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Giant Cells of *Escherichia coli*

HOWARD I. ADLER, CLAUDE E. TERRY,[1] AND ALICE A. HARDIGREE
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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A mutant strain of *Escherichia coli* K-12 produced amorphous cells when grown in a variety of media. The *lon*$^-$ allele, known to increase the radiation sensitivity of the cytokinesis mechanism, was introduced into the mutant by means of conjugation. Cells of this recombinant strain grew, after exposure to radiation, into giant amorphous cells, approximately 500 to 1,000 times the volume of a normal *E. coli* cell. These giant cells are analogous to the filaments formed after the irradiation of *lon*$^-$ rod-shaped cells.

Several recent reports have described properties attributable to the "*lon*" locus in *Escherichia coli* K-12 (4, 6, 10). The single most striking expression of mutation at this locus is enhanced sensitivity of the cytokinesis mechanism to a variety of injurious agents. If a "*lon*" mutant is exposed to an agent such as radiation, cell growth continues for many hours during the postirradiation incubation period but is not accompanied by cytokinesis. To date, this phenomenon has only been studied in rod-shaped cells, where it results in the production of long, multinucleate, nonseptate filaments. The filaments, like normal cells, grow only in length, not in width (5). It is the purpose of this report to describe the expression of the *lon*$^-$ allele in cells that are not rod-shaped.

MATERIALS AND METHODS

Organisms. All strains were derived from *Escherichia coli* K-12. Properties pertinent to this study are summarized in Table 1.

Media, conditions for genetic crosses, phase microscope techniques, and irradiation methods used were those described in previous papers (1, 4).

RESULTS

General properties of the mutant strain P678-7. P678-7 is a radiation-resistant mutant of P678 (Fig. 1), isolated after treatment of a logarithmic culture of P678 with triethylene melamine (2). Although we first isolated P678-7 and studied it because of its radiation resistance, it soon became apparent that a more interesting aspect of its phenotype was the unusual morphology of cells obtained from either liquid or solid culture media. The cells tended to be spherical but often had protuberances at one or more points. We do not know whether the unusual cell morphology and radiation resistance are a result of the same genetic alteration. These cells, although reminiscent of those seen in L-form cultures, did not require unusual osmotic conditions for their maintenance or growth, nor did they form colonies of unusual appearance on nutrient or synthetic agar. Nutrient broth cultures of P678-7 grew at the same rate as the parental strain but attained a slightly lower viable titer at stationary phase. P678-7 has retained the auxotrophic markers of the parental strain and is an effective genetic recipient in crosses with Hfr and F$^+$ donor strains. Although the morphology is similar to that reported for a temperature-sensitive mutant by Kohiyama et al. (7), our strain differs in that amorphous cells are produced in cultures grown at 25, 30, 37, or 43 C in a variety of complex and synthetic media. Time-lapse cinematography of individual cells growing and dividing on thin layers of nutrient agar revealed that cell growth and division may occur in a variety of planes; no obvious pattern was observed. This is in marked contrast to P678 which consists of rod-shaped cells of uniform length and width (Fig. 2A).

Introduction of the lon$^-$ *allele into P678-7.* To date, our attempts to map the gene(s) responsible for the unusual morphology of P678-7 have failed. The strain behaves as a recipient in crosses with a variety of Hfr and F$^+$ donors and shows normal segregation of auxotrophic markers, but all progeny retain the unusual cell morphology. The amorphous cell characteristic is not due to a single gene mutation in the lactose-galactose region of the K-12 chromosome. This fact makes it possible to introduce the *lon*$^-$ allele from a *lon*$^-$ donor. The strain 3.300-M6 was used for this purpose. In this donor strain, the *lon*$^-$ allele (located very close to the lactose locus) is asso-

[1] Atomic Energy Commission Postdoctoral Fellow under appointment from the Oak Ridge Associated Universities. Present address: Emory University, Atlanta, Ga. 30322.

TABLE 1. *Properties of Escherichia coli K-12 strains*

Organism	Requirements for growth	Fermentation of lactose	Streptomycin	Sex	Remarks	Source
P678	Threonine, leucine, B_1	−	Resistant	F^-	*lon*$^+$, cells rod-shaped	F. Jacob
P678-7	Threonine, leucine, B_1	−	Resistant	F^-	*lon*$^+$, cells amorphous	This study
P678-A4	Threonine, leucine, B_1	−	Resistant	F^-	*lon*$^-$, cells amorphous	This study
3.300-M6	None	+	Sensitive	F^+	*lon*$^-$, cells rod-shaped	A. Markovitz

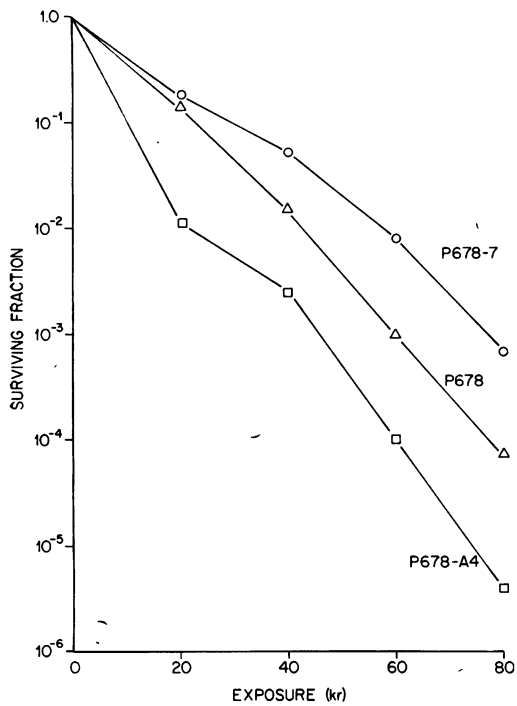

FIG. 1. *Survival curves for cultures grown to stationary phase in nutrient broth plus 1% glucose, exposed to X-irradiation, and plated on nutrient agar. Similar results have been obtained for ultraviolet irradiation (2,537 A).*

ciated with the production of mucoid colonies on synthetic media (8). We therefore mated the *lon*$^-$ donor with P678-7 (*lac*$^-$) in a nutrient broth and plated it on synthetic media that contained lactose as a carbon source and streptomycin to contraselect the donor organism. We picked lactose-positive, mucoid colonies for further purification by restreaking. One of these recombinants, P678-A4, was selected for further study. The recombinant strain exhibited the following properties which provide good evidence for the incorporation of the *lon*$^-$ allele: (i) the colonies of P678-A4 are always mucoid on synthetic media containing sugar; (ii) P678-A4 is more sensitive to both ionizing and ultraviolet (2,537 A) irradiation than its parent (Fig. 1); and (iii) P67δ-A4 cells have a morphology indistinguishable from that of P678-7 (Fig. 2B) and, after irradiation, grow but do not divide. All of the properties listed are identical to or analogous to expressions of the *lon*$^-$ allele in the rod-shaped organisms previously studied (4).

Properties of giant cells produced by the irradiation of a P678-A4 culture. When A4 cells were exposed to 10 to 20 kr of X-rays and placed on thin layers of nutrient agar on microscope slides, 50 to 90% grew into giant cells such as those shown in Fig. 2C, in a period of 3 to 4 hr.

Irradiated cells of the parental strain, P67δ-7, grew only slightly under the same conditions. The majority of these giant cells persisted for 24 hr or more at 37 C but did not divide. During the period of active growth, many optically dense internal structures appeared, coalesced, and frequently disappeared; these structures are as yet unidentified. In addition, a threadlike material filled much of the cytoplasm and persisted throughout the existence of the cell. We have estimated that the volume of the largest giant cells is 500 to 1,000 times that of a normal *E. coli* cell. The largest giant cells were several times larger than mammalian red blood cells, which have diameters of 7 to 8 μ.

The greatest yield of giant cells was obtained when an irradiated A4 population was grown on a thin layer of agar between a cover slip and microscope slide. Cultures grown on agar in petri plates or in nutrient broth yielded fewer giant cells, and they tended to be somewhat smaller.

DISCUSSION

The introduction of the *lon*$^-$ allele into cells that do not have the rod shape typical for *E. coli* has allowed us to produce giant cells that seem to be analogous to the filaments formed from "normal" *E. coli*. In both cases, we are observing the consequences of cell growth without cell division. In rod-shaped cells, growth results in the extension of cells along the long axis; in amorphous cells, such as those formed by P678-7, growth occurs in all directions. For either type of cell, the introduction of the *lon*$^-$ allele increases

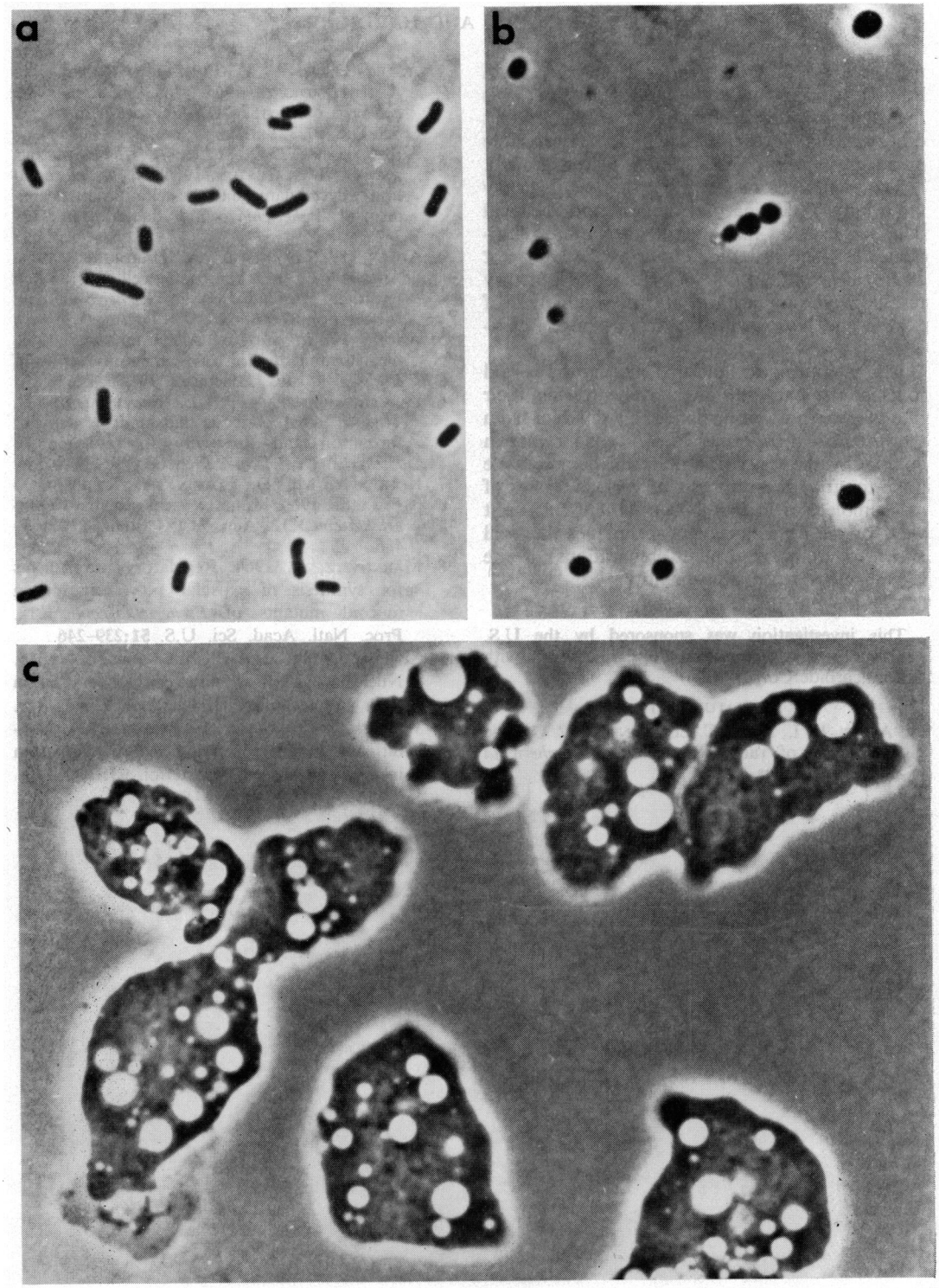

FIG. 2. *Phase microscope photographs of cells on nutrient agar. All sections of this figure are at the same magnification (approximately 2,000 ×). (a) Logarithmic-phase cells of the parental strain P678 (approximately 1 × 3μ). (b) Logarithmic-phase cells of P678-A4, the lon⁻ mutant derived from P678-7. The morphology of P678-7 is indistinguishable from that shown here. (c) Giant cells obtained from the growth of irradiated cells (10 kr) of P678-A4 for 3 hr on nutrient agar.*

the sensitivity of some step(s) of the mechanism leading to cytokinesis. In strains carrying this mutation, it is possible to stop (by radiation and other agents) cytokinesis without appreciable damage to the processes leading to cell growth.

The giant cells may prove to be cytologically interesting. Preliminary cytological and chemical data suggest that they, like filaments, are multinucleate. The distribution of nuclear bodies is not clear, since we have not yet obtained satisfactory results from application of the techniques of Mason and Powelson (9) or from Feulgen staining.

It is interesting to note that, during the period in which the giant cells are actively growing, their rate of growth is not demonstrably different from the rate at which normal *E. coli* cells increase in volume by the formation of a microcolony. We hope to study the synthesis of various classes of macromolecules in the giant cells, but this must wait for the development of techniques that will allow us to harvest pure preparations of the largest cells in reasonable quantity.

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LITERATURE CITED

1. ADLER, H. I., AND J. C. COPELAND. 1962. Genetic analysis of radiation response in *Escherichia coli*. Genetics **47**:701–712.
2. ADLER, H. I., W. D. FISHER, A. COHEN, AND A. A. HARDIGREE. 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. U.S. **57**:321–326.
3. ADLER, H. I., W. D. FISHER, A. A. HARDIGREE, AND G. E. STAPLETON. 1966. Repair of radiation-induced damage to the cell division mechanism of *Escherichia coli*. J. Bacteriol. **91**:737–742.
4. ADLER, H. I., AND A. A. HARDIGREE. 1964. Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720–726.
5. ADLER, H. I., AND A. A. HARDIGREE. 1965. Growth and division of filamentous forms of *Escherichia coli*. J. Bacteriol. **90**:223–226.
6. HOWARD-FLANDERS, P., E. SIMSON, AND L. THERIOT. 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. Genetics **49**:237–246.
7. KOHIYAMA, M., D. COUSIN, A. RYTER, AND F. JACOB. 1966. Mutants thermosensible d'*Escherichia coli* K12. Ann. Inst. Pasteur **110**:465–486.
8. MARKOVITZ, A. 1964. Regulatory mechanisms for synthesis of capsular polysaccharide in mucoid mutants of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S. **51**:239–246.
9. MASON, D. J., AND D. M. POWELSON. 1956. Nuclear division as observed in live bacteria by a new technique. J. Bacteriol. **71**:474–479.
10. WALKER, J. R., AND A. B. PARDEE. 1967. Conditional mutants involving septum formation in *Escherichia coli*. J. Bacteriol. **93**:107–114.