On Alternatives to Selection-induced Mutation in the Bgl Operon of Escherichia coli

Barry G. Hall
Biology Department, University of Rochester

Selection-induced mutations are nonrandom mutations that occur as specific and direct responses to environmental challenge. Examples of selection-induced mutations have been reported both in bacteria and in yeast. I previously showed (Hall 1988) that excisions of the mobile genetic element IS150 from within bglF are selection induced and argued that they occurred because they were potentially advantageous under the selective conditions employed. Mittler and Lenski (Mittler and Lenski 1992) have argued that such excisions are not selection induced but that they occur randomly in nondividing cells. Here I provide further evidence that IS150 excisions are induced by selection and that the excisions are immediately, rather than only potentially, advantageous to the cell. I also provide evidence that excisions, which Mittler and Lenski claim occur randomly in saturated broth cultures, actually occur after samples from those cultures are plated onto selective medium.

Introduction

Selection-induced, or "directed," mutations are nonrandom mutations that occur as specific and direct responses to environmental challenges (Cairns et al. 1988; Hall 1990, 1991a, 1992). Since 1988 many other examples of selection-induced mutations have been reported both in bacteria (Hall 1988, 1989, 1990, 1991a, 1991b; Böe 1990; Cairns and Foster 1991; Foster 1991; Foster and Cairns 1992; Thomas et al. 1992) and in yeast (Hall 1992; Steele and Jinks-Robertson 1992). At this point selection-induced mutations have been reported to occur in at least seven loci in Escherichia coli and in at least three loci in yeast, and to occur by base substitutions, by positive and negative frameshifts, and by excision of mobile elements from within coding sequences. Selection-induced mutations thus appear to be a general phenomenon, and their occurrence has potentially important implications for our understanding of a variety of biological processes, not the least of which is evolution.

Selection-induced mutations appear to occur primarily in nondividing cells during prolonged, intense selection. The key feature that distinguishes selection-induced from random mutations is that selection-induced mutations are specific, in the sense that they occur because they are beneficial to the cell under those selective conditions (Cairns et al. 1988; Hall 1990).

In 1988 I reported a case of selection-induced excision of a mobile element, in which the excision restored the function of the bglF gene of E. coli K12 (Hall 1988). Wild-type E. coli K12 cells cannot utilize the β-glucoside sugar salicin because the bgl operon is cryptic; that is, the operon cannot be expressed unless a mutation in the bglR site activates the inducible expression of the operon (Prasad and Schaefler 1974; Reynolds et al. 1981, 1984; Schnetz et al. 1987; Schnetz and Rak 1988). Strain χ342LD is further prevented from utilizing salicin by the presence of IS150 within bglF; thus strain χ342LD requires two events, a bglR+ mutation and excision of IS150 from within bglF, to be able to utilize salicin.

To measure the rate at which IS150 excisions occur spontaneously, fluctuation tests were conducted using strain 1011A, which is already bglR+ and thus requires only the excision event in order to utilize salicin. Those tests showed that in growing cultures, under nonselective conditions, excision of IS150 is so rare (<2 × 10⁻¹² cell division) that it cannot be detected. During prolonged selection on MacConkey-salicin plates, however, excision of IS150 was sufficiently common that after 1 week all strain 1011A colonies had produced salicin-positive (Sal+) papillae.

Even strain χ342LD, which requires two mutations to utilize salicin, produced Sal+ papillae on 60% of the colonies after 2 weeks on MacConkey-salicin medium. In contrast, when 28-day-old or 32-day-old χ342LD cul-
onies growing on MacConkey-base medium were tested by replating them onto salicin-minimal medium, no Sal\(^+\) mutants were detected. Because the Sal\(^+\) mutants of \(\chi_342LD\) arose in the presence, but not in the absence, of salicin, it was concluded that the excision mutations were selection induced. Because the Sal\(^+\) mutants of strain \(\chi_342LD\) were the result of two mutational events, it was important to determine whether the two events occurred simultaneously or sequentially. Mutants in which only the excision event had occurred were still Sal\(^-\), but they could be detected by their ability to form Sal\(^+\) papillae within 3 days when plated onto MacConkey-salicin medium. With that assay, it was shown that \(\chi_342LD\) colonies on MacConkey-salicin medium rapidly accumulated Sal\(^-\) excision mutants during the first 7 days of incubation and that Sal\(^+\) papillae arose as the result of secondary \(bglR^+\) mutations that occurred within the population of \(10^6-10^7\) Sal\(^-\) excision mutants per colony. Sal\(^-\) excision mutants of strain \(\chi_342LD\) are genetically equivalent to wild-type cells. Investigations of the \(bgl\) operon at the genetic, physiological, and molecular levels (Prasad and Schaeffer 1974; Reynolds et al. 1981, 1984; Schnetz et al. 1987; Schnetz and Rak 1988) had shown that wild-type \(E.\) coli are not able to utilize salicin; thus it was surprising to observe that excision mutants accumulated so rapidly within \(\chi_342LD\) colonies. A reconstruction test was used to determine whether such excision intermediates could, indeed, grow on MacConkey-salicin medium that had been depleted of other resources. The results of that test indicated that the excision intermediates could not grow, which implied that the excision intermediates accumulated as the result of millions of independent excision events within each colony.

Since the excision intermediates had no detectable selective advantage under the conditions employed, I concluded that the excisions occurred because they were potentially, rather than immediately, advantageous to the cell.

Mittler and Lenski (1992) have recently challenged the conclusions of that investigation, on two grounds. First, they argue that the excision mutants are indeed able to grow under the selective conditions employed. Second, they argue that excisions of IS150 occur in nongrowing populations whether salicin is present or not and that the excision events are therefore not “directed,” or selection induced. Recently they have used that claim to support the argument that selection-induced mutations in general do not occur (Lenski and Mittler 1993). In this communication I present the results of a reinvestigation of these issues and conclude that excision of IS150 is, indeed, selection induced.

Material and Methods

MacConkey medium (Difco) is an indicator medium that permits \(Escherichia coli\) to grow at the expense of small peptides and thus to form colonies, whether or not they can utilize a sugar that is added to the base medium. If cells can ferment an added sugar, such as salicin, colonies are red; if they cannot ferment the sugar, colonies are white. MacConkey medium with no added sugar is referred to as MacConkey-base (MacBase). MacConkey-salicin (MacSal) medium contained 1% (w/v) salicin and was prepared according to manufacturer’s instructions.

Minimal medium consisted of 423 mg sodium citrate, 100 mg MgSO\(_4\) \(\cdot\) 7H\(_2\)O, 1 g (NH\(_4\))\(_2\)SO\(_4\), 540 mg FeCl\(_3\), 1 mg thiamine, 3 g KH\(_2\)PO\(_4\), 7 g K\(_2\)HPO\(_4\), and 1 g carbon source per liter. When required, amino acids were added to a concentration of 100 mg/liter. L-broth (Luria Broth) (Miller 1972, p. 435) was used as a rich, complete medium.

\(Escherichia coli\) K12 Strains

Strain \(\chi_342LD\) is HfrC, \(bglR^0\), \(bglF^+:\)IS150, \(metB1\), \(DelacZ\) 4680, \(relA\) and is phenotypically Sal\(^-\) (Hall 1988; Hall et al. 1989). Strains XSW1, XSW2, XSW3, and XSW4 are spontaneous \(bglF^+\) excision mutants of strain \(\chi_342LD\) and are phenotypically Sal\(^-\) (Hall 1988). Strain XSR1 is a spontaneous \(bglF^+\) \(bglR^+\) mutant of strain \(\chi_342LD\) and is phenotypically Sal\(^+\) (Hall 1988). A \(metB^+\) allele was transduced from the wild-type strain W3110 into strains XSW1, XSW2, XSW3, XSW4, and XSR1 to generate strains XSW1M, XSW2M, XSW3M, XSW4M, and XSR1M. Strain 1011A is HfrC, \(bglR^+\), \(bglF^+:\)IS150, \(DelacZ\) 4680, lacY, \(relA\) \(Degbg1011\), \(rpsE\), \(ampC\) (Hall 1988; Parker et al. 1988; Hall et al. 1989) and is phenotypically Sal\(^-\). All the mutations that distinguish strain 1011A from strain \(\chi_342LD\) were spontaneous, and none (except the \(bglR^+\) mutation) is near the \(bgl\) operon. Strains FL1, FL2, FL3, FL4, and FL5 are spontaneous Sal\(^+\) mutants of strain 1011A.

Results and Discussion

Growth of Excision Intermediates on MacConkey-Salicin Medium

To determine whether \(bglR^0\) \(bglF^+\) excision intermediates could grow on the salicin in MacSal plates after other resources had been exhausted, I used strains XSW1M, XSW2M, XSW3M, and XSW4M, which are four independent \(metB^+\) \(bglR^0\) \(bglF^+\) excision-intermediate mutants of strain \(\chi_342LD\). For each of these strains, about 35 Met\(^+\) Sal\(^-\) cells, together with about 3 \(\times\) 10\(^4\) \(\chi_342LD\) (\(metB1\) \(bglF^+:\)IS150) cells, were spread
onto MacSal plates. The presence of the $\text{metB}^+$ allele allowed the descendants of the excision intermediates to be distinguished from the predominant $\chi^{342LD}$ population.

In early experiments, in agreement with Mittler and Lenski (1992), white ($\text{Sal}^-$) papillae appeared on the lawns, between days 7 and 9 (data not shown). When tested on salicin-minimal medium, those papillae often included a few $\text{Met}^+\text{Sal}^+ (bglR^+ bglF^+)$ cells that were descended from the seeded excision-intermediate cells.

The $\text{Sal}^+$ mutants might have arisen early, from the small population of seeded excision mutants that grew as the lawn grew, or, as Mittler and Lenski argued, the excision mutants might have continued to grow at the expense of salicin to form the observed papillae, and the $\text{Sal}^+$ mutants might have then developed from that population. To distinguish between those possibilities, the experiment was repeated, and the numbers of $\chi^{342LD}$ cells, of $\text{Met}^+$ excision cells, and of $\text{Met}^+\text{Sal}^+$ secondary-mutant cells were determined daily (fig. 1).

As a control, a mixture seeded with about 35 $\text{Sal}^+$ XSR1M ($bglR^+ bglF^+ \text{Met}^+$) cells was also spread onto MacSal plates and monitored daily.

The population of $\chi^{342LD} (bglR^0 bglF::\text{IS150})$ grew to about $3 \times 10^{10}$ cells/plate by day 1 and decreased slowly thereafter, declining to about $3 \times 10^9$ cells/plate by day 12. The seeded $\text{Sal}^+\text{XSR1M}$ cells grew rapidly and, by day 2, represented about 3% of the total population of cells on the plates. The number of $\text{Sal}^-$ excision-mutant cells per plate increased from 35 cells to about 2,000 cells by day 1 and remained at that level until day 4, at which time the cells began to grow and to form papillae that were visible by day 7. On day 8, the first $\text{Sal}^+$ mutants appeared, after which they grew (presumably at the expense of the salicin) so that by day 12 they had become a significant portion of the population of $\text{Met}^+$ cells.

The same pattern of results was seen in each of three similar reconstruction experiments. After a lag period of 4 days, the $\text{Met}^+\text{Sal}^-$ cells began to grow. The conclusion that those cells were growing at the expense of salicin is inconsistent with the observations (1) that they could not form colonies on salicin-minimal medium and (2) that they appeared to have grown as rapidly as did the XSR1M $\text{Sal}^+$ mutants, once they arose. However, it is clear that, as Mittler and Lenski (1992) as-

![Graph showing growth of excision intermediates on MacSal medium.](image-url)
serted, the genetically marked excision mutants do have a significant and reproducible growth advantage over *bglF::IS150* insertion cells on MacSal plates that are exhausted of nonsugar resources, even though the *bgl* operon is cryptic.

Excision of IS150 in the Presence of Salicin

To examine the dynamics with which IS150 excises in the presence of salicin, strain 1011A (*bglR bglF::IS150*) cells were spread onto MacSal plates, which were incubated at 30°C. In the first such experiment, papillae began to appear on day 7 and appeared at an increasing rate for the next several days. The first papillae to appear were red, indicative of salicin fermentation, but as the plates became more crowded, new papillae were pale pink or white. To determine the reliability of using the number of papillae as an estimate of the number of Sal+ excision mutants, each day one papilla was streaked for single colonies onto salicin-minimal medium, and the remaining papillae were transferred by toothpick to salicin-minimal medium as patches. Through day 10, when there were an average of 4.8 papillae/plate, only 3 of the 76 papillae tested were actually Sal+; thus the reliability was 96%. On day 12, there was a burst of papillae, so that each plate produced in excess of 100 new papillae, many of which were white. When replicated to salicin-minimal medium were designated "FL1" through "FL5" and were retained for use in later experiments.

There are two additional operons, the *cel* and the *asc* operons, in which mutations can lead to salicin utilization (Kricker and Hall 1984, 1987; Parker and Hall 1988, 1990a, 1990b; Hall and Xu 1992). Expression of the *cel* operon results in a cellobiose-positive phenotype, while expression of the *asc* operon results in a strong arbutin-positive but weak salicin-positive phenotype. Each of the FL mutants was strongly positive on both arbutin and salicin and negative on cellobiose, which is consistent only with expression of the *bgl* operon. In order to firmly establish that the Sal+ phenotypes of strains FL1–FL5 were the result of excision of IS150 from within the *bgl* gene, the region between bp 2423 and 2934 of the *bgl* operon (Schnetz et al. 1987) was polymerase chain reaction (PCR)–amplified from strains FL1–FL5 and from the parent strain 1011A. In strain 1011A, the 1,441-bp IS150 is inserted between bp 2713 and 2718 of the *bgl* operon (Parker et al. 1988; Hall et al. 1989). The PCR product from strain 1011A was 1.9 kb, while the products from FL1–FL5 were 0.5 kb, showing that IS150 had excised from within *bglF* in those strains.

The results of a second, similar experiment are shown in figure 2A. The population density rose to nearly 1011 cells/plate in 1 day and slowly declined thereafter. Salicin-utilizing papillae began to appear by day 6, and by day 11 all plates contained >150 Sal+ papillae. Although there was experiment-to-experiment variation in the day on which papillae first began to appear, the kinetics of appearance were quite consistent: a lag of about 1 week, after which the number of papillae increased at a fairly constant rate for about 5 days, then a dramatic burst in the number of Sal+ excision papillae such that each plate contained a few hundred Sal+ excision mutants.

**Excision of IS150 in the Absence of Salicin**

To estimate the rate of excision in the absence of salicin, 1011A cells (*bglR+ bglF::IS150*) were spread onto MacConkey medium lacking any added sugar (MacBase). At intervals (fig. 2B), cells were washed from those plates, and the entire suspension of cells was plated onto salicin-minimal medium to determine whether any Sal+ excision mutants were present. No Sal+ excision mutants were ever detected among the 32 suspensions that were tested during that experiment. The rate at which Sal+ excision mutants occur in the presence of salicin (fig. 2A) is such that if Sal+ excision mutants occurred at the same rate in the absence of salicin they certainly would have been detected, unless those Sal+ excision mutants were unable to form colonies on salicin-minimal medium.

A reconstruction experiment was conducted to determine the efficiency with which Sal+ mutants could

---

**FIG. 2.—**A, Accumulation of Sal+ papillae on lawns of strain 1011A on MacSal plates. Approximately 5 x 107 strain 1011A cells were spread onto 60-mm plates containing MacSal medium and incubated at 30°C. Sal+ papillae were counted each day. Daily, to estimate the number of viable cells per plate, cells were washed off one plate that contained no Sal+ papillae, diluted, and plated onto glucose-minimal medium. By day 11, each plate had over 250 papillae, and by day 12, there were over 1,000 papillae/plate. B, Viable 1011A cells on MacBase plates. Approximately 5 x 107 1011A cells were spread onto 60-mm MacBase plates, which were incubated at 30°C. At indicated intervals, cells were washed off plates, appropriate dilutions were plated onto glucose-minimal medium to estimate the number of viable cells per plate, and the remainder of the suspension was concentrated and spread onto a salicin-minimal plate to estimate the number of Sal+ excision mutants that were present. No Sal+ excision mutants were ever detected among the 32 plates tested.
Table 1
MacBase Reconstruction Experiment

<table>
<thead>
<tr>
<th>DAY AND Sal' STRAIN</th>
<th>Glucose-Minimal Medium</th>
<th>Salicin-Minimal Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cells Alone^a</td>
<td>Mixture^b</td>
</tr>
<tr>
<td>Day 6:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL1</td>
<td>127</td>
<td>173</td>
</tr>
<tr>
<td>FL2</td>
<td>97</td>
<td>86</td>
</tr>
<tr>
<td>FL3</td>
<td>101</td>
<td>83</td>
</tr>
<tr>
<td>FL4</td>
<td>110</td>
<td>93</td>
</tr>
<tr>
<td>FL5</td>
<td>109</td>
<td>100</td>
</tr>
<tr>
<td>Day 12:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL1</td>
<td>192</td>
<td>153</td>
</tr>
<tr>
<td>FL2</td>
<td>197</td>
<td>199</td>
</tr>
<tr>
<td>FL3</td>
<td>178</td>
<td>207</td>
</tr>
<tr>
<td>FL4</td>
<td>219</td>
<td>234</td>
</tr>
<tr>
<td>FL5</td>
<td>160</td>
<td>222</td>
</tr>
<tr>
<td>Day 15^c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL1</td>
<td>102</td>
<td>95</td>
</tr>
<tr>
<td>FL2</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>FL3</td>
<td>101</td>
<td>107</td>
</tr>
<tr>
<td>FL4</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>FL5</td>
<td>88</td>
<td>77</td>
</tr>
</tbody>
</table>

^a Duplicate 100-μl samples of an appropriate dilution of resuspended Sal' cells from a MacBase plate were spread onto the indicated medium. Values are average number of colonies per plate. Dilution for day 6 sample was 1.6 X 10^6-fold, and for day 12 and day 15 samples was 10^5-fold.

^b 100 μl of the diluted Sal' cell suspension was mixed with a suspension of 342LD cells from a MacBase plate. The entire mixture was concentrated, and one-half of the concentrated mixture was spread onto one plate of each of the indicated media.

^c 100 μl of the diluted Sal' cell suspension was mixed with a suspension of 342LD cells from two MacBase plates, the entire mixture was concentrated, and one-half of the concentrated mixture was spread onto one plate of each of the indicated media.

have been detected by plating entire suspensions from MacBase plates onto salicin-minimal medium. Each of the Sal' FL strains and the Sal' Met' strains 342LD were spread, separately, onto several MacBase plates, and the plates were incubated at 30°C. On days 6 and 12, cells were washed off the plates to produce dense suspensions. Appropriate dilutions of each of the FL suspensions were plated onto glucose-minimal and salicin-minimal medium to determine the plating efficiencies of the Sal' mutants on salicin relative to glucose (table 1). The same dilutions were mixed with 342LD cells to construct a suspension containing a specific number of Sal' cells. The mixture was concentrated, and one-half of the mixture was plated onto glucose-minimal medium, while the other half was plated onto salicin-minimal medium. Because the medium lacked methionine, required by strain 342LD, only the Sal' FL cells could form colonies on those plates. Indeed, when pure suspensions of 342LD cells were plated, no colonies appeared on the minimal plates. The number of FL cells added to each 342LD suspension was such that there were 100–200 colonies/plate, and the plating efficiencies of those Sal' mutants on salicin were about the same on salicin as on glucose (table 1). Because each minimal plate received only one-half of the 342LD cells that were on one MacBase plate, it could be argued that formation of Sal' colonies on salicin-minimal medium might have been inhibited if all of the cells from one MacBase plate were present. For that reason, on day 15 the experiment was repeated, except that the FL cells were mixed with a suspension of two MacBase plates, and that suspension was concentrated and divided between a glucose- and a salicin-minimal plate. The results were the same as on days 6 and 12.

The results of that reconstruction experiment showed that if Sal' excision mutants were present in populations of 1011A cells that had been incubated for prolonged periods on MacBase (fig. 2B), they would have been detected. The failure to detect any Sal' excision mutants of strain 1011A during more than 4 weeks of incubation on MacConkey medium lacking salicin contradicts Mittler and Lenski's (1992) claim that random excisions, unrelated to the presence of salicin, accounted for the results that I originally reported (Hall 1988).
Understanding Mittler and Lenski's Results

Mittler and Lenski (1992) grew cultures of strain χ342LD (bglR⁺ bglF::IS150) to saturation in rich medium and held them in that condition at 30°C for 15 days. These “treatment” cultures were then diluted 100-fold into fresh broth, grown overnight, and 1% of each subculture was plated onto MacSal. As a control, fresh single colonies of χ342LD were inoculated into L-broth, grown overnight, and plated onto MacSal plates. The median time for the first papilla to appear on the MacSal plates was 3 days for the “treatment” cultures and 6 days for the control cultures, which were assumed to be in the same physiological state as the “treatment” subcultures. Mittler and Lenski pointed out that the bulk of the papillae from the control cultures were neither Sal⁻ excision mutants nor Sal⁺ double mutants; that is, they were papillae that arose as the result of adaptive mutations unrelated to the bgl operon. In contrast, the bulk of the papillae from the “treatment” were either Sal⁻ excision mutants or Sal⁺ double mutants (Mittler and Lenski 1992).

It was necessary for them to distinguish Sal⁻ excision papillae from other Sal⁻ papillae that were unrelated to events within the bgl operon. To avoid that complication, I repeated their experiments using strain 1011A, for which excision mutations produce a clear Sal⁺ phenotype.

Five cultures of strain 1011A (bglR⁺ bglF::IS150) were grown to saturation in L-broth, which is not known to contain salicin or any other β-glucoside sugars, and maintained at 30°C. On days 7 and 14, samples were removed from the cultures and washed to eliminate any nutrients remaining in the L-broth. A portion of the washed culture was diluted 100-fold into fresh L-broth and grown overnight. Two 0.1-ml aliquots of the washed “original” cultures were spread onto salicin-minimal plates, and two 0.1-ml samples were spread onto MacSal. To be sure that the washing procedure itself did not affect the appearance of papillae, duplicate 0.1-ml samples were removed from the original unwashed cultures and spread onto MacSal. The following day, duplicate 0.1-ml aliquots of the washed cultures were spread onto salicin-minimal and MacSal plates. All plates were examined daily for 7 days following plating of the cells.

FL strains (the Sal⁺ derivatives of strain 1011A) formed colonies in 2 days on salicin-minimal medium. In contrast, neither original cultures nor subcultures of L-broth grown strain 1011A ever produced colonies on the salicin-minimal plates, even after the plates were incubated for 7 days.

In no case did any papillae appear on the MacSal plates on the first day after the cells were spread. Sal⁺
(red) papillae did begin to appear on the second day and continued to appear sporadically after that. The kinetics with which papillae appeared were indistinguishable whether or not the culture had been washed and whether or not the cells had been subcultured and were similar for the 7-day and 14-day cultures. Taken together these results show that excision mutations did not occur at a detectable level in old, saturated L-broth cultures.

In contrast to Mittler and Lenski's results, only a minority of plates produced papillae within the first week following plating. Nevertheless, some plates did produce "early" papillae, a result that was not seen when fresh cultures were spread onto MacSal plates (contrast fig. 3 with fig. 2A). It was possible that excision mutations did occur in the saturated LB cultures, but that those Sal⁺ excision mutants (1) were unable to form colonies on salicin-minimal medium and (2) exhibited delayed growth as papillae on MacSal plates. To test that possibility, reconstruction experiments were done by using the Sal⁺ mutants of strain 10¹¹A, strains FL1-FL5.

The reconstruction experiments were conducted in the same way as the broth experiments described above: five 10¹¹A cultures and cultures of the Sal⁺ strains FL1-FL5 were grown to saturation in L-broth and maintained at 30°C for 2 weeks. Again, the populations were sampled at 7 and 14 days. On day 14, 12 samples from each culture were spread onto salicin-minimal and MacSal plates, and 20 samples were similarly spread from each of the subcultures the following day. Because previous experience had shown that washing did not affect the rate at which papillae appeared, only washed samples were used in the reconstruction experiment. No colonies appeared on salicin-minimal medium, and no papillae appeared before 2 days of incubation on MacSal (fig. 3).

After the 10¹¹A samples were spread, appropriate dilutions of the washed FL cultures were mixed with the washed 10¹¹A cultures, and duplicate 0.1-ml samples were spread onto MacSal and salicin-minimal medium. Equivalent dilutions of the FL cultures were also plated onto MacSal and salicin-minimal medium in the absence of any 10¹¹A cells. Table 2 shows that for 14-day-old L-broth cultures, cells of the Sal⁺ FL strains formed colonies with roughly equal efficiency on salicin-minimal medium and on MacSal medium, when plated alone, and formed papillae on day 1 on MacSal and colonies on salicin-minimal medium on day 2, with about that same efficiency, when mixed with the 10¹¹A cultures. Seven day-old cultures behaved similarly (data not shown).

These results indicate that if Sal⁺ excision mutants had arisen during prolonged incubation of strain 10¹¹A in L-broth, those excision mutants would have been detected both as colonies on salicin-minimal plates and as papillae after 1 day of incubation on MacSal plates. No such mutants were detected. Since the "early" papillae
Conclusions

One of the more puzzling aspects of the original work on this topic (Hall 1988) was the conclusion that selection-induced excisions occurred because they were potentially, rather than immediately, advantageous to the cell. That conclusion was based on the failure to detect growth of a Sal− excision intermediate on MacSal medium in which other resources had been depleted. The design of the reconstruction experiment that led to that conclusion involved the use of an antibiotic, chloramphenicol, to inhibit the growth of the excision intermediate until the majority population of chloramphenicol-resistant cells had grown up. It now seems likely that Mittler and Lenski were correct when they suggested (1) that the design of that experiment was flawed thereby and (2) that the flaw led to an incorrect conclusion (Mittler and Lenski 1992). The observations presented here confirm Mittler and Lenski’s contention that excision-intermediate mutants are able to grow on MacSal medium after other resources are exhausted. The physiological basis of that growth is unclear. Nevertheless, it is now clear that the excision mutations are consistent with other selection-induced mutations, in that they occur when they are immediately, rather than only potentially, advantageous.

In contrast, Mittler and Lenski’s conclusion that excision mutations occur in aged saturated L-broth cultures is not supported. That conclusion depended on the untested assumption that “early” papillae on MacSal plates were the result of excision mutations that occurred in the broth cultures. The use of strain 1011A, in which excision mutations generate a selectable Sal+ phenotype, has shown that excision mutants could not be detected in the absence of salicin either on MacConkey medium, the condition most relevant to the original study, or in L-broth, the condition relevant to Mittler and Lenski’s study. The use of Sal+ derivatives of that same strain in-relevant, that the events can truly be called “selection induced.”

Acknowledgments

I am grateful to Shariff Bayoumy for his expert technical assistance. This work was supported by a grant from the National Science Foundation.

LITERATURE CITED

———. 1990. Spontaneous point mutations that occur more often when they are advantageous than when they are neutral. Genetics 126:5–16.


JULIAN P. ADAMS, reviewing editor

Received August 13, 1993

Accepted October 29, 1993