JOURNAL OF BACTERIOLOGY, May 1973, p. 882-884

Vol. 114, No. 2
Printed in U.S.A.

# Selective Autocytotoxicity in a Model System of *Escherichia coli* K-12 Recombinants

DOUGLAS MARTINEZ AND FRANK WHITEHOUSE, JR.

*Department of Microbiology, The University of Michigan Medical School, Ann Arbor, Michigan 48104*

Received for publication 23 January 1973

Autocytotoxicity was shown by Lac$^+$ recombinant strains of *Escherichia coli* K-12 when their growth was inhibited in media containing *o*-nitrophenyl-β-D-galactopyranoside. Lac$^-$ strains without lactose permease or β-galactosidase activity grew well. Selective autocytotoxicity was shown by simultaneous inhibition of Lac$^+$ cells and multiplication of Lac$^-$ cells grown together in this medium.

The term autocytotoxocity has been applied to the sequence of events which occur in a cell when a nontoxic substrate is degraded by an intracellular enzyme to release a toxic component which injures the cell. Selective autocytotoxicity occurs when injured cells are adjacent to uninjured cells lacking the same enzyme (13). Selective autocytotoxicity was demonstrated previously by the selective inhibition of Lac$^+$ *Escherichia coli* mutant cells (13) or transduced cells (F. Whitehouse, Jr., and M. Proctor, Bacteriol. Proc., 1969, p. 148) in medium containing phenolic β-D-galactopyranosides, whereas adjacent Lac$^-$ cells were not inhibited. In the present study recombinants were used to evaluate the requirement for lactose permease and β-galactosidase (EC 3.2.1.23) in autocytotoxicity (AC) of Lac$^+$ cells grown in medium containing *o*-nitrophenyl-β-D-galactopyranoside. Also, a mixture of Lac$^+$ and Lac$^-$ cells was used as a model system to demonstrate selective autocytotoxicity (SAC) in recombinant cells. (This work was presented in part at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minn., May 2-7, 1971.)

Characteristics of *E. coli* K-12 strains were verified by appropriate nutritional and biochemical tests including those for lactose permease (1, 8) and β-galactosidase (2). Hfr strains were isolated (3), and F$^+$ strains were converted to F$^-$ (7) as needed. A standard mating technique was used (4). Relevant genotypes are shown in Table 1. Recombinant Lac$^-$ R2 and Lac$^+$R3 were isolated from a single Hfr1 × F$^-$2 mating after 1 and 10 min, respectively, and were used in the model SAC system. Chemicals used were: *o*-nitrophenol (ONP) (Eastman Organic Chemicals, Rochester, N.Y.); *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Calbiochem, Los Angeles, Calif.); and proflavine (2:8-diaminoacridine) (National Biochemicals Co., Cleveland, Ohio.) A buffered salt solution (12) basal medium was designated BM. Supplemented media (BMGs) was BM plus glycerol (5 g/liter) plus required supplements of amino acids (15 μg/ml) and thiamine hydrochloride (2 μg/ml). BMGs was autoclaved at 121 C for 15 min, and ONP and ONPG were added below 40 C. BMGs + ONPG medium was free of contamination. Stock cultures were grown in nutrient broth (Difco, Detroit, Mich.).

In preliminary experiments, the minimal inhibitory concentration of ONP for 20 × 10$^6$ log-phase *E. coli* cells in 5 ml of BMGs medium at 24 h was 0.03 g/100 ml. Since the weight of

TABLE 1. *Autocytotoxicity: Inhibition of E. coli Lac$^+$ strains R3, Hfr1, and R1 in medium containing ONPG*[a]

| Medium | Cells/ml (×10$^6$)[b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Expt A strains: | | | | Expt B strains: | | |
| | F$^-$2[c] | R2 | R3 | Hfr1 | F$^-$1 | R1 | Hfr2 |
| BM | 5 | 5 | 15 | 7 | 8 | 6 | 8 |
| BMGs | 190 | 2370 | 2600 | 5300 | 4200 | 3000 | 200 |
| BMGs + ONPG | 209 | 2550 | 0.01 | 17 | 3700 | 87 | 189 |

[a] Both β-galactosidase and lactose permease are required.
[b] After 24 h of incubation in separate cultures.
[c] F$^-$2, by proflavin treatment of F$^+$ (AB312) *thr*$^-$, *leu*$^-$, *lacZ*$^-$*Y*$^+$. R2, from F$^-$2 × Hfr1, 1 min; *thr*$^+$, *leu*$^+$, *lacZ*$^-$*Y*$^+$. R3, from F$^-$2 × Hfr1, 10 min; *thr*$^+$, *leu*$^+$, *lacZ*$^+$*Y*$^+$. Hfr1, (AB309) *thr*$^+$, *leu*$^+$, *lacZ*$^+$*Y*$^+$. F$^{-1}$, (200U) *lacZ*$^+$*Y*$^-$. R1, from F$^{-1}$ × Hfr2, *lacZ*$^+$*Y*$^+$. Hfr2, spontaneous from F$^+$ (AB312) *lacZ*$^-$*Y*$^+$.

ONP is half that of ONPG, 0.06% (2 mM), ONPG was used in AC and SAC experiments. Log-phase cells ($1 \times 10^6$ to $7 \times 10^6$) were incubated in BM, BMGs, and BMGs + ONPG media on a reciprocal shaker, 22 cycles/min. at 37 C. Samples were removed at intervals, serially diluted in 0.85% NaCl at 4 C, and plated on Endo agar (Difco) to obtain viable cell counts as indicated by red $Lac^+$ and colorless $Lac^-$ colonies.

**Autocytotoxicity.** Recombinant strains $lacZ^-Y^+$ R2 and $lacZ^+Y^+$ R3 were incubated separately in BMGs and BMGs + ONPG media. Growth curves in Fig. 1 show AC of $Lac^+$ R3 cells in BMGs + ONPG medium where there was immediate and progressive inhibition, although R3 cells grew well in BMGs medium and $Lac^-$ R2 grew well in both media.

The role of β-galactosidase in this AC was evaluated by incubating $lacZ^-Y^+$ strains $F^-2$ and R2 and $lacZ^+Y^+$ strains R3 and Hfr1 separately for 24 h in BMGs and BMGs + ONPG media. Table 1, experiment A shows that only R3 and Hfr1 were inhibited in ONPG medium. In addition, $lacZ^-Y^+$ strain Hfr2, experiment B, grew well in both media. Therefore, even though all strains possessed lactose permease activity to transport ONPG into the cell, only R3 and Hfr1 cells, which have the additional basal β-galactosidase activity to release ONP from ONPG, showed AC. The fact that $F^-2$ and R2 and Hfr2 grew well in BMGs + ONPG medium also indicated that intact ONPG was relatively nontoxic when taken into the cell. ONPG remained intact after transport into constitutive $lacI^-Z^-Y^+$ mutants of *E. coli* and accumulated in an intracellular-extracellular ratio of 40:150, depending upon the extracellular concentration (14). Although ONPG apparently was not an inducer in $lacZ^-Y^+$ mutants, galactosides were still accumulated approximately 10-fold after pretreatment with ONPG due to basal permease activity (10).

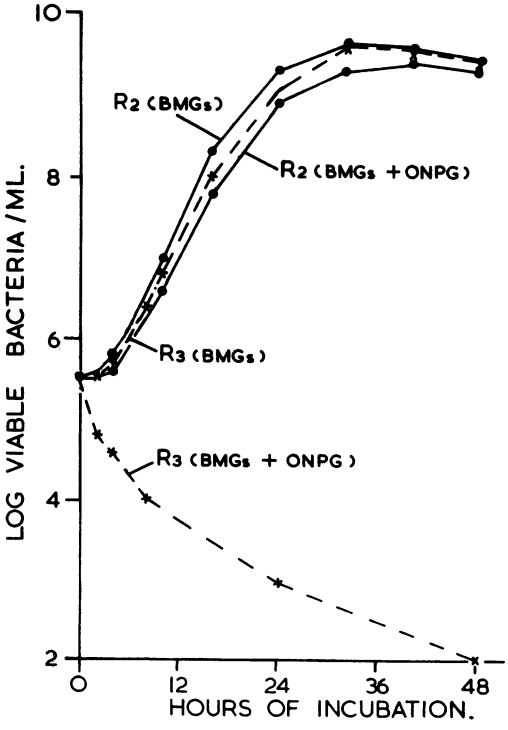


FIG. 1. *Autocytotoxicity. Inhibition of $Lac^+$ E. coli strain R3 in medium containing ONPG. $lacZ^+Y^+$ R3 and $lacZ^-Y^+$ R2 were incubated separately in BMGs and BMGs + ONPG media.*

The role of lactose permease in AC caused by ONPG was evaluated by incubating permease-positive recombinant strain R1 and its permease-negative parent strain $F^-1$ separately in BMGs and BMGs + ONPG media for 24 h. Table 1, experiment B shows that $Lac^+$ R1 grew well in BMGs but was markedly inhibited in BMGs + ONPG. The $Lac^-$ $F^-1$ strain grew well in both media. Therefore, even though both R1 and $F^-1$ possessed β-galactosidase to degrade ONPG into ONP, only R1 with additional permease activity to rapidly transport ONPG into the cell showed AC. Because permease activity is the rate-limiting factor in intracellular hydrolysis of ONPG (11), it is unlikely that the toxic effects observed in $Lac^+$ cells are due to intracellular accumulation of intact ONPG. Intracellular β-galactosidase is located at sites not readily accessible to substrates such as ONPG in the absence of the lactose-permease system (8). However, cryptic $lacZ^+Y^-$ mutants show minimal transport and hydrolysis which increases linearly with ONPG concentrations up to 20 mM (6). The slight inhibition of permease-negative strain $F^-1$ in BMGs + ONPG medium (Table 1, experiment B) probably results from similar transport of ONPG.

**Selective autocytotoxicity.** A mixture of $lacZ^-Y^+$ R2 and $lacZ^+Y^+$ R3 cells was incubated in BMGs and BMGs + ONPG for 72 h, and samples were taken at 24-h intervals. The viable cell counts shown in Table 2 indicate that both strains grew well in BMGs where $Lac^+$ R3 cells increased from 28% of the initial cell population to 37% during the 72-h incubation period. However, in BMGs + ONPG, only the $Lac^-$R2 cells grew well, and $Lac^+$R3 cells decreased from an initial 28% to 0.1% during the 72 h. Restated, the R2 strain was enriched to 99.9% of the population during the 72-h incuba-

TABLE 2. *Selective autocytotoxicity: Inhibition of lacZ⁺Y⁺ E. coli strain R3 grown in mixed culture with lacZ⁻Y⁺ strain R2 in BMGs + ONPG medium*

| Time grown (h) | Cells/ml (× 10⁶) | | | |
|---|---|---|---|---|
| | BMGs medium | | BMGs + ONPG medium | |
| | Strain R2 | Strain R3 | Strain R2 | Strain R3 |
| 0 | 5 | 2 | 5 | 2 |
| 24 | 2,330 | 1,572 | 1,950 | 105 |
| 48 | 3,000 | 1,740 | 2,600 | 18 |
| 72 | 3,300 | 1,900 | 2,200 | 2 |

tion period due to SAC of R3. The apparent slight inhibition of R2 cells by ONPG was probably due to release of ONP into the medium by R3 cells. In previous experiments with higher concentrations of ONPG, as Lac⁺ cells died increasing amounts of ONP appeared in the medium causing late death of some Lac⁻ cells (F. Whitehouse, Jr., and M. Proctor, Bacteriol. Proc. 1969, p. 148).

Therefore, as shown in model *E. coli* systems utilizing the *lac* operon, SAC can be used to select mutant, transduced, and recombinant cells more precisely in conjunction with other conventional techniques which use nutritional requirements and sensitivity to antibiotics or phage. As a general inhibitory method, SAC includes procedures such as selection of Lac⁻ *E. coli* after release of galactose from galactosides in Lac⁺ cells which are sensitive to galactose (5, 9). The concept of SAC provides a rational approach for designing agents to produce damage selectively in any type of cell based on the degradative enzymes of the cell. Agents which elicit their own degradative enzyme would be particularly effective. In some instances bacteria would be appropriate enzyme cell models of other cell types for testing such agents. Theoretically, SAC is applicable to any population of mixed cells which differ in one appropriate degradative enzyme. However, as shown in the present study, additional cellular factors such as transport activity may be required.

We gratefully acknowledge cultures from R. G. Greenberg and R. B. Helling, technical suggestions from Y. Nishioka and B. Wanner, and technical assistance from N. Beckley. This investigation was supported by National Science Foundation grant GU-3470, Project 117, administered by the Horace H. Rackham School of Graduate Studies.

## LITERATURE CITED

1. Beckwith, J. R. 1964. A deletion analysis of the *lac* operator region in *Escherichia coli*. J. Mol. Biol. **8**:427-430.
2. Beggs, W. H., and P. Rogers. 1966. Galactose repression of β-galactosidase induction in *Escherichia coli*. J. Bacteriol. **91**:1869-1874.
3. Berg, C. M., and R. Curtiss. 1967. Transposition derivatives of an Hfr strain of *Escherichia coli* K-12. Genetics **56**:503-525.
4. Curtiss, R., L. J. Charamella, D. R. Stallions, and J. A. Mays. 1968. Parental functions during conjugation in *Escherichia coli* K-12. Bacteriol. Rev. **32**:320-348.
5. Davies, J., and F. Jacob. 1968. Genetic mapping of the regulator and operator genes of the *lac* operon. J. Mol. Biol. **36**:413-417.
6. Herzenberg, L. A. 1959. Studies of the induction of β-galactosidase in a cryptic strain of *Escherichia coli*. Biochim. Biophys. Acta **31**:525-538.
7. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **46**:57-64.
8. Kennedy, E. P. 1970. The lactose permease system of *Escherichia coli*, p. 49-92. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, New York.
9. Malamy, M. 1966. Frameshift mutations in the lactose operon of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **31**:189-201.
10. Muller-Hill, B., H. V. Rickenberg, and K. Wallenfels. 1964. Specificity of the induction of the enzymes of the *lac* operon in *Escherichia coli*. J. Mol. Biol. **10**:303-318.
11. Rickenberg, H. V., G. N. Cohen, G. Buttin, and J. Monod. 1956. La galactoside-permease d'*Escherichia coli*. Ann. Inst. Pasteur **91**:829-857.
12. Vogel, H. L., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biochem. **218**:97-106.
13. Whitehouse, F., Jr., and S. Loyalka. 1967. Antibacterial properties of phenethyl alcohol and phenethyl-β-D-galactopyranoside. Antimicrob. Ag. Chemother. 1966, p. 555-562.
14. Winkler, H. H., and T. H. Wilson. 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biochem. **241**:2200-2211.