Isolation and characterization of autolysis-defective mutants of *Escherichia coli* that are resistant to the lytic activity of seminalplasmin

SANJAY N. CHITNIS, KOLLI S. N. PRASAD and PUSHPA M. BHARGAVA*

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

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Two temperature-sensitive autolysis-defective mutants of *Escherichia coli* were isolated and shown to be resistant to lysis induced by seminalplasmin, an antimicrobial protein from bovine seminal plasma, as well as to lysis induced by ampicillin, D-cycloserine and nocardicin, at 37 or 42 °C but not at 30 °C. The mutants were, however, sensitive to inhibition of RNA synthesis by seminalplasmin even at the nonpermissive temperature. Temperature-resistant revertants of the mutants were sensitive to lysis induced by the various antibiotics at 37 or 42 °C. The mutations in both strains were mapped at 58 min on the *E. coli* linkage map. The lysis resistance of the mutants was phenotypically suppressed by the addition of NaCl. Partial suppression of the lysis-resistant phenotype was also observed in a *relA* genetic background.

Introduction

Seminalplasmin, a 5411 Da basic protein originally isolated as an antibacterial protein from bovine seminal plasma (Reddy & Bhargava, 1979), is now known to have a wide variety of effects on biological systems (Shivaji & Bhargava, 1987; Shivaji *et al.*, 1989). We recently found that seminalplasmin lyses bacteria at concentrations higher than those required for bactericidal activity; characterization of the lytic activity of seminalplasmin led us to the hypothesis that the protein lyses bacteria by activating autolysis (Chitnis *et al.*, 1987).

In order to test our hypothesis and to get an insight into the mechanism of lysis of *Escherichia coli* by seminalplasmin, we have isolated mutants of *E. coli* deficient in autolysis by a procedure similar to that described by Harkness & Ishiguro (1983). The mutants thus obtained were found to be resistant to seminalplasmin-induced lysis. In this paper we describe our studies on these mutants and compare their properties with those of similar mutants isolated earlier (Harkness & Ishiguro, 1983; Shimmin *et al.*, 1984) in the context of regulation of autolysis in *E. coli*.

Methods

Bacterial and phage strains. The bacterial strains used in this study were all derivatives of *E. coli* K12. They are described in Table 1. Phage

P1-kc, used for transduction, and phage λ NK370, used in the Tn10 transposition experiments, were from our laboratory stocks.

Media. Media used in this study included minimal A, LB (Miller, 1972) and tryptic soy broth (TSB; Hi Media Laboratories, Bombay, India). Media were solidified by the addition of agar (Difco) to a final concentration of 1.5% (w/v). When used, the concentration of tetracycline was 5 $\mu\text{g ml}^{-1}$ in minimal medium and 15 $\mu\text{g ml}^{-1}$ in enriched medium.

Chemicals. L-Amino acids and D-cycloserine were purchased from Sigma. Nocardicin was a gift from K. Murayama of Fujisawa Pharmaceutical Co. Ltd, Japan. Ampicillin and tetracycline were purchased from local pharmaceutical sources. [^3H]Uridine was obtained from Bhabha Atomic Research Centre, Bombay, India. All other chemicals used were of analytical grade.

Seminalplasmin. Seminalplasmin was purified from bovine seminal plasma as described earlier (Reddy & Bhargava, 1979); the CM-Sephadex-purified fraction was used in the studies described here.

Isolation of mutants. Temperature-sensitive autolysis-defective mutants were isolated by a procedure similar to that described by Harkness & Ishiguro (1983). Strain C90 was mutagenized with ethyl methanesulphonate (Miller, 1972) and the survivors were grown in TSB overnight at 30 °C. The population of mutagenized cells was subcultured and grown at 30 °C to a density of about 1×10^8 cells ml^{-1} . The culture was shifted to 42 °C for 30 min before D-cycloserine was added to a final concentration of 50 $\mu\text{g ml}^{-1}$. Incubation at 42 °C was continued until no further decrease in OD_{600} could be seen. The surviving cells were collected by centrifugation, washed once with minimal A containing 0.9% NaCl, and grown overnight at 30 °C. The procedure described above was repeated the next day, with the modification that ampicillin at 1 mg ml^{-1} was used instead of D-cycloserine for inducing lysis at 42 °C, and yet again on the third day, with D-cycloserine.

The survivors of the final D-cycloserine treatment were grown overnight at 30 °C and plated on TSB plates at 30 °C. Individual colonies were then tested for temperature sensitivity (lack of colony

Abbreviations: TSB, tryptic soy broth; MTC, maximum tolerable concentration.

Table 1. *Bacterial strains*

Strain	Relevant characteristics	Source or reference
C90	HfrC <i>phoS9</i>	Bachmann (1972)
CSH57	F ⁻ <i>purE trp his arg met ilv leu thi ara lacY gal malA xyl mtl rpsL</i>	Miller (1972)
KL16	Hfr (PO45) <i>thi-1 relA1 spoT1</i>	Low (1972)
GJ157	F ⁻ $\Delta(\arg F-lac)U169 rpsL150 relA1 araD139 flbB5301 deoC1$ <i>ptsF25 *proU::lac</i> stabilized by p1(209) $\Delta putPA101 proP221$ $\Delta(pyr-76::Tn10)$	Gowrishankar (1985)
GJ216	F ⁻ $\Delta(\arg F-lac)U169 rpsL150 araD139 flbB5301 deoC1 ptSF25$ $\Delta putPA101 proP222 \Delta(pyr76::Tn10) recA srl::Tn10$	Gowrishankar (1985)
GJ46	F ⁻ $\Delta(\arg F-lac)U169 rpsL150 relA1 araD139 flbB5301 deoC1$ <i>ptsF25 zfi-900::Tn10</i>	Gowrishankar (1985)
NF161	<i>metB1 argA52 spoT1</i>	
NF162	<i>metB1 argA52 spoT1 relA1</i>	
CCMB1001	C90 <i>lyt-1(ts)</i>	This study
CCMB1002	C90 <i>lyt-2(ts)</i>	This study
CCMB1003	Temperature-resistant revertant of CCMB1001	This study
CCMB1004	C90 <i>zfi-905::Tn10</i>	This study
CCMB1005	CCMB1001 <i>zfi-905::Tn10</i>	This study
CCMB1007	NF161 <i>lyt-1(ts) zfi-905::Tn10</i>	This study
CCMB1008	NF162 <i>lyt-1(ts) zfi-905::Tn10</i>	This study

* *proU* is the new name for the *osrA* locus defined by Gowrishanker (1985).

formation at 42 °C); some of the temperature-sensitive colonies were further purified and screened for resistance to ampicillin- and D-cycloserine-induced lysis.

Genetic techniques. Conjugation was done as described by Miller (1972). Preparation of P1_{kc} lysates and P1_{kc} transduction were performed as described by Gowrishankar (1985). Random transpositions of the tetracycline-resistance transposon, *Tn10*, were obtained in C90 with the use of the λ NK370 lysate, essentially by the method of Kleckner *et al.* (1978).

Antibiotic-induced lysis. Bacterial cultures were initially grown at 30 °C in a gyratory shaking water bath to an OD₆₀₀ of 0.10–0.25 and then shifted to a shaking water-bath at the desired temperature. After 30 min, the antibiotic was added to the required concentration and lysis was followed by continuously recording the decrease in OD₆₀₀ of the culture incubated in a cuvette maintained at the desired temperature.

Maximum tolerable concentration (MTC). Bacterial cultures were grown at 30 °C in a gyratory shaking water-bath to an OD₆₀₀ of 0.1–0.2 and then shifted to a shaking water bath at 42 °C. After 30 min, the antibiotic was added to the desired concentration, incubation continued at 42 °C and lysis followed by measuring the decrease in OD₆₀₀ at various time intervals. MTC is defined as the highest concentration of the antibiotic not causing lysis within 2 h.

RNA synthesis. Exponentially growing *E. coli* cells (OD₆₀₀ 0.3) were incubated with [³H]uridine (sp. act. 14 Ci mmol⁻¹, 518 GBq mmol⁻¹) at a final concentration of 20 μ Ci ml⁻¹ in TSB. Samples (0.1 ml) taken at various times were treated with 1 ml cold 10% (w/v) trichloroacetic acid (TCA) followed by 0.5 mg bovine serum albumin. Precipitation was allowed to continued for 30 min and the TCA-insoluble material was collected on Whatman GF/C filter paper discs, washed with cold 5% (w/v) TCA, dried and counted in a toluene-based scintillation fluid.

Results

Isolation of mutants

A mutagenized *E. coli* culture was subjected to a three-step enrichment procedure to select for temperature-

sensitive, lysis-resistant mutants as described in Methods. The mutant culture thus obtained was plated to obtain single colonies, and the individual colonies were tested for temperature sensitivity. Initially, both temperature-resistant and temperature-sensitive colonies were tested for resistance to lysis by ampicillin. Whereas all the 20 temperature-sensitive colonies tested were resistant to lysis by ampicillin, none of the 20 temperature-resistant colonies tested were observed to be so. Two independent isolations were carried out and two mutants (one from each isolation), designated CCMB1001 and CCMB1002, were chosen for further study. The mutant loci in these two strains are tentatively designated as *lyt-1* and *lyt-2* respectively.

Mapping of *lyt-1* and *lyt-2*

A *Tn10* insertion cotransducible with *lyt-1* was obtainable as described below. Random transpositions of *Tn10* were obtained in *E. coli* C90 using the phage vector λ NK370, as described by Kleckner *et al.* (1980). A P1_{kc} lysate grown on a population of the Tet^r cells was used to transduce the mutant CCMB1001. One of the Tet^r, temperature-resistant transductants so obtained (CCMB1004) was shown to have a *Tn10* insertion with 85% linkage to the wild-type allele corresponding to the *lyt-1* locus.

Preliminary conjugation experiments between CCMB1004 (itself an HfrC derivative) and CSH57 indicated that the site of *Tn10* insertion was between *his* and *argG*. More precise mapping was done (i) by interruption mating after transfer of the *Tn10* insertion into the Hfr strain KL16, and (ii) by two-factor

Fig. 1. Lysis of the mutants and wild-type induced by ampicillin ($1000 \mu\text{g ml}^{-1}$) or seminalplasmin ($200 \mu\text{g ml}^{-1}$). Strains C90 (wild-type) (A), CCMB1001 (*lyt-1*, ts) (B) and CCMB1002 (*lyt-2*, ts) (C) were grown to the exponential phase in TSB at 30°C and lysis was studied at the indicated temperature by continuously recording the decrease in OD_{600} as described in Methods.

transductional linkage analysis with the loci *srl* and *proU* at 58 min on the *E. coli* map (Bachmann, 1972; Gowrishanker, 1985). In a cross between CCMB1004 (donor, *zfi*::Tn10) and GJ157 (recipient, *proU*::*lac*), 200 out of the 350 Tet^r transductants were *lac*, indicating that the Tn10 insertion in CCMB1004 is 57% cotransducible with *proU*. In two crosses with CCMB1001 (*lyt-1*, ts) as recipient and GJ46 (*zfi-900*::Tn10) or GJ216 (*srl*::Tn10) as donor, 55 out of the 90 Tet^r transductants (61%) were temperature resistant in the first case and 40 out of the 75 Tet^r transductants (53%) were temperature resistant in the second case, indicating that *lyt-1* is linked 61% with the *zfi-900*::Tn10 insertion near *proU* and 53% with a *srl*::Tn10 insertion. In accordance with the recommended nomenclature (Chumley *et al.*, 1979), the Tn10 insertion in CCMB1004 has been given the allele number *zfi-905*::Tn10. The above results suggest that *lyt-1*, along with the closely linked *zfi-905*::Tn10 insertion, lies between the *srl* and *proU* loci on the chromosome.

The *zfi-905*::Tn10 insertion was also shown to be 15% cotransducible with *lyt-2* in CCMB1002 (data not shown). Since *lyt-1* and *lyt-2* are, respectively, 85% and

15% linked with this Tn10 insertion, it is possible that the two *lyt* alleles represent different loci. Complementation tests between them have not been done.

Resistance to antibiotic-induced lysis

It is clear from the data shown in Fig. 1 that the mutants CCMB1001 and CCMB1002 were resistant to lysis by both seminalplasmin ($200 \mu\text{g ml}^{-1}$) and ampicillin ($1000 \mu\text{g ml}^{-1}$) at 37°C , the temperature at which the mutants could not form colonies, but not at 30°C , the temperature at which they could grow and form colonies. The results obtained at 42°C (data not shown) were essentially similar to those obtained at 37°C . Resistance to lysis could be seen in terms both of the time taken for the onset of lysis and of the rate of lysis. Mutant CCMB1002 appeared to be the more resistant to lysis, as it lysed only after about 90 min treatment with ampicillin. At lower concentrations of ampicillin the time taken for the onset of lysis was longer. It is noteworthy that the kinetics of seminalplasmin-induced lysis were very similar to those of ampicillin-induced lysis. The mutants were also similarly resistant to lysis

Fig. 2. Effect of ampicillin on viability of mutant CCMB1001 (*lyt-1*, *ts*) (○, □) and its revertant, CCMB1003 (●, ■). Strains were grown to the exponential phase in TSB at 30 °C, then incubated in a shaking water bath at 37 °C for 30 min, followed by addition of ampicillin (100 µg ml⁻¹) (*t* = 0). At various time intervals, a 1 ml sample was taken, chilled and the OD₆₀₀ read (□, ■), while another sample of 0.1 ml was immediately diluted as required, plated on TSB plates and the number of colonies that grew overnight at 30 °C counted to determine viability (○, ●). Initial viability, taken as 100%, was 6×10^7 cells ml⁻¹ in the case of CCMB1001 and 1.6×10^8 cells ml⁻¹ in the case of CCMB1003. No attempt was made to remove the antibiotic before plating as its concentration after the dilution would be too low to inhibit the growth of colonies on the plate.

Table 2. MTC values of ampicillin and D-cycloserine at 42 °C

The MTC is defined as the highest concentration of antibiotic not inducing lysis within 2 h.

Strain	MTC value (µg ml ⁻¹)	
	Ampicillin	D-Cycloserine
C90	10	<50
CCMB1001	75	2000
CCMB1002	150	4000

induced by two other antibiotics, namely nocardicin and D-cycloserine. Resistance to lysis induced by ampicillin and D-cycloserine could also be seen in terms of the MTC values (Table 2). Here again, CCMB1002 was more resistant than CCMB1001.

As the mutants were resistant to lysis induced by a variety of antibiotics, namely seminalplasmin, ampicillin, D-cycloserine and nocardicin, it was important to see if resistance to lysis induced by all the antibiotics was due to a single mutation. We therefore obtained temperature-resistant revertants of CCMB1001 and CCMB1002 by selecting for colonies that grew at 42 °C; CCMB1003, a revertant of CCMB1001, was studied in detail. The

Fig. 3. Effect of seminalplasmin on the synthesis of RNA in mutant CCMB1001 (*lyt-1*, *ts*). The strain was grown in TSB at 30 °C to an OD₆₀₀ of 0.15 and the culture was preincubated at 37 °C for 30 min before studying RNA synthesis at 37 °C in the presence (△) or absence (○) of seminalplasmin (100 µg ml⁻¹), as described in Methods.

revertant was as sensitive as the wild-type to both seminalplasmin- and ampicillin-induced lysis. Essentially similar results were obtained with D-cycloserine and nocardicin. Two other temperature-resistant revertants of CCMB1001 and three revertants of CCMB1002 were also found to be more sensitive to antibiotic-induced lysis than the mutants from which they were derived.

Since the mutants were resistant to lysis induced by ampicillin, we wanted to see if they would also be resistant to the bactericidal activity of ampicillin. The results in Fig. 2 show that mutant CCMB1001 was indeed resistant to the bactericidal activity of ampicillin at 37 °C. These results are in agreement with earlier observations (Shimmin *et al.*, 1984; Kitano & Tomasz, 1979).

Effect of seminalplasmin on synthesis of RNA by the mutants

Inhibition of RNA synthesis was shown to be one of the earliest effects of seminalplasmin in *E. coli* (Reddy & Bhargava, 1978). Our earlier studies (Chitnis *et al.*, 1987) suggested that the inhibitory effect of seminalplasmin upon RNA synthesis occurs independently of its bacteriolytic effect. We therefore studied the effect of seminalplasmin on RNA synthesis by the mutant strain CCMB1001 at 37 °C (Fig. 3). As one would expect for a temperature-sensitive mutant, the incorporation of [³H]uridine into RNA did not increase exponentially with time. Nevertheless, the inhibitory effect of seminalplasmin on RNA synthesis could be seen even at 3 min, the earliest time studied. This observation supports our view that the inhibition of RNA synthesis by seminal-

Fig. 4. Effect of NaCl on ampicillin-induced lysis of mutants CCMB1001 (*lyt-1*, ts). The strain was grown to the exponential phase in TSB at 30 °C and lysis studied at 37 °C as in Fig. 1. NaCl, when used, was added to a final concentration of 0.4 M just before adding ampicillin (1000 µg ml⁻¹).

plasmin is one of its earliest effects on *E. coli* and occurs independently of its lytic effect.

Suppression of the lysis-resistant phenotype of the mutants

Ishiguro and his co-workers had earlier isolated temperature-sensitive mutants of *E. coli* that were resistant to lysis induced by ampicillin and other antibiotics (Harkness & Ishiguro, 1983; Shimmin *et al.*, 1984). The lysis-resistant phenotype of the mutants isolated by Shimmin *et al.* (1984) was found to be suppressed either by the addition of salts such as NaCl or by the presence of a *relA*

genotype in the mutant strain (Kusser & Ishiguro, 1987). We therefore determined whether our mutants behaved similarly. The effect of NaCl on ampicillin-induced lysis of the mutant CCMB1001 is shown in Fig. 4. NaCl at a final concentration of 0.4 M suppressed the lysis-resistant phenotype of the mutant. Further, addition of NaCl to the ampicillin-treated mutant culture resulted in immediate lysis, provided the salt was added at least 20 min after the addition of ampicillin. Similar results were obtained with the mutant CCMB1002 (data not shown). In the case of either mutant, NaCl (0.4 M) had no effect on temperature sensitivity for growth. Similar studies could not be done with seminalplasmin-induced lysis because salts inhibit the lytic activity of seminalplasmin (unpublished results).

In order to study the role of the *relA* gene in antibiotic-induced lysis of the mutants, a P1_{kc} lysate grown on CCMB1005 (*lyt-1 zfi-905::Tn10*) was used to transduce the isogenic pair of strains NF161 (*relA*⁺) and NF162 (*relA*) to Tet^r. One temperature-sensitive, Tet^r transductant was chosen in each case and used to study antibiotic-induced lysis.

Fig. 5 shows seminalplasmin- and ampicillin-induced lysis of *relA*⁺ and *relA* strains carrying the mutant or wild-type *lyt-1* allele at 37 °C. While the *lyt-1*⁺ *relA*⁺ (NF-161) and *lyt-1*⁺ *relA* (NF-162) strains showed little difference in their sensitivity to lysis by seminalplasmin or ampicillin, the *lyt-1 relA* strain (CCMB1008) was much more sensitive than the *lyt-1 relA*⁺ strain (CCMB1007). However, the *lyt-1 relA* strain did not lyse as well as the *lyt-1*⁺ strains NF161 and NF162. Essentially similar results were obtained when the antibiotic-induced lysis was studied at 42 °C but, as

Fig. 5. Suppression of the lysis-resistant phenotype of the *lyt-1* mutation in *relA* strains. Strains NF161 (*lyt-1*⁺ *relA*⁺) (A), NF162 (*lyt-1*⁺ *relA*) (B), CCMB1007 (*lyt-1 relA*⁺) (C) and CCMB1008 (*lyt-1 relA*) (D) were grown to the exponential phase at 30 °C in TSB, and lysis induced by ampicillin (1000 µg ml⁻¹) or seminalplasmin (200 µg ml⁻¹) was studied at 37 °C as in Fig. 1.

expected, there was no difference among the four strains when lysis was studied at 30 °C. These results show that the lysis-resistant phenotype was partially suppressed in strains carrying a *relA* genotype and suggest the involvement of factors other than *relA* in the lysis resistance of the mutants. Further evidence for the involvement of the *relA* gene in the lysis resistance of the mutants was obtained by our observation that addition of gentamicin or chloramphenicol, which act as inhibitors of the stringent response, suppressed the lysis resistance of the mutants CCMB1001 and CCMB1002.

Discussion

Comparison with the mutants isolated earlier

The mutants isolated by us resembled those of Shimmin *et al.* (1984) in that (1) they were temperature-sensitive; (2) they were resistant to lysis only at the nonpermissive temperature (Fig. 1); (3) their degree of lysis resistance depended upon the concentration of the antibiotic used; and (4) their lysis-resistant phenotype was suppressed on addition of NaCl (Fig. 4). The *lyt-1* mutation mapped at 58 min, as did *lytA*, the locus to which one of the two sets of mutations obtained by Shimmin *et al.* (1984) has been assigned (Kusser & Ishiguro, 1987).

However, our mutants differed from those of Shimmin *et al.* (1984) in that they were much more resistant to D-cycloserine-induced lysis (Table 2); also, their lysis-resistant phenotype was suppressed only partially in the presence of a mutant *relA* allele or on addition of inhibitors of the stringent response. Thus, in contrast to the results obtained by Kusser & Ishiguro (1987), we found that *relA* strains carrying a mutant *lyt* allele were much less sensitive to antibiotic-induced lysis than those carrying the wild-type *lyt*⁺ allele (Fig. 5). It remains to be seen whether *lyt-1* defines a locus different from *lytA*.

The mutants isolated by Harkness & Ishiguro (1983) differ from our mutants and from those of Shimmin *et al.* (1984) in that they are not phenotypically suppressed and are not lysed at all, even at high concentrations of antibiotic.

Evidence suggesting defective autolysis in the mutants

As the temperature-resistant revertants showed wild-type characteristics with regard to lysis by the various antibiotics, a single mutation must be responsible for the temperature-dependent lysis resistance of the mutants to the various antibiotics. This observation strongly supports the view that the mutants were defective in autolysis.

We discount decreased permeability as an explanation for the observed lysis-resistant phenotype of the mutants

CCMB1001 and CCMB1002, because addition of NaCl to the mutant cultures treated with ampicillin resulted in immediate lysis provided the salt was added 20 min after the addition of the antibiotic. The fact that the mutants were sensitive to inhibition of RNA synthesis by seminalplasmin immediately on addition (Fig. 3) also rules out decreased permeability as an explanation for the lysis resistance of these mutants.

Resistance of these temperature-sensitive, autolysis-defective mutants to lysis induced by seminalplasmin at 37 or 42 °C but not at 30 °C provides strong evidence for our hypothesis that seminalplasmin, like penicillin, lyses *E. coli* by activating autolysis (Chitnis *et al.*, 1987). However, seminalplasmin-induced lysis differs from that of penicillin in that it does not require continued protein or RNA synthesis (Chitnis *et al.*, 1987). Further, the fact that the mutants were sensitive to inhibition of RNA synthesis by seminalplasmin supports our earlier observation (Chitnis *et al.*, 1987) which indicated that the RNA-synthesis-inhibitory activity of seminalplasmin was independent of its lytic activity.

Mechanism of autolysis deficiency in the mutants

The mutants described here were resistant to lysis induced by the various antibiotics only at the nonpermissive temperature, the temperature at which they failed to grow. Since non-growing cells autolyse poorly (Leduc *et al.*, 1982), there exists the possibility that the defective autolysis in these mutants is merely due to their defective growth as a result of mutation in an essential gene (not involved in autolysis). This trivial explanation is, we think, unlikely because the mutants were resistant to lysis induced by seminalplasmin, which lyses non-growing cells such as cells treated with chloramphenicol or rifamycin (Chitnis *et al.*, 1987). We therefore believe that the mutations are in a gene or genes involved in autolysis, and defective autolysis leads to impaired growth.

If these mutants are genuine autolysis-defective mutants, as we think they are, then the defect(s) may lie either in a gene(s) for an autolysin or in a gene(s) involved in regulation of the autolysin activity. We think it unlikely that the mutation(s) is in the gene for an autolysin because the lysis resistance was suppressed in *relA* strains (Fig. 5). It is most likely, therefore, that the mutants are defective in the regulation of autolysis.

According to Kusser & Ishiguro (1987), the mutants of Shimmin *et al.* (1984) were defective in autolysis because they exhibited the stringent response even without starvation for amino acids. This conclusion was based on the following observations: (1) the mutants showed the stringent response at the nonpermissive temperature only, that is, at the temperature at which they were

resistant to antibiotic-induced lysis; (2) autolysis is known to be inhibited under a stringent response (Kusser & Ishiguro, 1985); and (3) the lysis-resistant phenotype of the mutants was suppressed in a *relA* background or on addition of stringent-response inhibitors. Kusser & Ishiguro (1987) further hypothesized that the loci *lytA* and *lytB* identified by their mutants interact directly or indirectly with *relA* to prevent its activation under non-starvation conditions.

The above model is, however, inadequate to explain the behaviour of our mutants. If the function of the *lyt-I* product is only to keep the *relA* product under control (preventing the stringent response under non-starvation conditions), one would expect *relA lyt-I* strains to be temperature-resistant. However, the *relA lyt-I* strain CCMB1008 was temperature-sensitive, suggesting that the *lyt-I* product has a role in bacterial growth independent of *relA*. The *relA lyt-I* strain (CCMB1008), although more sensitive to antibiotic-induced lysis than the isogenic *relA⁺ lyt-I* strain, was much less sensitive to antibiotic-induced lysis than the *relA lyt-I⁺* strain, NF162 (Fig. 5). This partial suppression of *lyt-I* by *relA* suggests that *lyt-I* has a role in the regulation of autolysis independent of any role it may have through *relA*, and that antibiotic-induced autolysis in *E. coli* may have at least two pathways that differ in their regulation.

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