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# A COMPARISON OF DNA CONTENT BETWEEN TWO SUBSTRAINS OF *ESCHERICHIA COLI* B/r

John A. Hucul and Charles E. Helmstetter

Departments of Biology and Experimental Biology
Roswell Park Memorial Institute
Buffalo, New York 14263

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SUMMARY. The average DNA content per cell was measured in steady-state cultures of two substrains of *E. coli* B/r growing at various rates at 37°C. The DNA content of substrain B/r F was consistently lower than that of substrain B/r A. It is suggested that the differences in DNA contents are consequences of strain-specific differences in the relationship between chromosome replication and the division cycle of *E. coli*.

*Escherichia coli* strain B/r has been used extensively for investigating the relationship between chromosome replication and the division cycle of bacteria. This relationship has generally been quantitated through measurements of the time for a round of chromosome replication (C) and the time between the end of a round and the subsequent cell division (D) in cells growing at a variety of rates. Although there has been substantial agreement as to the values of C and D in cells growing in media which support rapid growth at 37°C, there are uncertainties concerning the behaviour of slowly growing cells (1-10). In early studies, Cooper and Helmstetter (1) found that C and D were approximately 40 and 20 min, respectively, in cells growing with interdivision times shorter than 64 min at 37°C, and that they increased to approximately two-thirds and one-third of the interdivision time, respectively, in cells growing with longer interdivision times. A number of other studies have also suggested a lengthening of C and D during slow growth (2, 5-10). On the other hand, Kubitschek and Freedman (3) concluded that C and D remained constant at 47 and 25 min, respectively, over a wide range of growth rates. Their conclusions were based on measurements of total DNA per cell in steady-state cultures, or in glucose-limited chemostat cultures with growth rates as

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low as 0.1 doublings/hr. Furthermore, a C value of 40 min has been reported for a K12 strain of E. coli growing with interdivision times between 20 and 200 min (11).

One possible explanation for the differing experimental findings with slow-growing cells is that C and D could be strain- and substrain-dependent. Although most of the studies summarized above were performed on the same presumptive B/r strain, variations may have developed in stocks of cells bearing the same strain designation during years of cultivation in different laboratories. In an effort to resolve this question, Helmstetter and Pierucci (5) used the membrane-elution technique to examine $^{14}$C-thymidine incorporation during the division cycle of the B/r substrain of E. coli used by Cooper and Helmstetter (designated B/r A), the substrain employed by Kubitschek and Freedman (designated B/r K) and a third substrain which was designated B/r F. They found differences in the relationship between chromosome replication and the division cycle in the three substrains. The C and D periods were shorter in substrains B/r F and B/r K than in substrain B/r A at growth rates below 1.0 doubling/hr.

Since substrain differences in the lengths of C and D could account for the discordant observations described above, and since the DNA content per cell is a function of C and D (1), it became necessary to compare the average DNA contents of the substrains during exponential growth. Without verification of the substrain dependency of DNA content, it would remain possible that the membrane-elution technique perturbs the growth of bacterial cells during slow growth, thereby introducing experimental errors in measurements of C and D. The results of measurements of DNA per cell in two substrains (B/r A and B/r F) are reported here. It is shown that the DNA contents are clearly different in the two substrains growing at the same rates, consistent with the prediction based on the earlier measurements of C and D.

METHODS. Cultures of E. coli B/r A and B/r F were incubated in a shaking water bath at 37°C in minimal salts medium (5) containing six different carbon sources (Table 1) for at least 16 hr until the concentration of cells reached approximately 1 x $10^8$ per/ml growing exponentially. Cellular concen-

TABLE 1

DNA Content of E. coli B/r A and B/r F Growing Exponentially at a Variety of Rates

| Carbon Source | Substrain | Doubling Time (min) | DNA/cell ± Standard Deviation ($g \times 10^{-15}$) |
|---|---|---|---|
| Acetate | A | 119 | 6.6 ± 0.2 |
| | F | 115 | 5.6 ± 0.2 |
| Succinate | A | 96 | 6.5 ± 0.1 |
| | F | 83 | 5.6 ± 0.1 |
| Glycerol | A | 54 | 7.4 ± 0.4 |
| | F | 55 | 6.6 ± 0.5 |
| Glucose | A | 43 | 8.2 ± 0.6 |
| | F | 40 | 7.3 ± 0.9 |
| Glucose plus 6 amino acids | A | 36 | 10.2 ± 0.6 |
| | F | 35 | 8.4 ± 0.6 |
| Glucose plus Casamino Acids | A | 26 | 14.4 ± 0.5 |
| | F | 26 | 11.2 ± 0.5 |

trations were determined with a model ZB Coulter Electronic Particle Counter. The average DNA contents per cell were determined with Burton's modification of the diphenylamine reaction (12), using deoxyadenosine as a standard and trichloroacetic acid (TCA) extraction as described previously (1). Thirty ml of culture was transferred to 10 ml of ice-cold 20% TCA and placed in an ice bath for 30 min. The samples were then centrifuged at 10,000 rpm for 10 min. The pellet was resuspended in 4 ml of 5% TCA and incubated at 90°C for 20 min. Dilutions of deoxyadenosine were heated simultaneously. The samples were centrifuged again and the supernatant was added to Burton's diphenylamine reagent and incubated at 25°C for 16 to 20 hr. The absorbances of the samples and the standards were then measured at 600 nm with a Zeiss PMQII spectrophotometer. Four separate determinations of DNA content were performed at each growth rate. E. coli substrains B/r A and B/r F were employed in this study because a significant difference in DNA content per cell would be predicted between these two, and because substrain B/r K formed filaments at the slower growth rates which affected measurements of cell concentrations.

RESULTS AND DISCUSSION. Table 1 shows the average DNA per cell in exponential-phase cultures of E. coli B/r A and B/r F growing at a variety of rates at 37°C. As expected, the DNA content increased as the growth rate was increased, but more importantly, substrain B/r A contained more DNA per cell than substrain B/r F at each growth rate. From these data, the average genome equivalents of DNA per cell ($\bar{G}$) were calculated for both substrains and these are shown as a function of growth rate in Figure 1. For comparison, Figure 1 also

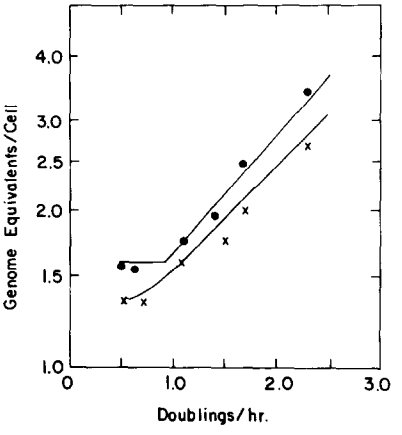


Figure 1. Genome equivalents of DNA per cell. The grams of DNA per cell shown in Table 1 were converted to genome equivalents of DNA per cell ($\bar{G}$) upon division by $4.2 \times 10^{-15}$ g, taken as the mass of one, non-replicating chromosome (1). ●, B/r A, x, B/r F. The lines show $\bar{G}$ based on previously reported values for C and D (5) substituted into the equation: $\bar{G} = \frac{\tau}{C \ln 2} (2^{C+D/\tau} - 2^{D/\tau})$, where $\tau$ is the doubling time.

shows the predicted $\bar{G}$ for substrains B/r A and B/r F based on the values for C and D reported previously (5). As can be seen, the genome equivalents of DNA per cell determined with the diphenylamine reaction are indistinguishable from those calculated from the values for C and D.

The following conclusions can be reached. First, the average DNA content per cell differed significantly in two substrains of E. coli B/r growing at the same rates. Second, the average DNA contents of E. coli B/r A and B/r F growing at various rates were consistent with the predicted DNA contents based on previous measurements of the length of the C and D periods. Thus, the variation in cellular DNA contents among substrains of E. coli B/r is probably due to differences in C and D, if it is assumed that the molecular weight of the chromosome is not significantly different. Since the values of C and D change to differing extents in substrains grown at low rates, values determined for one strain or substrain cannot be assumed to apply to all strains or substrains of E. coli.

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