiaca* DNA appeared to be 1.94 times that of the *E. coli* standard (Fig. 1b). There was little difference in genome size between *M. xanthus* and *S. aurantiaca* (Fig. 1c). Since 50°C is below the optimal reassociation temperature for *M. xanthus* and *S. aurantiaca* DNA, the apparent genome sizes for these organisms are probably overestimated relative to the *E. coli* standard.

At 58°C, which is near to the optimal reassociation temperature for *M. xanthus* and *S. aurantiaca* DNA, the measured kinetic complexities were reduced relative to *E. coli*. As calculated from the $C_0t_{1/2}$ of the unique sequence portion of their renaturation curves, the apparent genome sizes of *M. xanthus* and *S. aurantiaca* are, respectively, 1.28 (Fig. 2a) and 1.33 (Fig. 2b) times that of *E. coli*. Measured against each other, the kinetic complexities of *S. auran-*

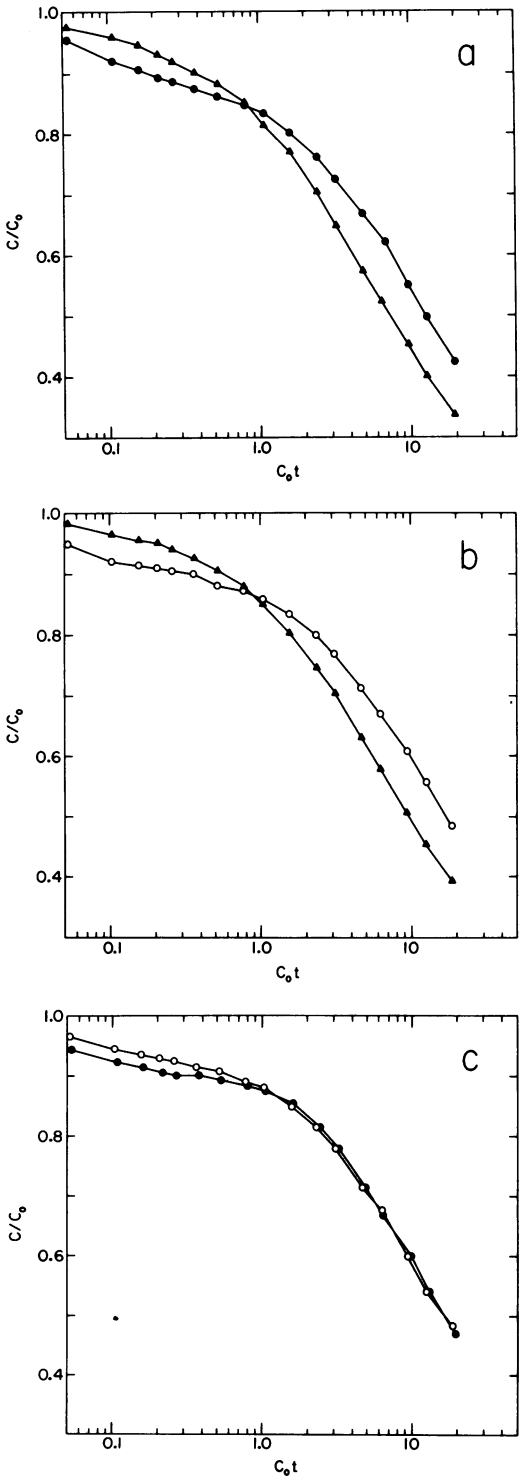

FIG. 1. *Optically determined reassociation of bacterial DNA samples at 50°C in 0.2 M PB–8 M urea. (a) M. xanthus DNA compared with E. coli DNA; (b) S. aurantiaca DNA compared with E. coli DNA; (c) S. aurantiaca DNA compared with M. xanthus DNA. Symbols: (▲) E. coli DNA; (●) M. xanthus DNA; (○) S. aurantiaca DNA.*

tiaca and *M. xanthus* appeared virtually identical (Fig. 2c).

There is considerable controversy concerning the relationship between base composition and intrinsic renaturation rate. For example, Gillis et al. (7) describe an inverse relationship between percent G+C and renaturation rate, such that at their respective optimal renaturation temperatures, a DNA of 68% G+C content will renature only 69% as fast as DNA of equivalent complexity, but containing only 50% G+C. On the other hand, Wetmur and Davidson (21) and Seidler and Mandel (19) give a direct rather than inverse relationship, whereas Bak et al. (1) conclude that no relationship exists.

It is unfortunate that accurate, noncontroversial measurements of genome size for bacteria with G+C contents more closely comparable to those of *M. xanthus* and *S. aurantiaca* do not seem to be available. For example, it would seem that *P. aeruginosa* DNA with its 67% G+C content would provide an ideal reference against which to measure the kinetic complexity of myxobacteria. However, independent reports using various methodologies for measurement of genome size give estimates for this DNA ranging from 2.1×10^9 to 7.0×10^9 daltons (1, 16, 17). The lowest estimate may be the most accurate, since Pemberton (17) used direct measurement of the length of the DNA as observed in the electron microscope.

At 58°C, the kinetic complexity of *M. xanthus* DNA indicated a genome size which is 1.47 times that of *P. aeruginosa* (Fig. 2d). If *P. aeruginosa* has a genome size of 2.1×10^9 daltons, then the genome size of *M. xanthus* is 3.1×10^9 daltons, or only 24% greater than that of *E. coli*.

Renaturation at 68°C in 0.12 M PB buffer, followed by analysis of renatured fractions using hydroxylapatite chromatography, yielded results corresponding to the optical reassociation data (Fig. 3). Up to 10% of the DNA strands of *M. xanthus* showed immediate reassociation. Since hydroxylapatite chromatography gives number fractions of renatured DNA strands rather than weight fractions of renatured DNA, the computed ratio of total genome size to *E. coli*, 1.97, must be considered an overestimate caused by incorrect positioning of the point of 50% renaturation.

Figures 1 to 3 suggested the existence of rapidly renaturing material in the genomes of both *M. xanthus* and *S. aurantiaca*. No such fraction was seen in the renaturation curve of *P. aeruginosa* (Fig. 2d), from which it appeared that this

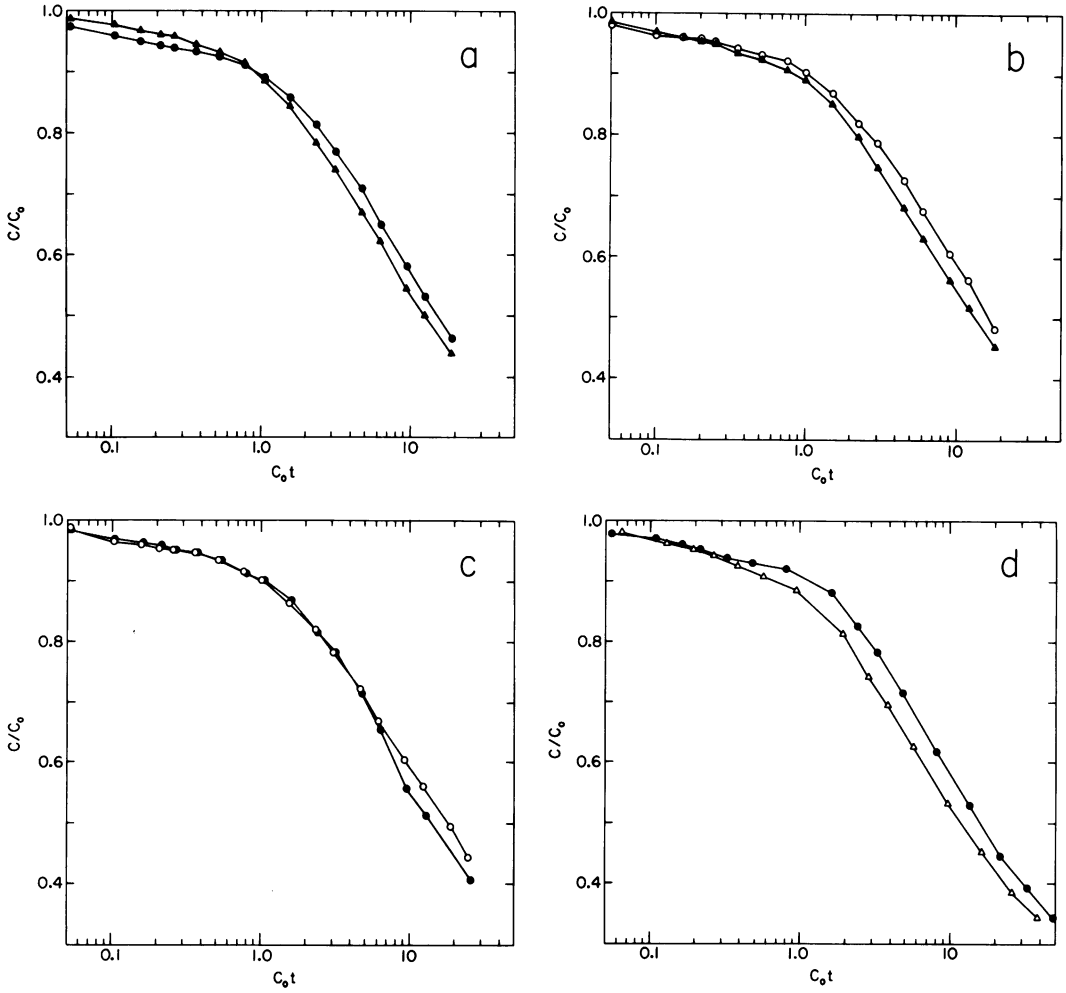

FIG. 2. *Optically determined reassociation of bacterial DNA samples at 58°C in 0.2 M PB–8 M urea. (a) M. xanthus DNA compared with E. coli DNA; (b) S. aurantiaca DNA compared with E. coli DNA; (c) S. aurantiaca DNA compared with M. xanthus DNA; (d) M. xanthus DNA compared with P. aeruginosa DNA. Symbols:* (▲) *E. coli DNA;* (●) *M. xanthus DNA;* (○) *S. aurantiaca DNA;* (△) *P. aeruginosa DNA.*

fraction was not simply an artifact due to the high G+C content of those DNAs. An effort was made to characterize and to isolate the rapidly renaturing material. If the presence of these rapidly renaturing fractions represented true specific associations, then we expected that denaturation of native, unsheared DNA followed by brief renaturation and S1 nuclease treatment would yield discrete size fragments of DNA which would be separable by agarose electrophoresis, such as has been demonstrated in *Shigella* and *E. coli* (15).

Surprisingly, although a few faint S1 nuclease-resistant bands were detected, the bulk of the rapidly renaturing material could not be resolved into distinct size classes (data not shown). This was despite repeated efforts using a variety of protocols for renaturation and for S1 nuclease digestion of the renatured material.

A second expectation, if these rapidly renaturing fractions represented true specific associations, was that restriction enzyme digests of rapidly renatured material would show a less complex pattern than a digest of bulk DNA. We were unable to demonstrate unambiguously whether this was so.

When crude lysates of *M. xanthus* prepared according to Mickel et al. (14) were sized on 0.7% agarose gels, at least three species of DNA exhibiting mobilities ranging from 60% to less than 20% of the band of broken chromosomal DNA were observed (data not shown). These bands may have represented high-molecular-weight plasmids. The presence of these putative plas-

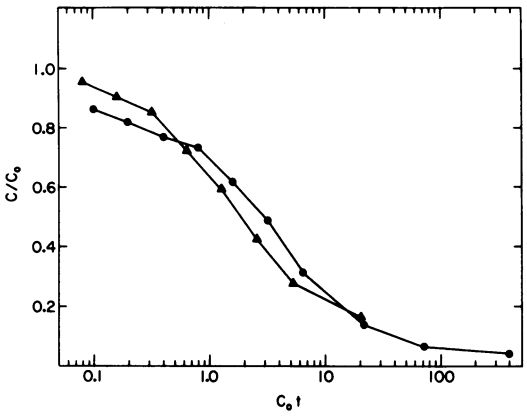

FIG. 3. *Reassociation of M. xanthus and E. coli DNA at 68°C in 0.12 M PB as determined by hydroxylapatite chromatography. Symbols:* (●) *M. xanthus DNA;* (▲) *E. coli DNA.*

mids is insufficient to explain the origin of the anomalously renaturing fraction. However, their presence could require a revision of our genome size estimate.

Restriction enzyme fragment analysis. The large difference between our measurement of the genome size of *M. xanthus* and previous reports which indicated a genome size to three to four times that of *E. coli* led us to consider a second means of measurement. If DNA from a stationary-stage culture is subjected to a restriction enzyme digest and a known quantity of the digest is then subjected to agarose gel electrophoresis followed by ethidium bromide staining, individual bands of single-copy sequence will exhibit a fluorescence intensity which is inversely proportional to genome size and directly proportional to the molecular weight of the fragments. By mixing together known quantities of two digests of different organisms and adjusting the proportions until equivalent fluorescence intensities are obtained, it is possible to get an estimate of the relative genome sizes of the two organisms. Adjusting the concentrations to equivalent band intensities partially avoids errors due to minor nonlinearities in the fluorescence measurements.

This was done for DNA from *M. xanthus*, *E. coli*, and phage λ. *E. coli* DNA was digested with *Sau*3AI, *M. xanthus* DNA was double-digested with *Rsa*I and *Hin*dIII, and λ DNA was digested with *Hpa*I. Figures 4 and 5 illustrate a typical experiment in which digests of *M. xanthus* DNA and *E. coli* DNA were titrated with various amounts of the λ DNA digest. Figures 6 and 7 illustrate densitometer scans of selected regions of negative photographs taken of these gels. The ratios of the average peak areas normalized by their corresponding molecular weights of the λ fragments versus *M. xanthus* and *E. coli* fragments are plotted in Fig. 8. The ratio r is defined as

$$r = \frac{\sum_i A_{\lambda i} / \sum_i M_{\lambda i}}{\sum_j A_{tj} / \sum_j M_{tj}}$$

where $A_{\lambda i}$ represents the area under the i^{th} λ fragment, $M_{\lambda i}$ represents the molecular weight of the i^{th} λ fragment, A_{tj} represents the area under the j^{th} fragment of the DNA being tested, and M_{tj} represents the molecular weight of the j^{th} fragment of the DNA being tested.

Both least-squares best-fit lines intersected fairly close to the origin, indicating that the exposures were approximately linear. The points at which the best-fit lines cross unity ($r = 1$) indicate where the λ fragments have fluorescence intensities equivalent to those of the *E. coli* and *M. xanthus* fragments. Each agarose well had 2.01 μg of *E. coli* or *M. xanthus* DNA, as determined by an ethidium bromide fluorimetric assay using *E. coli* DNA as a standard, mixed with successive 1.25X dilutions of a λ DNA digest originally supplying 29 ng per slot. In the titration of *M. xanthus*, $r = 1$ when 13.5 ng of λ DNA is present per slot. In the titration of *E. coli*, $r = 1$ when 20.9 ng of λ DNA is present per slot. Assuming that λ DNA has a molecular weight of 3.1×10^7 (6), the genome size of *M. xanthus* is estimated to be 4.6×10^9 daltons, and the *E. coli* genome size is estimated to be 3.0×10^9 daltons. This second estimate is about 20% over the generally accepted value for the *E. coli* genome (2.5×10^9 [4, 10, 20]). If the same systematic errors which led to this overestimation of the *E. coli* genome size are operative on the *M. xanthus* determination, then the most probable size of the *M. xanthus* genome, as measured by this method, is 3.8×10^9 daltons.

DISCUSSION

From the present experiments, the most probable size of the *M. xanthus* genome is considered to lie in the range of 3.1×10^9 to 3.8×10^9 daltons. These values are considerably smaller than several previously reported estimates, which include (i) viscoelastic measurements of the largest DNA molecules in gently lysed cells, yielding a size estimate of 8.2×10^9 daltons (24) and (ii) chemical measurement of the DNA content of newly replicated cells in an exponentially growing population of cells, yielding a genome size of 8.4×10^9 daltons (9, 24).

We are unable to offer any completely satisfactory explanation for the discrepancy between our genome size measurements and those previously reported for *M. xanthus*. It should be noted, however, that (i) direct chemical mea-

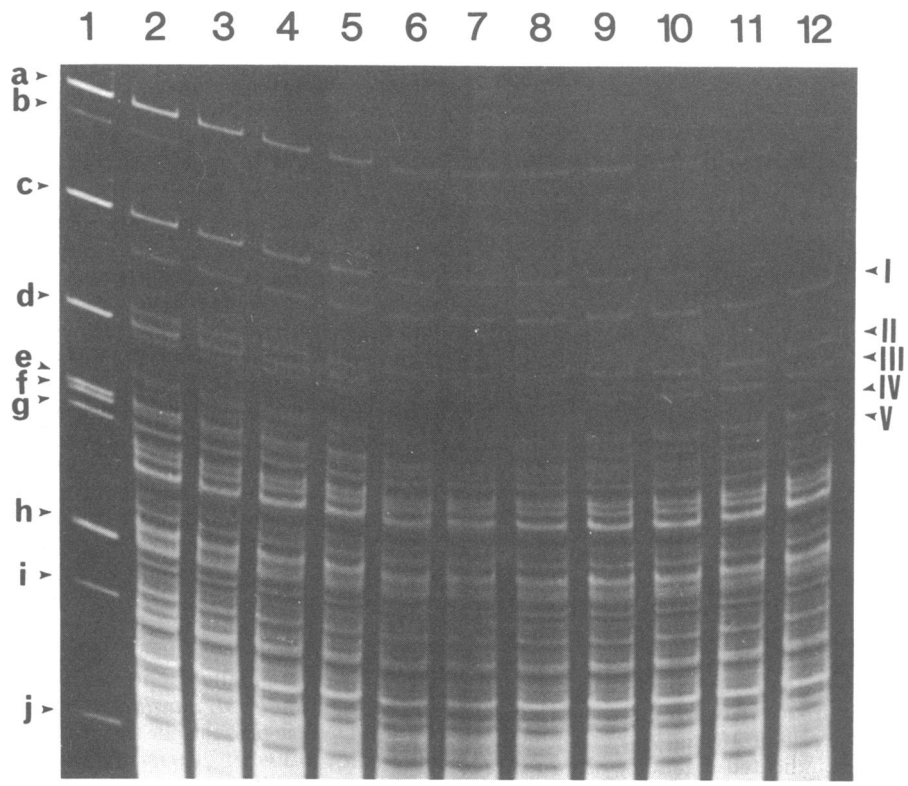

FIG. 4. *Genome size determination of M. xanthus DNA by titration of an (RsaI + HindIII) double digest with an HpaI digest of λ DNA. Slot 1 contains λ DNA. Slot 12 contains M. xanthus DNA. Slots 2 through 11 contain 2.01 μg of M. xanthus DNA mixed with, respectively, 29, 23.2, 18.6, 14.8, 11.9, 9.5, 7.6, 6.1, 4.9, and 3.9 ng of λ DNA. The faint λ fragment (b) is the result of cohesion between fragment c and an 0.7-Kilobase-pair fragment not seen on this gel. Fragments a through j are, respectively, 10.7, 9.4, 8.6, 5.3, 4.6, 4.5, 4.4, 3.4, 3.0, and 2.3 Kilobase pairs in length. The bands of M. xanthus DNA labeled I through V are identified in the legend to Fig. 6.*

surement of the DNA content of bacterial cells is imprecise and involves assumptions concerning copy number and the state of cell replication (18) and (ii) viscoelastic measurement preferentially measures the size of the largest DNA molecules in a solution (10), which may indicate that, if even a relatively small fraction of molecules in a population are partially replicated, this technique may overestimate the size of a single genome. In addition, viscoelastic measurements are sensitive to artifactual DNA aggregation. On the other hand, the methods used in this study can also be criticized as follows: (i) the precision of renaturation techniques is hampered by the lack of availability of high-G+C reference DNA of well-defined genome size and (ii) analysis of restriction fragments may be sensitive to the state of DNA replication and is subject to various other systematic errors as discussed below.

Accurate genome size determination by the analysis of restriction fragments requires that the chromosomes be completely replicated. DNA was routinely extracted from stationary-phase cultures, but it has not been shown that this guarantees that all of the DNA in *M. xanthus* will be completely replicated. Accurate genome size determination by this technique also requires that, after extraction, the DNA be reasonably intact and unsheared. The usual laboratory operations (pipetting, mixing, and pouring) involved in phenol extration procedures may result in breaking the DNA into pieces of about 2×10^7 daltons (18). When using such an extraction, it can be predicted that approximately one of every five restriction fragments of 4×10^6 daltons will be lost, resulting in a corresponding overestimate of genome size unless the reference DNA has also been subjected to the same shear conditions. This may be responsible for the systematic overestimate of the size of the *E. coli* genome seen when restriction fragments

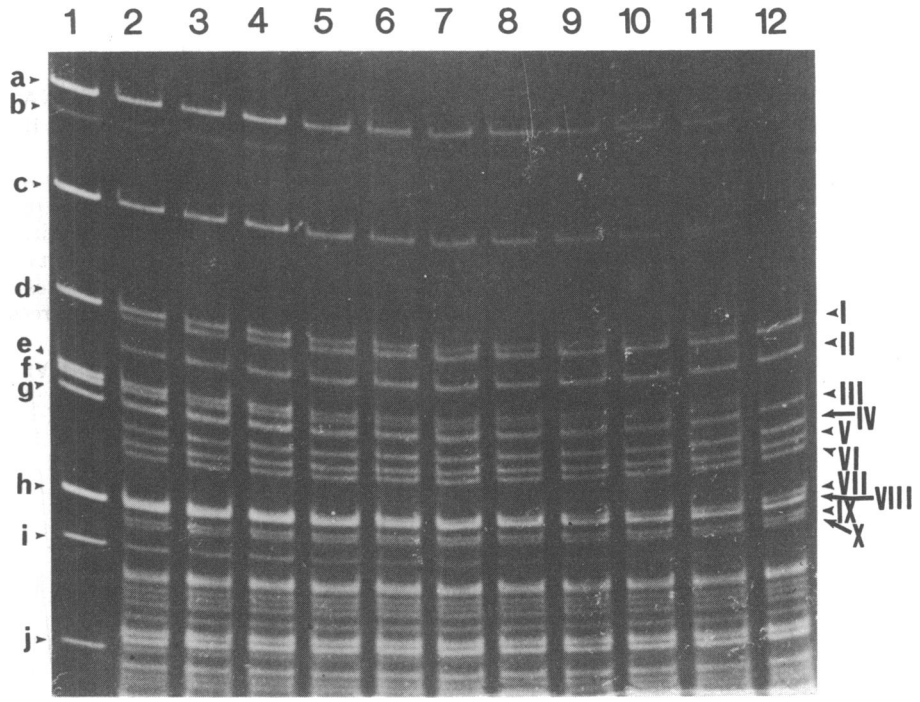

FIG. 5. *Genome size determination of E. coli DNA by titration of a Sau3AI digest with a HpaI digest of λ DNA. Slot 1 contains λ DNA. Slot 12 contains E. coli DNA. Slots 2 through 11 contain 2.01 μg of E. coli DNA mixed with, respectively, 29, 23.2, 18.6, 14.8, 11.9, 9.5, 7.6, 6.1, 4.9, and 3.9 ng of λ DNA. The λ fragments a through j are identified in the legend to Fig. 4. The bands of E. coli DNA labeled I through X are identified in the legend to Fig. 7.*

are directly compared with λ fragments, and we presume that these same effects are operative on our estimate of the *M. xanthus* genome size. Despite these reservations, quantitation of individual restriction fragments appears to be a highly useful technique, providing data which can corroborate measurements made by other means.

On various occasions we have observed in crude lysates of *M. xanthus* analyzed according to the technique of Mickel et al. (14) DNA bands which may represent plasmids of up to possibly several hundred million daltons. Different preparations exhibited different sets of putative plasmid bands. The source of the variability in our observations is not clear at present. The presence of large plasmids may require a significant downward revision of our own estimates of the *M. xanthus* genome size.

Our finding that the *M. xanthus* genome may be considerably smaller than has been previously reported (9, 24) may require a considerable change in our viewpoints concerning the number of genes required for cooperative behavior and the fruiting process of myxobacteria (8). It is already known that a large genome size is not necessary for the mere ability to sporulate, since *Bacillus subtilis*, which also is able to sporulate, has a genome which is slightly smaller than that of *E. coli* (7, 10). Neither does it seem that a large genome size is required for the growth of differentiated bacterial cells, since *Caulobacter crescentus*, which exhibits a primitive form of cell differentiation into stalked and swarmer cells, has a kinetic complexity which is probably not much greater than that of *E. coli* or, indeed, may even be somewhat less than that of *P. aeruginosa* (22).

The significance of the rapidly renaturing fractions which we consistently observed in *M. xanthus* and *S. aurantiaca* is not clear at present. DNA from *P. aeruginosa* with a G+C content of 67% did not show a rapid renaturing fraction when renatured under our conditions, indicating that the observation for myxobacteria DNAs is not simply a phenomenon related to their high G+C content. On the other hand, we were unable after repeated trials to prove the specificity of the observed interactions.

Inverted repeat sequences have been widely

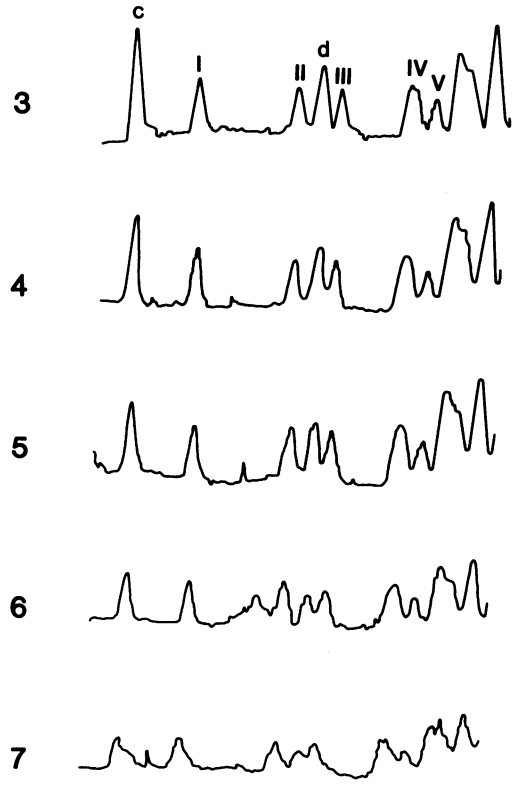

FIG. 6. *Microdensitometer scan of selected areas of a negative photograph of the gel illustrated in Fig. 4. Scans 3 through 7 correspond to well slots 3 through 7 in Fig. 4. c is the 8.6-kilobase-pair HpaI fragment of λ, and d is the 5.3-kilobase pair fragment of λ. I, II, and III are single-copy fragments of an M. xanthus (RsaI + HindIII) double digest of 7.3, 5.6, and 5.0 kilobase pairs. These five fragments were used for replotting in Fig. 8. IV appears to be an unresolved double peak of M. xanthus fragments, and V is a single copy peak, but neither was used in the genome size determination due to the difficulty in assigning a baseline. The 8.6-kilobase-pair λ fragment is present in about 10% less than single-copy sequence since it is an end piece and joins to form a 9.4-kilobase-pair fragment. This has been taken into account in the molecular weight computation.*

reported in the DNA of both procaryotes and eucaryotes. However, only one procaryote, *C. crescentus*, has been reported to have enough inverted repeat sequences to show up in C_0t analysis (22). *Caulobacter*, like the myxobacteria, has a complex life cycle involving the formation of differentiated cells. In addition, like the myxobacteria, its DNA has a high G+C content of approximately 67%. Neither *B. subtilis*, which is capable of a primitive form of differentiation, but which has a G+C content of 45 to 48%, nor *Xanthomonas perlargonii* or *P. aeruginosa*, which both have G+C contents of 67% but do not differentiate, has been reported to have any significant fraction of DNA possessing distinct reassociation kinetics (7, 16, 22; this paper).

If the rapid renaturing fractions which we have observed represent inverted repeat sequences, then our inability to identify specific fragments after S1 nuclease treatment or restriction enzyme digests probably indicates that the regions of uninterrupted homology responsible

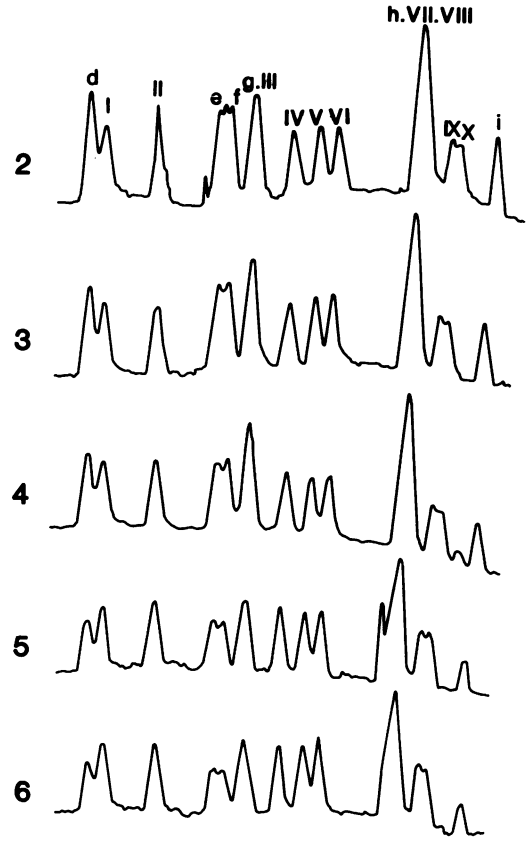

FIG. 7. *Microdensitometer scan of selected areas of a negative photograph of the gel illustrated in Fig. 5. Scans 2 through 6 correspond to well slots 2 through 6 in Fig. 5. d, e, f, and i are, respectively, the 5.3-, 4.6-, 4.5-, and 3.0-kilobase-pair HpaI fragments of λ. I, II, IV, V, VI, IX, and X are single-copy fragments of a Sau3AI digest of E. coli of 5.2, 4.9, 4.1, 3.9, 3.8, 3.3, and 3.2 kilobase pairs, respectively. These 11 fragments were used for replotting in Fig. 8. g.III is an unresolved double peak of a λ fragment superimposed on an E. coli fragment, and h.VII.VIII is an unresolved peak containing a λ fragment and at least three E. coli fragments. Neither g.III nor h.VII.VIII was used in the genome size determination.*

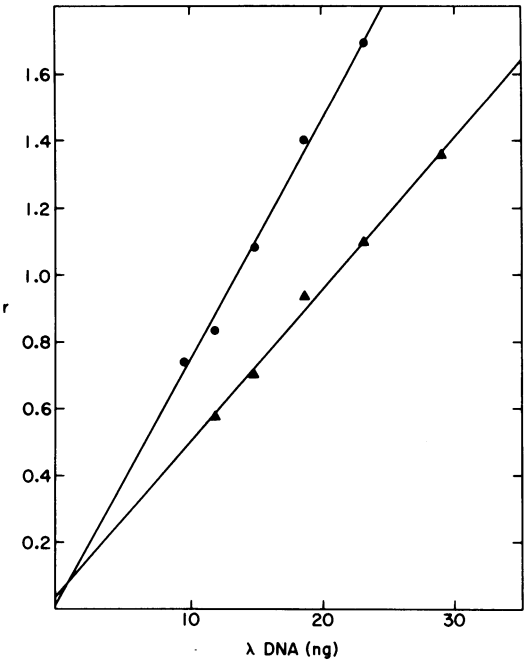

FIG. 8. *Relative fluorescence intensities of bands of λ DNA versus M. xanthus and E. coli DNA, derived from the densitometric scans presented in Fig. 6 and 7. The ratio r is as defined in the text, plotted against nanograms of λ DNA used per slot. Symbols:* (●) *λ DNA versus M. xanthus DNA;* (▲) *λ DNA versus E. coli DNA.*

for this renaturation behavior are short and polydisperse.

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