

# CHARACTERISTICS OF A STAPHYLOCOCCAL PHAGE CAPABLE OF TRANSDUCTION

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## ABSTRACT

MORSE, M. L. (University of Colorado Medical Center, Denver) AND J. W. LABELLE. Characteristics of a staphylococcal phage capable of transduction. *J. Bacteriol.* **83**:775-780. 1962—The growth of staphylococcal phage 53, a temperate phage, has been studied. The latent periods in nutrient broth are: infected sensitive cells, 45 min; ultraviolet-induced lysogenic cells, 83 min. The burst size, as indicated by one-step growth studies and single-burst experiments, is 25 to 30 particles per lysing cell. Phage particles transducing streptomycin resistance and novobiocin resistance are produced at the same time as non-transducing particles. Some observations have been made on the host, *Staphylococcus aureus* NCTC 8511.

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It has been shown that staphylococcal typing phage 53 is capable of transducing, independently, resistance to both streptomycin and novobiocin (Morse, 1959). These transductions have been confirmed, and additional transductions by phage 53 also observed: for ability to ferment lactose, maltose, and mannitol (Korman, 1960), and for various nutritional requirements (Edgar and Stocker, 1961). Transductions by other staphylococcal phages have also been reported (Ritz and Baldwin, 1958; Pattee and Baldwin, 1960; Stocker, *personal communication*). The purpose of this paper is to give some characteristics of phage 53 and its host cell.

## MATERIALS AND METHODS

The methods of growing and handling the staphylococcal cultures have been described previously (Morse, 1959). Nutrient broth cultures of the various mutant strains (all derived from NCTC strain 8511) of *Staphylococcus aureus*

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were grown at 37 C on a stationary wheel rack for a period of 4 hr of growth with the wheel rack rotating. With proper timing of the harvest of the culture, most of the cells in the culture occur either singly or in pairs; the number of cells in clumps usually does not exceed 1 to 5% of the total.

A General Electric germicidal lamp was used as a source of ultraviolet radiation. The cell suspensions, in saline or phosphate buffer, were irradiated with gentle agitation at a distance of either 44 or 90 cm from the source (depending upon the need). After irradiation, a one-tenth volume of 10 × concentrated broth was added and the cultures reincubated on the rotating rack.

Phage assays and transduction assays were performed as before (Morse, 1959). Phage 53 (NCTC 8406) rapidly adsorbs (> 95% in 5 min at 37 C) and produces millimeter-size plaques on nutrient agar. The frequency with which it lysogenizes NCTC 8511 cells has not been measured, but is estimated at about 10%.

## RESULTS

Figure 1 shows the survival of ultraviolet-irradiated staphylococcal cells (both lysogenic and nonlysogenic for phage 53). In the experiment shown, the lysogenic cells were Lac<sup>+</sup> and the nonlysogenic cells were Lac<sup>-</sup>. Equal numbers (1.0 × 10<sup>5</sup>) of each genotype were resuspended in a single volume of saline and the mixture irradiated. Platings were made on a differential medium (Morse and Alire, 1958) containing lactose. After 24 hr of incubation at 37 C, the Lac<sup>+</sup> and Lac<sup>-</sup> colonies were counted. At the low concentration of cells employed, there was no loss of phage-sensitive cells because of phage released from the induced lysogenic cells. The survival curves for cells lysogenic for phage 53 and for cells nonlysogenic for this phage show exponential survival to 0.1% survival. This is generally the case if the conditions for culture growth are carefully con-

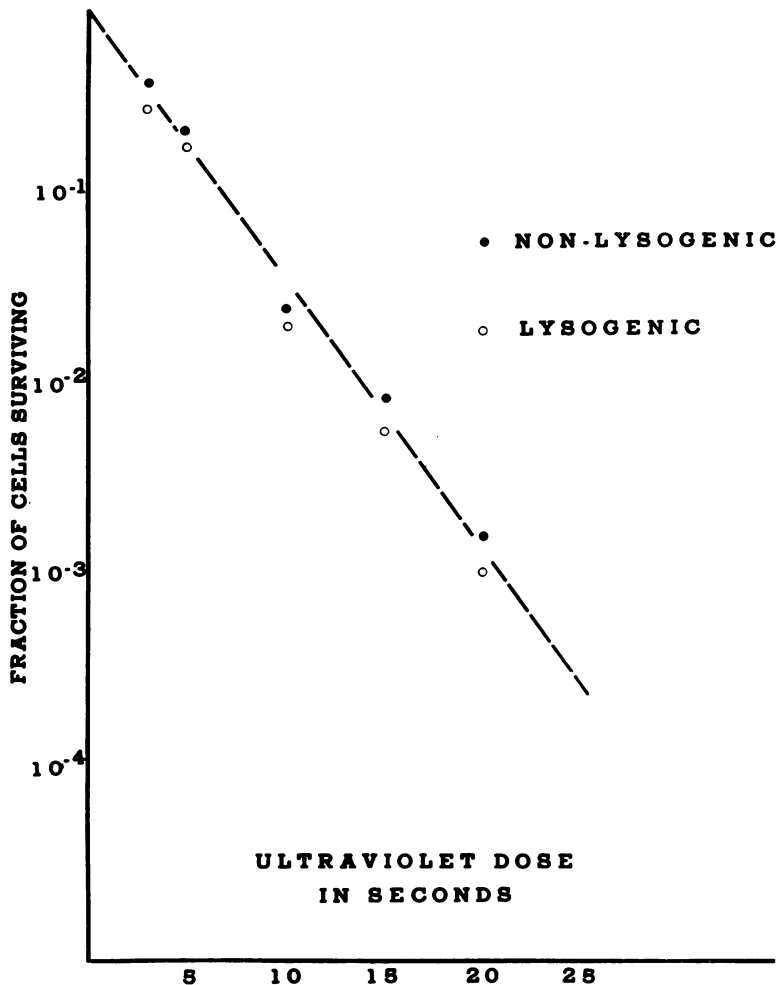


FIG. 1. Survival of *Staphylococcus aureus* cells lysogenic and nonlysogenic for phage 53 after ultraviolet irradiation. Irradiation in phosphate buffer at a distance of 44 cm from a General Electric germicidal lamp.

trolled. Occasional cultures show upward curvature at about the 1% level of survival. Such cultures are usually found microscopically to contain 1 to 2% of clumped cells. Suspensions of optical density of 2.0 and higher in saline or buffer usually show a delay in beginning the exponential phase, suggesting the shielding of some cells by others.

Figure 1 indicates little difference in ultraviolet sensitivity between cells lysogenic for phage 53 and those nonlysogenic for this phage. Examination of all the irradiation experiments performed, however, shows consistently higher survival of phage 53 nonlysogenic cells at every dose, which indicates that nonlysogenic cells are slightly less sensitive to ultraviolet.

Induction of the growth of phage 53 in lysogenic cells is probably not the sole mechanism of lysis of these cells, since nonlysogenic cells also lyse ultimately after exposure to ultraviolet (Fig. 2). This strain of *S. aureus* is known to be lysogenic for another phage which attacks NCTC 8331 (host for typing phage 47) and another phage probably unrelated to NCTC 8406 or the phage attacking NCTC 8331 (Morse, 1959). Neither of these other phages has been found in numbers greater than  $10^4$  per ml in lysates of ultraviolet-treated phage 53 nonlysogenic cells, which indicates that induction and growth of these phages are not the cause of cell lysis after ultraviolet induction.

The lysogenic and nonlysogenic cultures shown

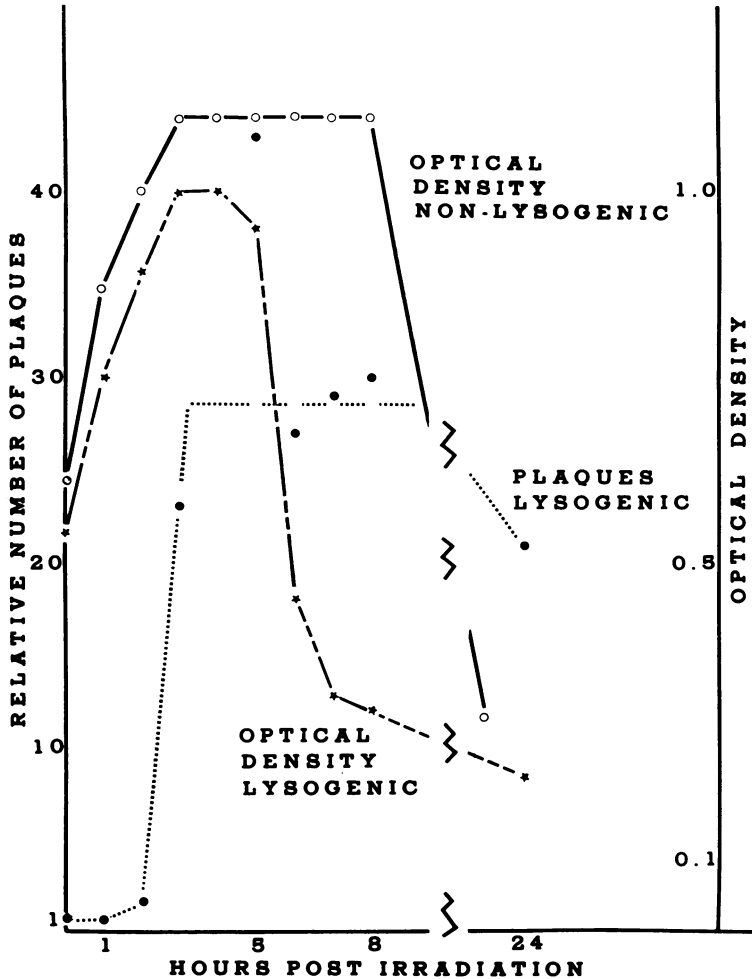


FIG. 2. Lysis of lysogenic and nonlysogenic cultures after ultraviolet irradiation.

in Fig. 2 were given the same dose of ultraviolet, which produced a kill of about 60%. It is to be noted that lysis is almost complete (at 24 hr the cultures are often perfectly clear) and that the ultraviolet survivors have not grown to produce turbidity. Viable cell counts on such cultures at 24 hr generally show only a few thousand cells per ml. The lysis of the ultraviolet survivors is not observed in cultures diluted after irradiation. Under these circumstances the survivors multiply and ultimately contribute sufficient phage spontaneously to complicate one-step growth curves.

The disappearance of the radiation survivors in dense cultures and not in dilute is probably an example of what Welsch, Cavallo, and Cantelmo (1953) have called "collateral lysis," and which

is probably caused by a virolysin (Ralston et al., 1955) released by the irradiated cells. [For a general discussion of lysis of staphylococci, see Elek (1959).]

About 30% of cells lysogenic for phage 53 can be induced to form phage. Maximal induction occurs at about 30% cell survival.

The survival of phage 53 irradiated in buffer suspensions with ultraviolet has also been measured, and the phage was found to be five- to six-fold more resistant than its host cell.

One-step growth curves have been performed on dilute cell suspensions, both on ultraviolet-induced cultures and on exogenously infected phage-sensitive cultures. Such one-step growth curves are similar to those published for other

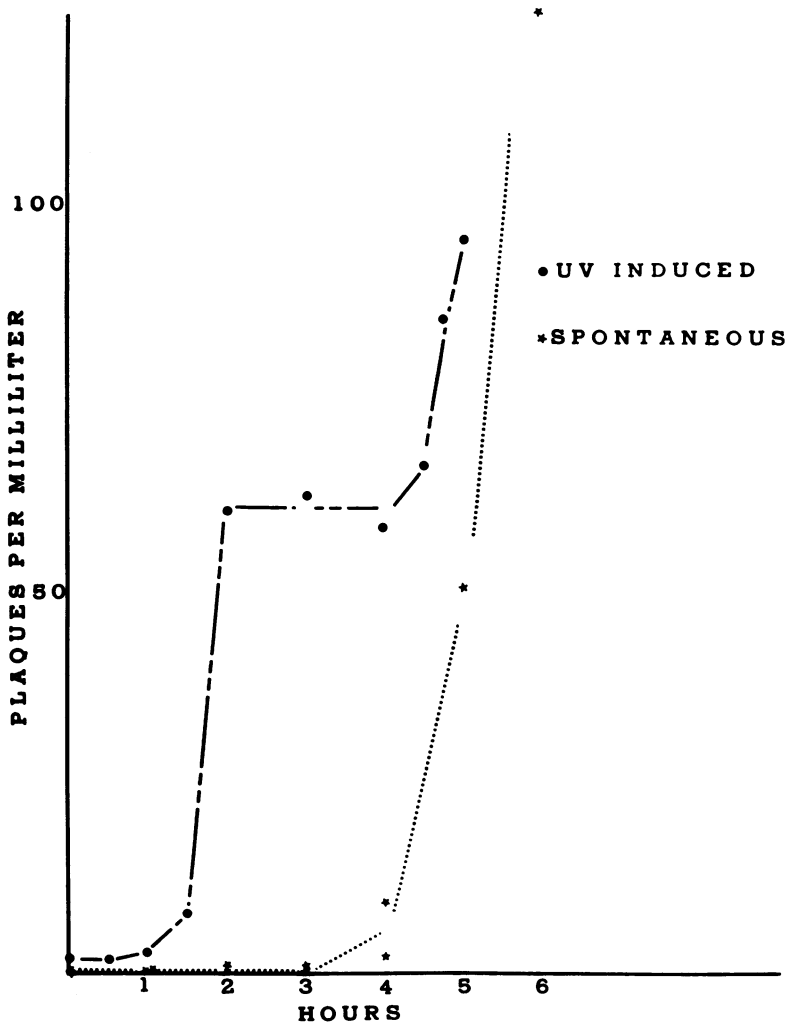


FIG. 3. Comparison of phage yield from ultraviolet-induced and noninduced cultures lysogenic for phage 53. The number of cells in the inoculum of the noninduced culture was equivalent to the number of survivors in the induced culture. The time of spontaneous production of phage by the unirradiated culture indicates that the secondary rise in plaques in the induced culture is caused by spontaneous production of phage by the survivors.

phages, except for a secondary increase in phage at 4 to 5 hr in the case of dilute ultraviolet-treated cultures. The late secondary increase can be adequately explained on the basis of spontaneous production of phage by the descendants of the survivors (Fig. 3).

In the experiment shown in Fig. 3, phage production in an ultraviolet-induced lysogenic culture is compared with that produced in an unirradiated culture started from a number of cells equivalent to the survivors in the irradiated

culture. The ultraviolet-induced culture shows two periods of phage production, one at about 90 min and the other at about 4 to 5 hr. The culture simulating the ultraviolet survivors shows a single rise in phage numbers at 4 to 5 hr. At this time, there are approximately  $10^4$  cells per ml. From these figures it can be calculated that the probability per lysogenic cell per division of spontaneously producing phage is between  $10^{-4}$  and  $10^{-5}$ .

The characteristics of growth of phage 53 in

TABLE 1. *Growth characteristics of phage 53*

Host	No. of expts.	Latent period	Rise period	Burst size
		<i>min</i>	<i>min</i>	
Infected sensitive cells.....	5	45	45	27.3
Ultraviolet-induced lysogenic cells.....	15	83	60	28.1

ultraviolet-induced lysogenic cells and in infected sensitive cells are given in Table 1. The mean burst sizes are the same, within experimental error. Latent and rise periods are longer in the case of ultraviolet-induced lysogenic cells than in the case of sensitive cells. These increases are presumably the results of damage to cell metabolism which results in a decreased rate of phage synthesis (Welsch et al., 1953).

Two single-burst experiments were performed on ultraviolet-treated lysogenic cells, and they gave mean burst sizes of 17 and 18 with a range from 1 to 44 particles per phage-yielding cell.

The production of phage 53 particles with ability to transduce streptomycin resistance and novobiocin resistance was studied in the following ways. (i) Novobiocin-resistant streptomycin-sensitive ( $N^rS^s$ ) cells ( $5 \times 10^8$ /ml) were irradiated in saline, and sufficient concentrated nutrient broth was added to make up a single-strength broth. This culture was sampled periodically and the samples plated with an excess of  $N^sS^r$  cells on nutrient agar containing 100  $\mu$ g streptomycin per ml. After sampling, 3 hr were allowed for phenotypic expression. Plates were then overlaid with 2.0 ml of agar containing 1  $\mu$ g of novobiocin. The samplings on streptomycin agar discriminate between intra- and extracellular phage particles, and the time of release of phage particles with  $N^r$ -transducing activity can be ascertained. The first such particles appear at 90 min; the number continues to rise, and reaches a plateau at about 120 min. Thus,  $N^r$ -transducing particles appear at the same time nontransducing phage particles appear. (ii) Similar experiments were performed using  $N^sS^r$  donor cells and  $N^rS^s$  indicator cells on novobiocin agar. This experiment gave results qualitatively similar to the first experiment, but not as clear-cut, apparently because novobiocin does not discriminate clearly between intracellular and extracellular phage.

## DISCUSSION

The system, staphylococcal phage 53 with its host NCTC 8511, offers a good opportunity to study the genetics and biochemistry of staphylococci. Phage 53 is a temperate bacteriophage, which in the lysogenic state is inducible by ultraviolet light. It adsorbs rapidly to cells. It multiplies rapidly, either by exogenous infection or when induced by ultraviolet. The host cell itself can be grown in a defined medium (Korman, 1960; Edgar and Stocker, 1961), so it is possible to select additional nutritional characteristics for study.

Some observations on the phage 53 NCTC 8511 system have already been reported. Dye (1959), in a study of small-colony variants obtained from NCTC 8511 after a variety of treatments, reported that four of five such variants had requirements for long-chain fatty acids, the first suggestion that staphylococci might have a metabolism involving fatty acids. The fifth small-colony mutant required a factor from the pyridoxal group of vitamins. Edgar and Stocker (1961), from a study of this system, and from study of phage 53 and other staphylococcal strains, stated that the biosynthetic pathways to tryptophan and threonine in staphylococci are similar to the pathways in *Salmonella typhimurium* and *Escherichia coli*. These authors also reported the first evidence for linked genes in staphylococci. Korman (1960) presented evidence for a heterogenetic state among the descendants of the initial transductant.

We have made preliminary studies of the ability to produce coagulase by this strain. The results obtained were not satisfactory, owing to our inability to obtain precise classification of the coagulase character on fibrinogen agar plates. We are currently proceeding satisfactorily in the analysis of some complex mutations resulting in the loss of ability to ferment a number of unrelated carbohydrates.

It is to be hoped that a continued investigation of this and other systems will lead to greater knowledge of the genetics and biochemistry of staphylococci. The comprehensive treatment by Elek (1959) of *S. aureus* indicates the deficiencies of our knowledge in these areas.

## ACKNOWLEDGMENTS

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