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Involvement of Recombination Genes in Growth and Viability of *Escherichia coli* K-12

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We have studied the growth properties of 17 isogenic strains of *Escherichia coli* K-12 differing only in the *recA*, *recB*, *recC*, and *sbcA* alleles. We have observed the following. (i) All recombination deficient strains have decreased growth rates and decreased viabilities compared with recombination proficient strains. The large populations of nonviable cells in Rec$^-$ cultures may arise by spontaneous lethal sectoring (9). (ii) A *recA* mutant strain which is entirely recombination deficient and which shows high ultraviolet sensitivity and "reckless" deoxyribonucleic acid (DNA) breakdown has approximately the same growth rate and twice the viability as *recB* and *recC* mutant strains which have residual recombination proficiency, moderate ultraviolet sensitivity, and "cautious" DNA breakdown. (iii) Indirectly suppressed (*sbcA*$^-$) recombination proficient (Rec$^+$) revertants of *recB* and *recC* mutant strains have approximately normal growth rates and are three times as viable as their Rec$^-$ ancestors (but not as viable as *rec*$^+$ cells). We suggest the following hypothesis to account for the low viability of Rec$^-$ *E. coli*. Single-strand breaks in the DNA duplex, necessary for normal bacterial growth, may be repaired in a Rec$^+$ cell. Failure of Rec$^-$ cells to repair this normal DNA damage may lead to the observed loss of viability.

Mutants of *Escherichia coli* K-12 have been isolated which are deficient in their ability to form recombinants (5, 6, 8, 11, 19, 20). Genetic mapping and complementation data places the mutants in three groups: *recA*, which lies between *cysC* and *pheA*, and *recB* and *recC*, both cotransducible with *thyA* (8, 19, 20). *recA* mutant strains are characterized by high ultraviolet (UV) sensitivity, "reckless" breakdown of their deoxyribonucleic acid (DNA) after UV irradiation, low spontaneous production of λ phage, and no detectable recombination proficiency. *recB* and *recC* mutant strains, on the other hand, are characterized by moderate UV sensitivity, "cautious" breakdown of DNA after UV irradiation, normal spontaneous production of λ phage, and reduced recombination proficiency (18). Extracts of strains mutant in *recB* or *recC* genes lack an adenosine triphosphate (ATP)-dependent nucleolytic activity which is present in extracts of *rec*$^+$ and *recA*$^-$ strains (1, 15). There are at least two classes of recombination proficient, UV-resistant revertants of *recB* and *recC* mutant strains: those which map in or near the originally mutant locus and those which indirectly suppress the original mutation. One suppressor gene involved in the second class of revertants has been designated *sbcA* (suppressor of *recB*$^-$ and *recC*$^-$; reference 2).

Several investigators have found that *E. coli* strains mutant in one or another of the *rec* genes grow more slowly and have fewer viable cells per unit of optical density than do their *rec*$^+$ ancestors (9, 10, 20; Kramer and Clark, *unpublished results*). Analogous results have been obtained with *Salmonella* (21). In most instances nonisogenic strains were used. The possibility remained that observed differences in growth properties were the result of differences between the strains other than the *rec* mutations. One study of two isogenic strains showed a decreased growth rate and viability of a *recA*$^-$ strain compared with the *recA*$^+$ strain (10).

We have studied the relationship between specific *rec* genes and recombination proficiency, on the one hand, and growth rate and viability (fraction of microscopically observed cells able to form colonies on Luria agar) on the other. We have done this by measuring (in three media of varying richness) the rate of increase of optical density, cell protein, viable cells, and total cells in cultures of 17 strains isogenic except for the particular *rec* and *sbc* allele(s) present. Mutations in *recA*, *recB*, and *recC* genes cause the growth rate

and viability of *E. coli* to decrease. Recombination proficient, indirectly suppressed revertants of *recB* and *recC* mutants recover growth rate and viability similar to genotypically wild type (*rec*$^+$) strains.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used were *E. coli* K-12. Their derivation and their relevant properties are described in Table 1. Figure 1 shows the pedigree of the strains we have used. Nomenclature conforms to that of Demerec et al. (7). The transduction procedure has been described previously (19).

Media. M9 salts consisted of 1g of NH_4Cl, 11 g of Na_2HPO_4-H_2O, 3 g of KH_2PO_4, 5 g of NaCl, 120 mg of $MgSO_4$, 10 mg of $CaCl_2$, and water to 1 liter. M9 medium consisted of 1 liter of M9 salts, 4 g of glucose, and 0.1 mg of thiamine. EM9 medium consisted of 1 liter of M9 medium plus 2.5 g of Casamino Acids. Luria broth has been described (19). Plates consisted of 2% agar added to liquid medium. Where required, histidine and thymine were added at a final concentration of 50 mg per liter. NaCl (0.85% saline) was used as a diluent.

Growth of strains. Each strain was grown overnight (37 C, on rotating wheel) at least two successive times in 5 ml of the same medium which would be used for the experiment. The culture was stored in the refrigerator and used as the stock for experiments with that particular strain and medium. The night before an experiment, a 1:25 or 1:50 dilution of the stock was made into 5 ml of fresh medium and incubated overnight at 37 C on a rotating wheel. On the morning of the experiment the overnight culture was diluted (between 1:6 and 1:50, depending on the strain and medium) into 5 ml of fresh medium and again incubated at 37 C on a rotating wheel for 2 to 4 hr (pregrown cells). The pregrown cells were diluted (1:15 to 1:75) into 75 ml of fresh medium in a 250-ml flask to give an initial concentration of viable cells of approximately 5×10^6 per ml. During the actual growth experiment flasks were incubated in a shaking 37 C water bath for at least 5 hr.

Optical density measurements. The optical density of the culture at various times in its growth was measured at 650 nm by using a Beckman DU spectrophotometer with a Gilford digital absorbance meter. Fresh medium of the same type was used as a blank. Medium which has sustained at least 5 hr of growth of either a *rec*$^+$, *recA*$^-$, or *recB*$^-$ *recC*$^-$ strain shows no change in absorbance compared with fresh medium.

Cell protein measurements. Two milliliters or 4 ml of the culture was sampled and centrifuged at 8,000 rev/min for 10 min (at 0 C for cells grown in Luria broth). The pellet was washed once (twice for cells grown in Luria broth) with saline and resuspended in 2 ml of saline. When samples were not being centrifuged or washed, they were chilled in an ice bucket. Protein was determined by the method of Lowry et al. (13).

Viable cell measurements. The number of viable cells per milliliter of culture has been defined in this paper as the number of cells in a liquid culture which, when diluted and plated as described below, give rise to *visible* (by eye) colonies on solid medium. A 0.1-ml sample of the culture was diluted 1:100 in saline and this dilution was vortexed for 60 sec. The sample was then further diluted serially through saline and plated in duplicate on Luria agar. Dilutions and plating were done at room temperature. The plates were incubated at 37 C for 24 hr and the colonies counted. With longer incubation times no further colonies appeared.

TABLE 1. *Bacterial strains*

Strain no.	Rec Phenotype	*rec* Genotype	*sbc* Genotype	Phenotype[a]		Parent	Derivation or reference
				Thy	His		
JC4583	+	+	+	+	−	HF4733[b]	(1)
JC4584	−	B21 C22	+	+	−	HF4733	P1 cotransduction with *thy*$^+$
JC4585	−	C22	+	+	−	HF4733	(1)
JC4586	−	B21	+	+	−	HF4733	P1 cotransduction with *thy*$^+$
JC4588	−	A56	+	+	−	HF4733	(1)
JC5170	+	C22	(A)4	+	+	JC6721	(2)
JC5174	+	B21	(A)1	+	+	JC6722	(2)
JC5176	+	B21 C22	(A)6	+	+	JC6724	(2)
JC6720	−	B21 C22	+	+	+	HF4733	(1)
JC6721	−	C22	+	+	+	HF4733	(1)
JC6722	−	B21	+	+	+	HF4733	(1)
JC6724	+	+	+	+	+	HF4733	(1)
JC6802[c]	−	B21 C22	+	−	+	JC6720	Trimethoprim selection
JC6803[c]	−	C22	+	−	+	JC6721	Trimethoprim selection
JC6804[c]	−	B21	+	−	+	JC6722	Trimethoprim selection
SDB1101	+	+	+	+	+	JC6803	P1 cotransduction with *thy*$^+$
SDB1102	+	+	+	+	+	JC6803	P1 cotransduction with *thy*$^+$

[a] All strains are in addition F^-, Gal^-, B_1^-, Sm^s, and endonuclease I deficient.
[b] HF4733 is F^-, Gal^-, B_1^-, Sm^s, and endonuclease I deficient.
[c] We thank Richard Unger who carried out the trimethoprim (17) selection and kindly sent us these strains.

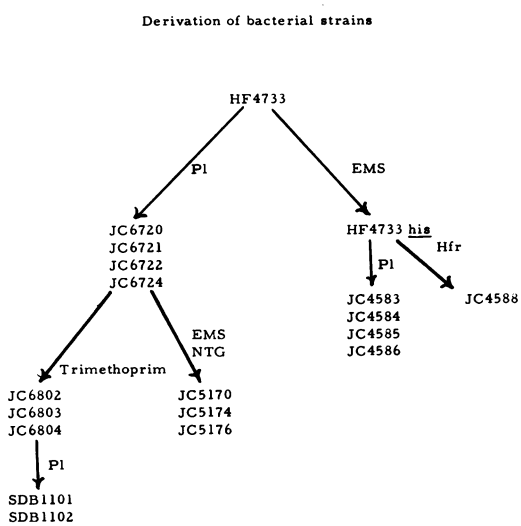

FIG. 1. *Pedigree of bacterial strains we have used. Details are given in Table 1.*

Total cell measurements. The total number of cells per milliliter of culture was determined directly by counting samples in a Levy-Hauser counting chamber at a magnification of 430×. Where necessary, samples were diluted through saline.

RESULTS

Doubling times. The strains surveyed in this paper may be divided on the basis of genotype and growth characteristics into four classes: (I) strains which are *rec*$^+$ *sbcA*$^+$, (II) strains which are *recA*$^+$ *sbcA*$^+$ but which carry either *recB*$^-$, *recC*$^-$, or both *recB*$^-$ and *recC*$^-$ mutations, (III) strains which are *recA*$^-$ *recB*$^+$ *recC*$^+$ *sbcA*$^+$, and (IV) strains which are *recA*$^+$ *recB*$^-$ or *recC*$^-$ or *recB*$^-$ *recC*$^-$ and which are *sbcA*$^-$. Doubling times were determined based on growth curves fitted to data by eye. Slopes between zero and 4 hr of growth were measured. In growth curves where an inflection point was routinely observed the doubling time given is the average slope between zero and 4 hr for the curve. Table 2 lists all strains used and their doubling times as determined by measurements of optical density, cell protein, viable cells, and total cells. Measurements were made in three different types of media. When more than one measurement of a parameter has been made for a particular strain, the doubling time indicated is the mean of all measurements. In these cases, standard deviations have been calculated. Standard deviation = $[1/(n-1)(\Sigma x_i^2 - (\Sigma x_i)^2/n)]^{1/2}$. It is clear that, with the exception of JC6720 and possibly JC6722 and JC6802, within a class the doubling times determined by measurements of a particular parameter are nearly identical. There is, however, some difference for any particular strain in the doubling time as determined by measurements of different parameters; protein and optical density measurements generally give longer doubling times than viable cell and total cell measurements. The reason for this is not clear. The data presented in Table 2 and summarized by Table 3 show the following. (i) In EM9 medium the doubling times of class II and class III strains are 35 to 40% longer than the doubling times of the isogenic *rec*$^+$ strains of class I. Moreover, the doubling times of class IV strains are similar to those of class I strains. (ii) In Luria broth, the doubling times of strains in classes I, II, and III are all shorter than in EM9 medium; however, classes II and III doubling times are still significantly longer (25 to 35%) than class I doubling time. (iii) In M9 medium the doubling times are all longer than in either of the other media, and classes II and III strains again have longer doubling times than the class I strain. Furthermore, the doubling times of the class II and class III strains, indistinguishable in EM9 medium and Luria broth, appear clearly different in M9 medium, class II doubling time being 20 to 30% longer than class III doubling time.

Figure 2 shows representative growth curves for strains belonging to each of the four classes and grown in EM9 medium. The inflection point occurring in the class III curves (optical density and viable count) after approximately 1½ hr of growth is reproducible and also occurs when the cells are grown in Luria broth. We do not know what causes the inflection, but it has also been observed by Hertman (10).

Rec$^-$ cells are more radiation sensitive than Rec$^+$ cells (6, 8, 11, 12, 18, 20). Therefore, it seemed possible that atmospheric radiation might be causing cell damage which Rec$^+$ but not Rec$^-$ cells could repair. If this were the case, the Rec$^-$ culture, by continually losing viable cells, would necessarily have a longer doubling time than the Rec$^+$ culture. This is made unlikely by the observation that *recA* mutant cells are more UV sensitive and at the same time more viable than *recB* and *recC* mutant cells. It also seemed possible that Rec$^-$ strains, having lost a *rec* repair system, might rely to a greater extent on the remaining mechanisms for repair of radiation-damaged DNA such as photoreactivation in order to maintain growth rate. If this were true, Rec$^-$ cells grown under conditions which prevent photoreactivation (i.e., darkness) might grow even more slowly than under standard growth conditions (i.e., in the light). To test these possibilities we grew JC4583, JC4584, and JC4588 (representatives of classes I, II, and III, respectively) in EM9 medium in the dark in flasks

TABLE 2. *Doubling times*

Medium	Class	Strain	OD[a] 650	Cell protein	Viable cells	Total cells
EM9	I *rec*$^+$	JC4583	40.2 ± 2.5[b] (11)[c]	39.0 ± 3.0 (3)	34.9 ± 4.0 (9)	33.0 ± 1.4 (2)
		JC6724	41.0 ± 1.4 (2)	42.0 (1)	40.0 (1)	
		SDB1101	43.5 ± 0.7 (2)	42.0 (1)	42.0 (1)	
		SDB1102	41.5 ± 0.7 (2)	42.0 (1)	42.0 (1)	
	II *recB*$^-$, *recC*$^-$, or both	JC4584	56.1 ± 2.9 (7)	55.0 ± 1.7 (3)	48.7 ± 5.0 (3)	52.0 (1)
		JC4585	56.5 ± 3.5 (2)	57.0 (1)	52.0 (1)	
		JC4586	57.0 ± 4.2 (2)	57.0 (1)	54.0 (1)	
		JC6720	75.7 ± 5.0 (3)	69.0 (1)	69.0 ± 4.2 (2)	
		JC6721	56.0 ± 3.5 (3)	57.0 (1)	51.0 ± 1.4 (2)	
		JC6722	65.0 ± 5.7 (2)	65.0 (1)	54.0 (1)	
		JC6802	67.0 (1)		66.0 (1)	
		JC6803	57.0 (1)		54.0 (1)	
		JC6804	59.0 (1)		60.0 (1)	
	III *recA*$^-$	JC4588	57.8 ± 3.7 (5)	51.0 (1)	54.2 ± 6.4 (4)	48.0 (1)
	IV *SbcA*$^-$, and *recB*$^-$, or *recC*$^-$, or both	JC5170	41.7 ± 1.2 (3)	42.0 (1)	37.0 ± 7.1 (2)	
		JC5174	42.3 ± 1.2 (3)	50.0 (1)	38.0 ± 5.7 (2)	
		JC5176	40.2 ± 1.5 (4)	42.0 (1)	36.0 ± 5.3 (3)	37.0 (1)
Luria	I	JC4583	34.6 ± 3.6 (5)	33.0 (1)	31.5 ± 7.8 (2)	28.0 (1)
	II	JC4584	44.8 ± 5.9 (5)	44.0 (1)	41.5 ± 2.1 (2)	36.0 (1)
	III	JC4588	43.4 ± 5.5 (5)	43.0 (1)	45.5 ± 0.7 (2)	38.0 (1)
M9	I	JC4583	55.0 ± 1.7 (3)	48.0 (1)	49.0 ± 1.4 (2)	52.0 (1)
	II	JC4584	73.3 ± 6.8 (3)	64.0 (1)	77.0 ± 7.1 (2)	76.0 (1)
	III	JC4588	61.7 ± 3.2 (3)	54.0 (1)	59.5 ± 2.1 (2)	54.0 (1)

[a] Optical density.
[b] Doubling times and standard deviations are expressed in minutes.
[c] Number in parentheses is the number of experiments upon which the mean and standard deviation are based.

completely enclosed by aluminum foil. The sampling, diluting, and plating were done in a dimly-lit room, and the plates were incubated in light-tight cardboard boxes. The doubling time of these strains was the same as when the strains were grown in the light.

Viability. When we compared the number of viable cells per milliliter with the total number of cells per milliliter in any sample, we observed striking differences between the *rec*$^+$, the *recA*$^+$ *recB*$^-$ *recC*$^-$, and the *recA*$^-$ *recB*$^+$ *recC*$^+$ strains. These results are summarized in Table 4, part 1. For all strains the per cent viability is not affected by the type of medium. Although *recA*$^-$ has only 50 to 60% the viability of *rec*$^+$, *recB*$^-$ *recC*$^-$ is even more sharply reduced in viability, that being 23 to 34% of *rec*$^+$. This is consistent with results which Haefner obtained using a different technique and nonisogenic strains (9). Furthermore, the viability of these strains does not appear to vary significantly with the stage in the growth cycle (Fig. 3A).

It is clear that the *sbcA* reversion restores wild-type doubling time (Table 2, class IV as compared with class II) as well as recombination proficiency and UV resistance (2). We then asked whether *sbcA*$^-$ also restores normal viability (Table 4, part 2). The class IV strain is three times as viable as an isogenic but unreverted class II strain but is, nevertheless, only 75% as viable as the isogenic *rec*$^+$ *sbcA*$^+$ strain. Again, the viability does not vary significantly with the stage in the growth cycle (Fig. 3B).

We considered the possibility that the lower viability of Rec$^-$ strains might not be a characteristic of the population of cells in liquid culture but might rather have resulted from our treatment of them during diluting and plating. To eliminate changes in ionic strength, temperature, and richness of media as possible causes of the

TABLE 3. *Relationship between doubling times*[a]

Medium	Class[b]	OD[c] 650	Cell protein	Viable cells	Total cells
EM9	I	1.00	1.00	1.00	1.00
	II	1.40	1.41	1.40	1.58
	III	1.44	1.31	1.55	1.45
	IV	1.00	1.08	1.03	1.12
Luria	I	1.00	1.00	1.00	1.00
	II	1.29	1.33	1.32	1.28
	III	1.25	1.30	1.44	1.36
M9	I	1.00	1.00	1.00	1.00
	II	1.33	1.33	1.57	1.46
	III	1.12	1.12	1.21	1.04

[a] For each parameter and medium, the class I doubling time has been set at 1.00, and the doubling time of each other class has been normalized to this value.

[b] Calculations in this table are based on the following strains: class I, JC4583; class II, JC4584; class III, JC4588; class IV, JC5176.

[c] Optical density.

decreased viability, we grew JC4583, JC4584, and JC4588 in EM9 medium, diluted through prewarmed (37 C) M9 salts and plated on warm EM9 plates. The viability observed was identical with results obtained using the standard procedure. It appears, therefore, that a Rec$^-$ culture consists of at least two populations of cells: viable cells, i.e., those which when plated give rise to a visible colony, and nonviable cells, i.e., those which when plated fail to give rise to a visible colony. This is consistent with results which Haefner obtained in single-cell pedigree studies (9).

The nonviable cells of the *recB*$^-$ *recC*$^-$ *sbcA*$^+$ strain grown in EM9 liquid do not appear to undergo appreciable lysis since the optical density 260/280 ratios measured on supernatants of JC4583 and JC4584 cultures at intervals during the growth are the same. Moreover, Lowry protein assays of these supernatants show no more reactive material than is present in fresh EM9 medium.

The total number of particles (observed microscopically) per unit of optical density is nearly identical for strains belonging to each of the four classes, I, II, III, and IV (Fig. 4). Therefore, we assume that the average optical density per cell is the same for these four classes of strains.

DISCUSSION

It is clear that *recA*$^+$, *recB*$^+$, and *recC*$^+$ genes normally are necessary to maintain the growth rate and viability characteristic of *E. coli* K-12. We propose two models which might account for this: (i) recombination proficiency per se is required for normal growth properties and (ii) the products of the *known rec* genes function in at least two different pathways, one of which is required for recombination and another which affects growth; several steps in these two pathways may either be identical or similar in terms of substrates and products. In either model, the decreased growth rate and viability may result *either* from the absence of steps required for growth *or* from damage sustained in the absence of particular reactions.

Our results do not eliminate either model. However, we stress that the *recA* mutant which is entirely recombination deficient and which exhibits high UV sensitivity and reckless DNA breakdown is twice as viable as *recB* and *recC* mutants which have residual recombination proficiency, moderate UV sensitivity, and cautious DNA breakdown. This fact appears to be more consistent with model (ii). Our results with the *sbcA*$^-$ revertants are compatible with either model. If model (ii) is correct, it may be possible to isolate recombination deficient mutants with normal growth rate and viability. Most likely, such mutations would affect *rec* genes other than *recA*, *recB*, and *recC*.

The biochemical functions of the products of the recombination genes have not been determined. To account for the low viability of Rec$^-$ strains we would like to suggest the following working hypothesis. Single-strand breaks in the DNA duplex may be required during the course of normal cell metabolism and growth, perhaps to permit transcription (16) and replication (4, 14). Because the *rec* products have been shown to be involved in the repair of X-ray induced single-strand breaks (12), they might also be expected to participate in the repair of normal metabolically produced single-strand breaks. Inability of the *rec* mutants to repair this normal DNA damage may lead to a loss of viability. However, the relation between repair of strand breaks and the Rec system is not clear. Earlier data (3) do not agree with those of Kapp and Smith (12) referred to above. Also if our hypothesis above is correct, one might expect to see a difference between the alkaline sucrose sedimentation profiles of DNA from unirradiated Rec$^-$ and Rec$^+$ cells. Such a difference has not been found (12).

Rec$^-$ cultures contain a population of cells which are unable to form *visible* colonies when plated on solid medium. We have observed that these nonviable cells do not lyse during the growth experiments. We have found that the total number of particles per unit of optical density is the same for Rec$^+$ and Rec$^-$ cultures. It therefore follows that the nonviable cells are very similar to

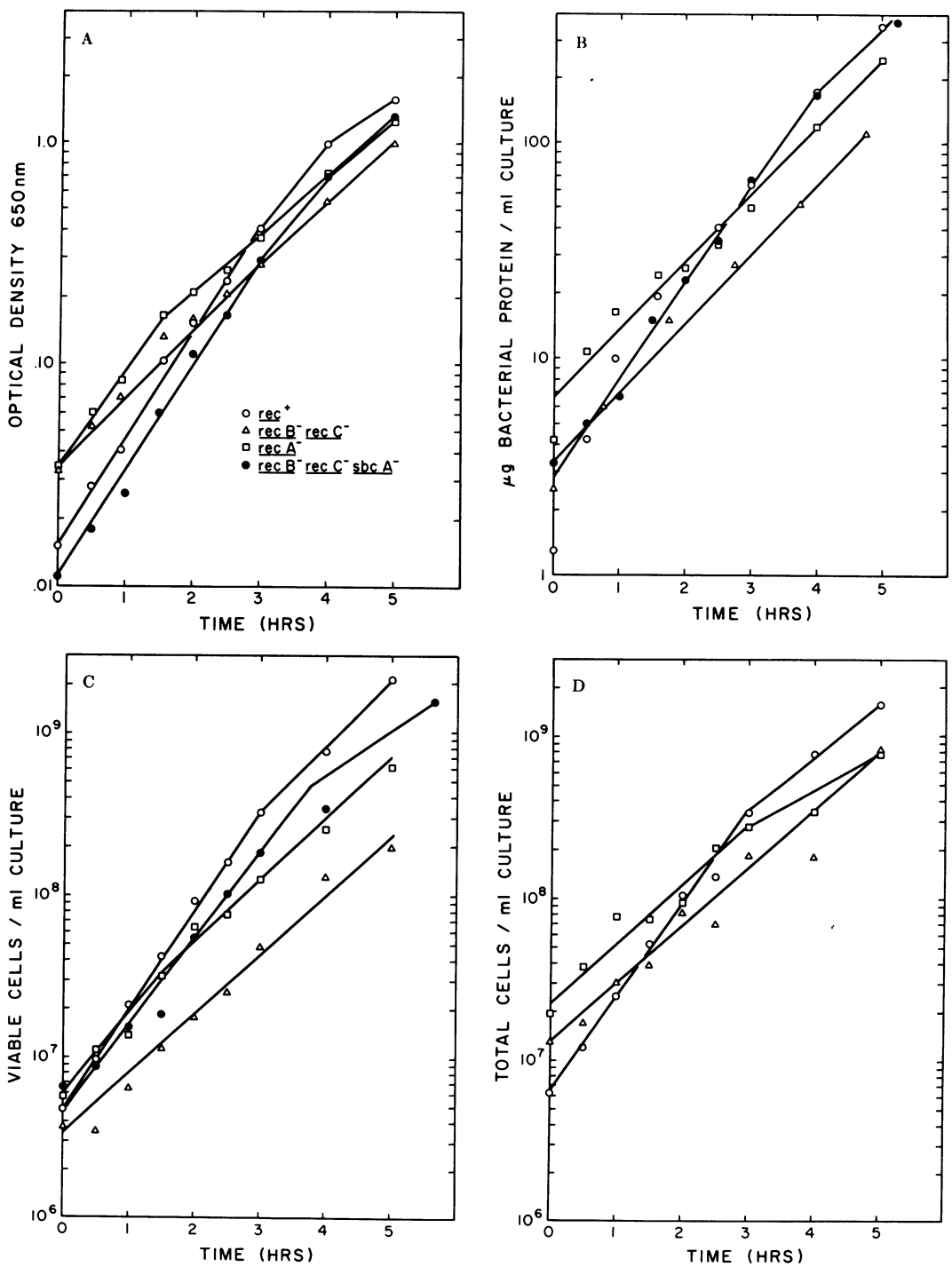

FIG. 2. *Growth curves in EM9 medium of strains representative of each of the four classes.* (○) *Class I, JC4583;* (△) *class II, JC4584;* (□) *class III, JC4588;* (●) *class IV, JC5176.*

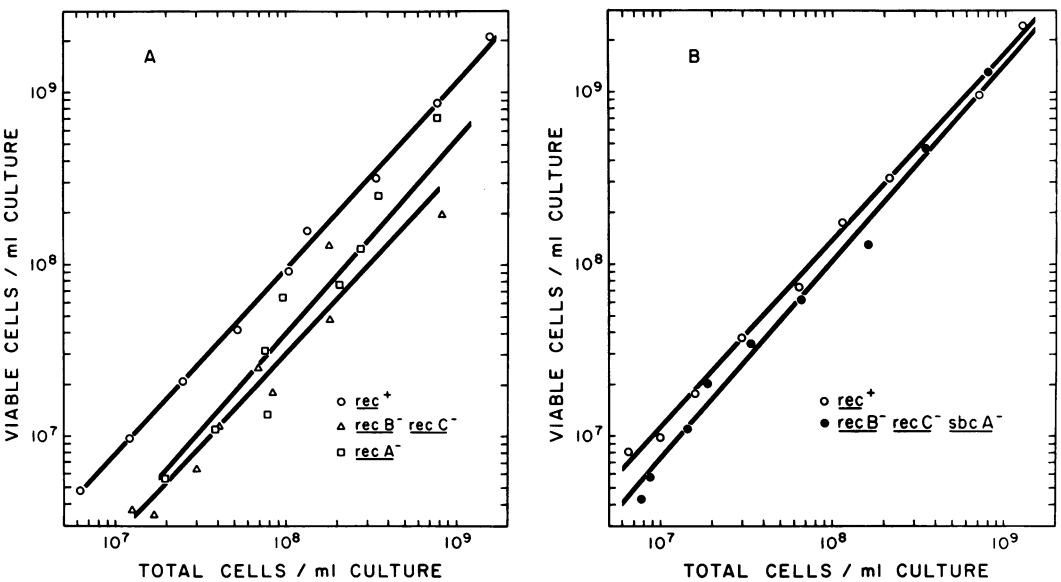

FIG. 3. *Viability of strains belonging to each of the four classes during growth in EM9 medium.* (○) *Class I, JC4583;* (△) *class II, JC4584;* (□) *class III, JC4588;* (●) *class IV, JC5176. Lines were drawn to fit the points based on linear regression analysis.*

TABLE 4. *Viability*

Class	Strain	M9		EM9		Luria	
		% Viable[a]	Normalized[b]	% Viable	Normalized	% Viable	Normalized
I	JC4583	95.0	100	95.0	100	118.2[c]	100
II	JC4584	22.2	23.4	25.9	27.2	40.0	33.8
III	JC4588	50.6	53.4	46.8	49.2	71.0	60.1
I	JC4583			130.7[c]	100		
IV	JC5176			98.0	75.3		

[a] Per cent viable is calculated as the average of the ratios of viable cells to total cells (multiplied by 100) taken at points throughout the growth of the culture.

[b] Per cent viable of the *rec*$^+$ strain has been set at 100, and the per cent viable for each of the other strains in each experiment has been normalized to this value.

[c] The presence of more viable cells than total cells in certain experiments resulted from a delay between the time cells were counted microscopically and the time the sample was diluted and plated for viable count. This delay was constant for the individual parts of the experiment. The data are from two separate experiments.

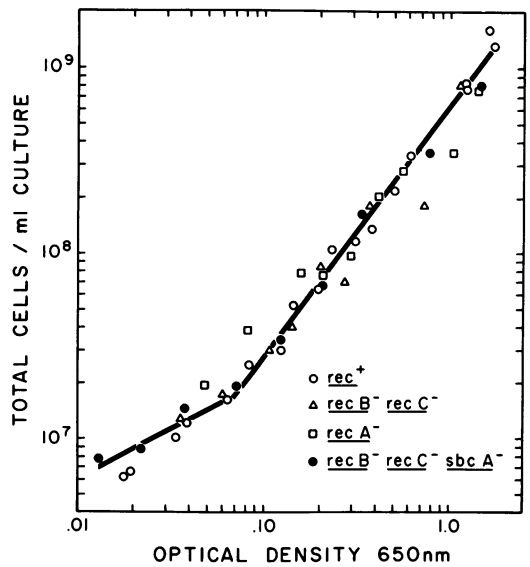

FIG. 4. *Relationship between optical density and total number of microscopically observed particles in cultures of strains belonging to each of the four classes, growing in EM9 medium.* (○) *Class I, JC4583;* (△) *class II, JC4584;* (□) *class III, JC4588;* (●) *class IV, JC5176.*

viable cells in those properties which cause light scattering as measured spectrophotometrically (i.e., size, shape, refractility).

Haefner (9) found that spontaneous lethal sectoring occurs at a high frequency in *rec*⁻ strains. In his experiments he reached his conclusion by determining single-cell pedigrees using micromanipulation. Our results are consistent with Haefner's findings. We must in fact assume that the size (number of cells) of lethal sectors is large enough to account for the growth rates of Rec⁻ strains. Since viability measures only those cells which on plates form colonies visible to the eye, we are determining microscopically whether smaller colonies (lethal sectors) can also be found (as would appear likely).

We have isolated a population of cells from a Rec⁻ culture which are refractile but do not form colonies on solid medium. We are carrying out experiments to determine the biochemical nature and capabilities of this population of defective cells. Study of these cells may clarify the in vivo function of the *rec* products.

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