Strains of *Salmonella typhimurium* and Other *Salmonella* Species Used in Genetic Analysis

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135

HISTORICAL PERSPECTIVE

The strains of *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium) which have been subjected to the most rigorous genetic analysis were originally collected and studied by Lilleengen (36). He tested 703 different strains of *S. typhimurium* for sensitivity to a set of 26 different phages and separated most of these strains into 25 different phage sensitivity types. Strains representing many of these phage types were obtained by Zinder and Lederberg (79) and used for a study of genetic exchange. From 20 of these strains, which were labeled LT1 through LT22 (LT representing Lilleengen type), they isolated mutants which required amino acids or which failed to ferment specific sugars; pairwise combinations of different strains were mixed and then plated on minimal medium to select prototrophic recombinants. Some of the combinations yielded prototrophs, but none or few prototrophs were found on the control plates inoculated with a single strain. Combinations involving auxotrophs derived from strain LT22, especially in combination with auxotrophs derived from strain LT2, produced the largest numbers of prototrophs, yielding up to 10^{-5} prototrophs per cell plated. Crosses in which LT22 was not involved gave infrequent and sporadic prototrophs. LT22 was shown to be lysogenic for a virus (called PLT22 by Zinder and Lederberg but now called P22). Extensive analysis (79) revealed much about this process of genetic exchange, which Zinder and Lederberg dubbed transduction but which is now called generalized transduction (see chapter 130) to distinguish it from the related process called specialized transduction (see chapter 131). Though many combinations were tested, only crosses involving strains LT2, LT7, and LT22 were extensively reported (79).

Experiments on adsorption of the transducing phage P22 indicated a correlation of adsorptive abilities of cells and their immunological specificities. Many different *Salmonella* serotypes were tested, and only those containing O factor 12 adsorbed the filterable agent (phage P22). Strains not containing O factor 12 gave no transductants. Factor 12, found in *Salmonella* spp. of O groups B (which includes *S. typhimurium*), D1, and A, is one of the antigenic factors associated with the O-specific polysaccharide side chain of the lipopolysaccharide (LPS) and is determined in part by rhamnose, a sugar in the repeat unit of the O chain (41). Phage P22 adsorbs to this specific component; digestion of the LPS by an endorhamnosidase enzyme in the phage tail facilitates entry of the phage DNA into the cell (37).

The initial studies of Zinder and Lederberg (79) concentrated on transductants obtained by intercrosses of different strains such as LT2 and LT22, but these investigators also revealed that phage-mediated crosses of different auxotrophs isolated from a specific strain, either within LT2 or within LT7, also yielded transductants, provided that phage P22 was present to mediate genetic transfer.

M. Demerec obtained the LT strains of *S. typhimurium* from Zinder and Lederberg and began an exhaustive series of studies reported initially in the mid-1950s (18, 20, 21, 23) and continuing until his death in the mid-1960s. The most important materials used were mutant strains derived from *S. typhimurium* LT2 and, to a lesser degree, from strain LT7. Phage-mediated transduction was effected by use of phage P22, derived from strain LT22.

Strain LT7 was not used extensively, because many isolates have high spontaneous rates of mutation, which are due at least in part to a mutator gene (22, 48) later designated *mutL* and located near *purA* at 96 min on the linkage map (31); alleles in these genes were also isolated in LT2 (65, 66). LT7 also carries a

colicin factor (8) of type ColIa (54); the presence of this factor has been confirmed by E. Eisenstadt (personal communication). Demerec and others largely abandoned the use of LT7, so that most strains of *S. typhimurium* presently used for genetic analysis derive from strain LT2. The entire set of LT strains was provided by J. Lederberg and is maintained at the *Salmonella* Genetic Stock Centre (SGSC) as kit 14A.
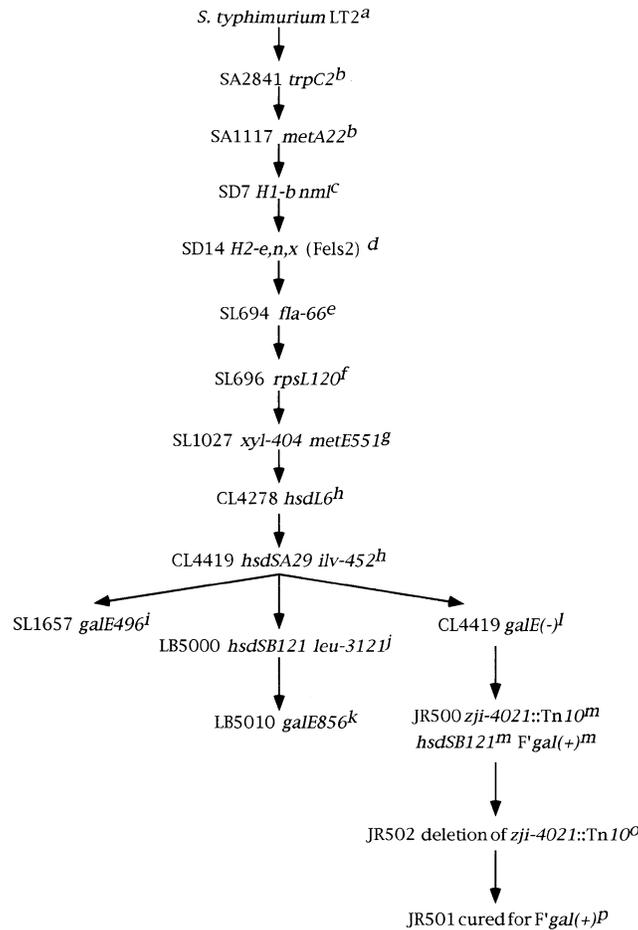
FIGURE 1 Origin of lines of *S.typhimurium* LT2 leading to SL1027 strains, used for isolation of numerous other mutants, including strains which are deficient in host restriction systems. These strains are useful in intergeneric transfer of chromosomal genes and plasmids. The genotype of each strain includes all the mutations shown in the strains from which it originated. Thus, LB5010 is *trp*C2 *met*A22 H1-*b* H2-*en,n,x nml (Fels2)* *fla*-66 *rps*L120 *xyl*-404 *met*E551 *hsd*L6 *hsd*SA29 *hsd*SB121 *ilv*-452 *leu*-3121 *gal*E856. See Sanderson and Roth (61) for a description of the genetic symbols used. Footnotes in the figure are as follows. [a]The original wild-type LT2 line was obtained by N. Zinder from K. Lilleengen. [b]*trp*C and *met*A mutants were isolated in the laboratory of M. Demerec. The *trp*-2 allele was originally labeled *trp*B, then placed in *trp*E, and finally changed to *trp*C to correspond to the nomenclature of E. coli K-12(61). [c]The *fli*C allele, determining phase 1 flagellin of antigenic character *i* of group B *Salmonella* spp., was replaced by the *fli*C(b) allele of *S.abony* by P22-mediated transduction. This strain simultaneously lost activity for *nml*, a gene located close to *fli*C, so that the flagellins, phase 1 or phase 2, made by SD7 and its descendants lack the methyl radical attached to the epsilon-amino group of about half the lysine residues in flagellin (45, 70). [d]The wild-type *fli*B (formerly H2) allele of SD7, which determines flagellin of antigenic type 1,2, was

replaced with the *flj*B allele of S. abony by P22-mediated transduction. SD14 was found to be accidentally cured of (and thus sensitive to) phage Fels2, which along with Fels1 is carried by most other LT2 lines. Loss of Fels2 causes no change in phenotype except for sensitivity to phage Fels2 and ES18 (34). However, lysates made from most other lines of LT2 contain Fels2, which will form plaques on SD14 and its descendants. eSelection for resistance to flagellotropic phage Chi (30). The fla-66 mutation is contransducible with *fli*C but has not been assigned to any of the several *fli* genes originally classified as *fla*A. [f]Selection for resistance to 1 mg of streptomycin per ml. The mutation was originally called *strA*120, but this gene for high-level streptomycin resistance, which determines the 30S ribosomal subunit protein S12, is now called rpsL (Stocker, unpublished data). [g]Treatment with ethyl methanesulfonate and isolation of a non -xylose-fermenting strain. SL1027 was discovered to have also a "leaky" mutation, *met*E551, causing partial requirement for methionine or vitamin B12 (thus distinguishable from the nonleaky mutation at *met*A which causes a requirement satisfied by methionine or homocystine) (Stocker, unpublished data). hCL4419 was isolated from SL1027 by two independent treatments with nitrosoguanidine with selection for loss of the host restriction activity of genes hsdL (which controls the LT restriction system) and *hsd*SA alleles are both r-m+ (15; C. Colson, personal communication). This strain has also acquired the ilv-452 mutation, which causes a leaky and revertible requirement for valine. This requirement sometimes reappears in the descendants of variants selected as valine independent; therefore, it is advisable to supplement defined medium for descendants of CL4419 with an isoleucine-valine mixture. iA spontaneous *gal*E (galactose epimerase) mutant was isolated by selection for resistance to phages FO (=Felix O) and P22. Thus, this strain, like other *gal*E mutants, makes galactose-deficient LPS and hence is resistant to phage P22 if grown on nutrient broth, but it is sensitive to phage P22 if grown on the same medium supplemented with galactose (and glucose, to prevent galactose toxicity). It is sensitive to phage P1 on medium not supplemented with galactose; on such medium, an incomplete LPS core of chemotype Rc is available for adsorption of phage P1, whose adsorption site is not masked by long O side chains (53). [j]The *hsd*SB121 allele, isolated after treatment with nitrosoguanidine, is r-m+ (16). The *hsd*SB gene controls the SB restriction system. The leu mutation originated in the same isolation step. kThe galE856 allele arose by spontaneous mutation. lA spontaneous *gal*E (galactose epimerase) mutant was isolated by selection for resistance to phage FO (72). mThe zji-4021::Tn10 allele from a pool of Tn10 insertions was inserted into LB5000 and then transduced by Pl into CL4419 accompanied by hsdSB121 as an unselected marker (72). F'gal(+) was introduced by conjugation. oA loss of Tn10 resulted from selection for Tet sensitivity on Bochner's medium (10). pSpontaneous curing of the F' gal(+). Note: For unknown reasons, the modification phenotype of the hsdSA29 allele in LB5000 and LB5010 was unstable, but the same allele in CL4419 was stable. Therefore, Tsai et al. (72) transferred the hsdSB121 allele from LB5000 into CL4419 to produce JR502 and JR501. These strains are stably r-m+ for each of the three restriction systems and thus are more suitable than LB5000 and LB5010 as hosts for the transfer of DNA into S. typhimurium LT2.

Kauffman (30a) designated all strains of *Salmonella* which differ in either the O (somatic) antigen or the H (flagellar) antigen as different species, and this has resulted in over 2,000 named species; for *Escherichia coli,* serotype designations (e.g., O1, O7, etc.) are used to distinguish strains with equivalent variations. Using DNA-DNA hybridization methods, Crosa et al. (17) showed that all strains of *Salmonella* belong to the same DNA-DNA hybridization group; thus, all *Salmonella* spp. might also be considered a single species. The species name *Salmonella enterica* was proposed to incorporate all serotypes (35); some modifications were suggested later (52). However, as an interim measure, we have chosen in this review to continue to use the serotype names as species; thus, we use *S. typhimurium* rather than *S. enterica* subsp. *enterica* serotype Typhimurium or other possible names. We have two reasons for this: first, downgrading the species designation to a serotype may mean that part of the name is not maintained, and thus, e.g., strains of *Salmonella typhi* might be known only as *S. enterica,* with consequent risk to their handlers; second, though there is agreement that the DNA-DNA reassociation group appears to form a distinct group,

here categorized at the genus level as *Salmonella,* there is no consensus as to how frequently it should be subdivided, and the decision not to greatly subdivide the *Escherichia* group is no easier to justify than the decision to do so in *Salmonella.*

CHARACTERISTICS OF STRAIN LT2 AND SOME OF ITS DERIVATIVES

Strain LT2 has proven to be suitable for use as a representative of the wild type of *S. typhimurium.* It is prototrophic, growing well, though less rapidly than on rich medium, on defined medium with ammonia as nitrogen source and with its carbon and energy needs satisfied by glucose, glycerol, and various simple organic acids, including citrate (which cannot be utilized by *E. coli*). It has the smooth LPS normal for the species, including O factor 5 (determined by an acetyl group on the abequose branch of the O repeat unit and absent in some "wild" isolates of *S. typhimurium*). Very useful derivatives of strain LT2 were derived by Ames et al. for the widely used tests of mutagens and carcinogens (2). Analysis of wild-type strains of *S. typhimurium* by multilocus enzyme electrophoresis shows that LT2 belongs to electrophoretic type Tm1, the most common of the electrophoretic types of this species (6).

However, LT2 has certain characteristics which require attention when it is used in genetic analysis. First, it carries a plasmid of about 90 kb, which has been called the virulence plasmid, the "cryptic plasmid," MP10, and the LT plasmid (68, 69); we use the term pSLT (60). This plasmid, derived from the original LT2 line, is present in practically all the strains of LT2 used in genetic analysis. A plasmid of similar size is present in about half of the *S. typhimurium* lines isolated directly from nature (3). In most circumstances, pSLT has no detectable effect on phenotype. A virulence region on this plasmid, designated *spv,* is involved in multiplication of bacteria which have invaded the mesenteric lymph nodes and spleen from the intestines of orally inoculated mice (26, 57).

pSLT is Fin$^+$ (F-fertility inhibiting), for it inhibits the fertility of F-factor-mediated conjugation; methods to circumvent this effect are outlined in chapter 128, where various F$^+$ and Hfr strains are described. The plasmid is in a different incompatibility (Inc) group from plasmid vectors used in molecular genetics, so maintenance of the vector or of a hybrid plasmid in *S. typhimurium* is not difficult. When DNA of a vector such as pBR322 is isolated, a small amount of pSLT DNA is also obtained. However, there are many more copies of the smaller, multicopy plasmids, so that after endonuclease digestion, fragments of pSLT are barely visible on the gel and do not confuse restriction analysis. Sources of plasmid-free strains and methods for isolating plasmid-free strains are summarized by Sanderson in chapter 128 of this volume.

Strain LT2 is sensitive to (and can be lysogenized by) P22, which is used extensively as a general transducing phage; LT2 is likewise sensitive to the related phages of group A1-A2 of Boyd (13) and the less closely related phages A3 and A4, all of which can effect general transduction. All the A phages are also converting: the A1-A2 phages, including P22, cause the addition of an α-1-6-linked glucose to the galactose of the O repeat unit of LPS and thus the development of antigenic factor O1 and a reduced ability to adsorb A phages; phages A3 and A4 cause acetylation of the rhamnose of the repeat unit, with almost complete loss of adsorbing capacity but no change in O antigenic character (74). Other *S. typhimurium* strains vary in respect to their reactions with P22 or other A1-A2 phages. A considerable proportion of isolates are lysogenic for phages of the A1-A2 group (and in consequence make the form-variable O factor 1). Such lysogeny confers immunity to the corresponding phage but not necessarily to other A1-A2 phages; the pattern of cross-immunity of these phages is complex. For instance, strain C5, widely used in mouse infection experiments, has factor O1 and releases a corresponding phage but supports multiplication of phage P22. Some *S. typhimurium* isolates adsorb P22 but, for unknown reasons or because of the presence of a prophage or plasmid which interferes with phage multiplication, do not support its multiplication. Strain LT2 is lysogenic for phages Fels1 and Fels2 of Boyd's group B, less heat resistant than the A phages, and not known to have any converting ability or transducing ability (76). Fels2 prophage is inserted into the chromosome near 57 min (75), and Fels1 lies between 18 and 21 min (K. K. Wong and M. McClelland, personal communication). The generalized transducing phage P22 can recombine with

prophages Fels1 and Fels2 to produce hybrid phages, but only at extremely low frequencies (76, 78).

Strain LT2, like other *S. typhimurium* isolates (and like strains of nearly all subspecies 1 serotypes), is sensitive to phage FO (Felix O, or O1), which uses the *Salmonella* type of complete LPS core as its site of adsorption.

Various phages which adsorb to surface components other than the O side chain of LPS act on strain LT2 and many other *S. typhimurium* isolates. These include Chi, which adsorbs only to functional flagella and so can be used for selection of nonflagellate or "paralyzed" mutants, and phage ES18, which adsorbs to both smooth and rough strains (34), providing they have the wild-type product of the gene equivalent to *fhuA* (formerly *tonA*) of *E. coli* (25, 71). Adsorbtion of P1, the most efficient generalized transducing phage of *E. coli* K-12, is blocked by smooth side chains of the LPS, but rough mutants of *S. typhimurium,* especially those with *galE* (UDP-galactose epimerase) mutations, producing LPS of Rc (galactose-deficient) chemotype, are sensitive to P1 and can be used for generalized transduction by this phage. Several rough-specific phages, including 6SR, Br2, Ffm, and C21, are of use for at least tentative recognition of the type of incomplete core made by nonsmooth mutant strains.

The presence of smooth LPS modifies the genetic properties of LT2 for transduction, conjugation, and transformation. The strains most commonly used in genetic analysis of *E. coli* are K-12 and B; both are rough mutants lacking the O (somatic) side chain of the LPS (55, 63). However, *S. typhimurium* LT2 possesses O side chains on the LPS and thus is characterized as a smooth strain. Genetic analysis of O side chain synthesis and assembly in *S. typhimurium* has been reviewed by Mäkelä and Stocker (43) and by Schnaitman and Klena (64).

For phage-mediated transduction, the side chains are essential for adsorption of P22, the most convenient and adaptable phage for generalized transduction in *S. typhimurium,* or for adsorption of its high-frequency transducing mutant P22 HT105/1 (62); Zinder and Lederberg (79) noted that rough mutants do not adsorb P22 and are not transducible. The *rfb* and *rfc* genes of *S. typhimurium* on a cosmid allow expression of the O side chain of group B salmonellae (51); this cosmid permits transduction by P22 in *E. coli, Klebsiella* spp., and other bacteria and is available from the SGSC as kit 9B. Phage KB1, a generalized transducing phage (11, 46) which is able to propagate on P22 lysogens (B. A. D. Stocker, unpublished data), is likewise O specific for adsorption. Rough mutants lacking the side chains, though not transducible by P22, can be transduced by phage ES18, ES18*h1,* or P1. The presence of smooth side chains also reduces the efficiency of strains as recipients in F-factor-mediated conjugation; for a review, see chapter 128.

LPS also affects transformation efficiency. Wild-type (smooth) strains of *S. typhimurium* and most rough mutants are transformed at low frequency by plasmid DNA in the $CaCl_2$ shock procedure. Rough mutants with LPS of specific chemotypes, however, especially *galE* mutants with Rc LPS or *rfaF* mutants, are transformed at a rate 10- to 100-fold greater than the rate for wild-type strains; transformation rates of up to 5×10^5 transformants per μg of pBR322 DNA have been obtained (42). Electrotransformation by the same plasmids gives much higher frequencies (10^8 to 10^9 transformants per μg of DNA); smooth as well as rough strains give this high rate (9).

Thus, smooth strains are optimal for P22-mediated transduction, while rough mutants are best for conjugation, and rough mutants of specific classes are best for transformation. Strains carrying mutations in the *galE* gene are especially suitable for all of these types of exchange. If *galE* mutants are grown on medium containing glucose plus galactose, they make smooth LPS (24), but if the cells are grown on a medium not supplemented with galactose but with traces of glucose added to repress the *gal* operon and so prevent uptake of traces of galactose present as a contaminant in complex media, LPS of the Rc (galactose deficiency) phenotype is produced. The *galE* mutants can be isolated by selecting for resistance to phage Felix O and screening for non-galactose-fermenting mutants (73) or by selecting for resistance to 2-deoxygalactose and screening Gal⁻ mutants for the GalE phenotype (1). They can also be introduced by joint transduction with Tn*10* insertions which map close to the *galE* locus, as described elsewhere (42).

Most of the strains used in the laboratory of M. Demerec were derived by selection of mutations directly in strain LT2, so they carried only single mutations. Double or triple mutants were obtained by selecting further mutations in a strain already carrying one or two mutations. These strains were used in transduction studies, but strains with complex sets of markers were seldom selected, so the pedigrees of most of the mutants isolated and studied were very simple. For example, *his-1, his-2,* etc., represent mutant strains isolated directly from LT2 and having no other (known) genetic divergence from LT2. These genetic designations were later used as the basis for recommendations concerning genetic nomenclature (19) and are also the basis for recommendations in "Instructions to Authors" in *Journal of Bacteriology* and other publications. Many of these strains were designated only by the gene designation of their mutant gene and the allele number and were not given a stock designation. In recent years, most mutant strains have a more complex genealogy, and records of this are facilitated by the use of stock designations by each individual laboratory. Stock designations for use by a laboratory and allele numbers for designation of mutations in specific genes can be obtained from the SGSC; equivalent information for *E. coli* can be obtained from the *E. coli* Genetic Stock Center, Department of Biology, Yale University, New Haven, CT 06510.

Thousands of mutant strains of *S. typhimurium* LT2 (and a few of strain LT7) were isolated in the laboratory of M. Demerec by numerous investigators who came to his laboratory, initially at Cold Spring Harbor, later at Brookhaven National Laboratory, and briefly at C. W. Post College. These strains were widely disseminated and have become the starting point for much of the genetic work in this species. The derivation of a specific set of strains of LT2 is described in Fig. 1. Strain SL1027, shown in Fig. 1, has been used as the parent for the isolation of many other mutants (e.g., see reference 73). In addition, mutants described in this figure are deficient in the host restriction systems which restrict acceptance of DNA transferred from other species. Some of these nonrestricting strains are also *galE* mutants and hence are effective recipients for plasmid-mediated transformation by Ca^{2+} shock (16, 42). Thus, they are good recipients for intergeneric transformation of plasmids. Moreover, they retain wild-type modification ability for all three systems and are good donors of plasmid DNA of *E. coli* origin to LT2 lines with wild-type restricting abilities.

Investigators who are terminating genetic work with *S. typhimurium* are invited to contact the SGSC to have their most important strains stored and made available to other investigators.

Many smooth sublines of *S. typhimurium* LT2 remain virulent for mice (28). A strain of LT2 has been stated to be mildly infectious at high doses for humans (5), but the strain involved in this incident was actually an LT7 derivative, which is more virulent (for mice) than LT2; in this incident, the worker swallowed a large dose of cells while mouth pipetting a thick, resuspended cell paste. Many years of experience free from overt infections in our own and many other laboratories, involving many thousands of work-years, convince us that simple, standard clinical microbiological procedures offer appropriate protection to personnel against strain LT2. Care in the handling, pipetting, and disposal of all materials is stressed as a general safety precaution. Mouth pipetting while working with *S. typhimurium* LT2 or any other *Salmonella* strain should be forbidden. The complete, smooth-form LPS is considered a prerequisite for normal virulence (58), and LPS-defective rough mutants are therefore safer to use.

Strain LT2 has proven to be unsuitable for the study of certain processes, and other wild-type strains have been used instead. For example, strain LT2 does not grow on a medium containing L-histidine as a sole source of nitrogen, though mutants of LT2 able to use it as a source of nitrogen and of carbon were obtained (47). Another strain of *S. typhimurium,* 15–59, which can be readily distinguished from LT2 by differences in susceptibility to bacteriophages, can use histidine as the sole source of nitrogen (14), and this strain has been used as starting material for studies on the *hut* genes for histidine utilization.

Most LT2 lines tested are less virulent for mice than are some *S. typhimurium* lines isolated from nature; these more virulent lines are frequently used in pathogenesis studies in place of LT2. One such is the parent of a set of strains called, collectively, the FIRN line, because the wild-type ancestor, strain M7471, is of the *S. typhimurium* biotype FIRN. FIRN is an acronym indicating that the strain is permanently

nonfimbriate (in respect of common type 1 pili, which cause mannose-sensitive hemagglutination), inositol nonfermenting, and rhamnose nonfermenting (50). Strain M7471 was given colicin factor ColE1-P9 because of the fertility-enhancing effect of this plasmid in ColI-mediated conjugational crosses; it was made, by successive exposure to ethyl methanesulfonate, *leu-1051 malB479 cysI1173* and was finally made *hisC527*(Am) by transduction. The multiply marked product, strain SL4522 (=SL3201 after mouse passage), retained its ancestral mouse virulence. Derivatives made either *galE* (and so phenotypically "rough") or *aroA* (and so dependent on an external supply of *p*-aminobenzoate, not available in host tissues) or cured of the 90-kb plasmid of *S. typhimurium* were nonvirulent or greatly reduced in virulence (29, 32; M. F. Edwards, Ph.D. thesis, Stanford University, Stanford, Calif., 1985). In recent years, the genetic work initiated in *S. typhimurium* LT2 has been extended to many other strains and species of *Salmonella* (see the information below under Kit 1E.)

KITS OF STRAINS AT THE SGSC

Over a period of years, kits of strains have been developed for solving specific genetic problems in genetic analysis. Most of the materials were generously provided by other investigators for use by the whole community. These kits of strains and/or phages are listed below with brief descriptions. These kits are available from the SGSC.

1. **Transposon Kits**

   Kit 1A. **Transposons in the genome of *S. typhimurium*.** Insertions of transposons into and between known genes of *S. typhimurium*. This includes over 1,000 strains, including insertions of Tn*10,* Mu*d*A, Mu*d*J, Mu*d*K, Mu*d*P22, and others. Strains were provided by many investigators. They are described in Table 2 in chapter 135 of this volume; an earlier version appeared in reference 61.

   Kit 1B. **Transposons used to insert specific mutations into strains.** Some strains in the transposon kit carry mutations which are linked by P22 transduction to a transposon such as Tn*10,* which has a selectable marker; thus, this strain can be used to transfer the mutation into new backgrounds. There are many such examples, including strains which can be used to transfer *galE* or *recA* alleles.

   Kit 1C. **Random Tn*10* insertion mutations in the chromosome.** A group of strains with random Tn*10* insertions into the chromosome of *S. typhimurium* LT2 was constructed (33); of these, 276 are available from the SGSC. Many of these insertions have been located on the chromosome and are listed in kit 1A, but about half are not yet located.

   Kit 1D. **The Mu*d*P and Mu*d*Q (Mu*d*-P22) Mapping Set.** Youderian et al. (77) developed a P22 phage with Mu ends which lysogenizes at many sites but cannot detach from the chromosome so is "locked in"; when induced, it replicates and packages about 100 to 200 kb of chromosomal DNA in the insertion region. Benson and Goldman (7) developed a set of about 50 strains, each of which has Mu*d*P22 inserted at different sites around the chromosome. Induced lysates from these lysogens are highly amplified for a specific part of the chromosome; they are a source of DNA for probing, cloning, or sequencing or a source of P22 phage for mapping auxotrophic mutations or Tn*10*s to chromosomal regions (transduction requires that the phage be "tailed," which can be done by using kit 7).

   Kit 1E. **Sets of Tn*10* insertions in other *Salmonella* species.** As part of a program at the SGSC to determine the physical maps of the genomes of several *Salmonella* species, Tn*10*s inserted in specific genes of *S. typhimurium* were transferred by P22 transduction into other species with selection for Tet resistance. Tn*10* has *Xba*I and *Bln*I sites, which facilitate physical map construction by pulsed-field gel electrophoresis. These strains carry the chromosome of the recipient strain except for a few kilobases around the Tn*10* which contain *S. typhimurium* DNA from the donor. Tn*10* insertions have been transferred into the following species: *Salmonella enteritidis,* 60 different sites (39); *S. typhi,* 70 different sites (40a, 40b); *Salmonella paratyphi* A, 60 sites (40c); *S. paratyphi* B, 40 sites (38); *S. paratyphi* C,

75 sites (A. Hessel and K. E. Sanderson, unpublished data); *Salmonella bongori,* 40 sites (S.-L. Liu and K. E. Sanderson, unpublished data).

2. **Mutants Organized by Gene or Operon**

    Kit 2A.   LPS mutants. Mutations in *rfa, rfb, rfc, pmi, oaf, galU,* and *galE* genes. Many mutants are part of an isogenic set constructed by transduction.

    Kit 2B.   Histidine mutants. *S. typhimurium* LT2 strains affecting the *his* (histidine) biosynthetic genes. Source: P. E. Hartman, Johns Hopkins University. There are three kits: kit 2B.1 contains mutants with point mutations in genes of the histidine biosynthetic operon, kit 2B.2 contains mutants with deletion mutations in the histidine biosynthetic operon, and kit 2B.3 contains a set of F′ strains with mutations in the *his* genes of *E. coli* K-12.

    Kit 2C.   Deletion mutants with mutations in the tryptophan operon. Source: P. Margolin. See reference 4. A set of deletion mutants, most of which were originally selected for their SupX (=TopA) phenotype; the deletions remove *topA* and part or all of the *trp* genes.

3. **Hfr Strains of *S. typhimurium* and *Salmonella abony***

    These strains and their use are reported on in detail in chapter 128 of this volume.

4. **F′ Factors with *E. coli* DNA**

    Strains of *S. typhimurium* with F′ factors which carry chromosomal genes of *E. coli* K-12, representing between them a large part of the chromosome. Many of the factors carry transposons Tn*5* or Tn*10* in the F part of the F′, which allows selection for their transfer by using antibiotic resistance. Many were reported by Sanderson and Hartman (59), or were provided by Laszlo Csonka.

5. **Delivery Systems for Construction of Transposition Strains**
    in *S. typhimurium*

    Kit 5A.   Tn*5* delivery systems

    5A.1. P22 generalized transduction of Tn*5*

    5A.2. Delivery system using lambda::Tn*5* for transduction into lambda-sensitive lines of *Salmonella* spp. or *E. coli.*

    Kit 5B. Tn*10* delivery systems. Material provided by John Roth, University of Utah. See reference 17. These systems are described in chapter 141.

    Kit 5C.   Mu*d*lac (Mu*d*J)delivery systems. Material provided by John Roth, University of Utah. See reference 35. A complementation method uses P22 to transduce a defective Mu element which has the operon fusion *lacZ* reporter gene.

    Kit 5D.   Tn*phoA* delivery systems. Material provided by Ron Taylor. See reference 52. The plasmids pRT291 and pRT733 are used for delivery of Tn*phoA* into gram-negative bacteria. In *S. typhimurium,* it is necessary to use a Pho⁻ mutant, lacking acid phosphatase, as a recipient strain.

6. **Transducing Phages for *S. typhimurium***

    Wild-type forms and (in some cases) mutants of phages which give generalized transduction. The phages include P22 and its derivative P22 HT105 *int*1, ES18 and ES18*h*1, and P1-Cm c_{ts} phage.

7. **System for Adding Tails to P22**

    Source: Phil Youderian. Some stocks of P22 phage contain intact heads with DNA but no tails. Tails can be produced by growing cells containing a plasmid which carries the tail gene; these can be added in vitro to tailless P22.

8. **Banks of Cloned *Salmonella* DNA**

    Kit 8A.   Cosmid library of *S. typhimurium* DNA in pHC79. Source: E. Vimr, University of Illinois. DNA of *S. typhimurium* LT2 was *Sau*3A-digested, ligated into pHC79, and infected into HB101 to give ca. 40-kb inserts. About 576 independent colonies were isolated and are maintained on agar plates by a replica plate system.

    Kit 8B. *Sau*3A library packaged in P22. Source: N. M. Kredich. See reference 49. A library of pBR322 derivatives was generated by ligating partial *Sau*3A digests of wild-type *S. typhimurium* LT2 into the *Bam*HI site of the vector, yielding about 15-kb inserts. These were transformed into *S.*

typhimurium LT2 LB5000, and cells were stored in glycerol at low temperature; cells are available on request. In addition, a P22 lysate was produced on these cells, and this lysate can be used to transfer hybrid plasmids into cells by transduction.

Kit 8C. **Lambda 1059 library.** Source: Russ Maurer. See reference 44. *S. typhimurium* DNA was *Sau*3A digested and ligated into lambda 1059, packaged in vitro, transfected into *E. coli* cells, and amplified as five separate pools. These pools were reamplified at the SGSC. *Salmonella* DNA for several genes has been isolated from plaques, e.g., see reference 67.

9. **Systems To Extend the Host Range of Bacteriophages**

    Kit 9A. **Systems to extend the host range of phage lambda.** Source: E. T. Palva, Helsinki, Finland. See reference 27. This plasmid expresses the *lamB* gene of *E. coli* K-12; the lambda receptor protein is displayed on the cell surface.

    Kit 9B. **P22 receptor site: an *rfb* and *rfc* expression system.** Material provided by Peter Reeves, University of Sydney. See reference 51. A cosmid carrying the *rfb* and *rfc* genes of *S. typhimurium,* when present in members of other genera (*E. coli, Klebsiella* spp.) or other species of *Salmonella,* expresses these genes, and the host cells display on their surface the LPS of *Salmonella* group B, which is the receptor for bacteriophage P22. The strains can be used as P22 transduction recipients; in some cases, they also propagate the phage and can be used as donors.

10. **Displacement of pSLT from *S. typhimurium***

    The 90-kb resident of *S. typhimurium* (pSLT; also called virulence plasmid) replicates very stably and is invariably found in LT2 lines unless it has been intentionally eliminated. pLL6, a plasmid constructed by Rod Kelln, University of Regina, permits displacement and curing of plasmid pSLT (17).

11. **Streptomycin-Resistant Point Mutants in *S. typhimurium***

    One method of locating a gene on the chromosome if the gene has a selectable phenotype is to transfer the gene by conjugation and look for linkage to known genes. The strain with a selectable marker, e.g., Tn*10* with Tet resistance, can be made F*lac*⁺ by conjugation and selection for Lac+; this strain can be mated as donor to a strain with a known auxotrophic mutation (e.g., *trp*) with selection for Tet resistance (from the donor chromosome) and Str resistance (counterselection against the donor cell), and the linkage between Tetr and the Trp– phenotype can be determined. A set of about 40 Strr strains, each with a single auxotrophic mutation at locations around the chromosome, has been constructed.

12. **Strains for Homologous Recombination of Genes Transferred**
    into *S. typhimurium*

    Genes transferred into *S. typhimurium* from organisms such as *E. coli* give a low frequency of homologous recombination because of nucleotide differences; many genes remain extrachromosomal or insert ectopically. Strains of *S. typhimurium* with *mutS, mutL,* or *mutH* mutations and that are defective in mismatch repair give a greatly increased frequency of homologous recombination. This permits the transfer of chromosome segments including mutant alleles in genes in *E. coli* or other genera into *S. typhimurium* by conjugation or transduction (56). These *mut* strains are also host restriction deficient.

13. **A Partial Set of Cloned Genes of *S. typhimurium***

    Some of the genes which have been cloned by several other investigators, who used a variety of cloning vehicles, have been assembled as a set to permit probing of portions of the chromosome; part of these clones have been reported (40).

14. **Sets of Wild-Type Strains of *Salmonella* Species**

    Kit 14A. **LT strains.** The 22 strains collected by Lilleengen were analyzed by Zinder and Lederberg, and lyophils from early transfers were provided by J. Lederberg to the SGSC.

    Kit 14B. SARA (Salmonella Reference A) reference collection. Beltran et al. (6) determined the electrophoretic types of *Salmonella* strains by using multilocus enzyme electrophoresis and then

established a set of 72 strains of *Salmonella* which contain "clones" representing the part of subgenus I of *Salmonella* which includes *S. typhimurium, Salmonella saintpaul,* etc.

Kit 14C.   SARB reference collection. Boyd et al. (12) established this set, also by using multilocus enzyme electrophoresis, which contains clones representing the remainder of subgenus I.

Kit 14D.   SARC reference Collection. This kit, assembled and provided by R. K. Selander, contains electronic types representing all the subgenera of *Salmonella*.

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