CURING OF LYSOGENIC SALMONELLA TYPHOS A VI-PHAGE TYPES BY ULTRAVIOLET IRRADIATION

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The dual nature of Vi-phage type specificity of lysogenic Vi-types of Salmonella typhosa was first expressed by Anderson and Fraser (1955). These workers felt that two factors played a part: (i) the nature of the precursor nonlysogenic parent culture of the Vi-type; (ii) the character of the temperate or type-determining phage which infects the precursor culture. This duality was expressed by Anderson and Fraser by structural formulas in which the precursor type culture is designated by capital letters and the type-determining phage by lower case letters or numerals, in parentheses. Thus Vi-phage type D₁ is expressed as A(d₄), type D₄ as A(d₄), type 25 as A(25'), etc.

Proof of the correctness of the formulas has, with but one exception, been obtained by infecting nonlysogenic Vi-types with type-determining phages and noting the character of any type transformations. The exception occurred in the case of type F₂. Old stock cultures of type F₂ were observed by Felix and Anderson (1951) to give rise to nonlysogenic mutants that were found to be type F₁. Lysogenization of a type F₁ culture with the temperate phage carried by F₂ cultures converted F₁ into F₂. The formula, therefore, for type F₂ is expressed as F₁(F₂).

Additional proof of the correctness of the structural formulas of Anderson and Fraser would require two steps: (i) derivation from lysogenic type cultures of nonlysogenic mutants that correspond to precursor types of the structural formulas; (ii) conversion of the mutants to the original lysogenic type by treatment with the appropriate temperate phage. In the present study, this has been carried out successfully with nine lysogenic Vi-phage types of S. typhosa, employing ultraviolet light radiation as the mutagenic agent in step (i). Reconversion of the "cured" culture to its original Vi-type (step (ii)) has been accomplished by lysogenization with temperate phage induced from the parent Vi-type culture.

So far as we are aware this is the first report of the use of a mutagenic agent for the purpose of selecting nonlysogenic Vi cultures of S. typhosa.

MATERIALS AND METHODS

Curing. Petri plates, containing a modified eosin methylene blue medium (EMB; Zinder, 1958), were dried at 37 C for 1 hr with covers off. Veal infusion broth cultures of lysogenic Vi-phage types containing approximately 2 × 10⁶ cells per ml were flooded over the indicator medium and excess culture removed. The inoculated plates were dried for 10 min at room temperature and then exposed to ultraviolet light (Westinghouse Sterilamp) at a distance of 9 in., with a measured intensity of 15 μw per cm². Exposure time was varied to yield plates with 50 to 300 survivor colonies. Postirradiation handling and incubation at 37 C were carried out in the dark to avoid photoreactivation. When survivor cells developed into colonies 1 mm in diameter, the plates were sprayed (Stolp, 1957) with clear plaque mutants (Read and Ferguson, 1959) of the temperate phages previously isolated from the lysogenic cultures. All temperate phages were propagated on type A culture. The efficiencies of plating on nonlysogenic types C₁, D₄, and E₁ were 1.0. The phage preparations were filtered through HA Millipore pads and tested for bacterial sterility. The quantity of phage sprayed was predetermined to be sufficient to put about five plaque-forming particles on each colony. Spraying was done with a DeVilbiss Nebulizer in an isolated room to localize aerosol spread of phage.

After further incubation for about 12 hr, the phage-sprayed colonies were examined with 10× magnification. Lysogenic colonies were unaffected by sprayed phage due to self-immunity. Phage-sensitive (i.e., nonlysogenic) colonies showed red-colored peripheral sectors of

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lysis on EMB medium. Growth from unlysed areas of these colonies was streaked to obtain isolated phage-free colonies. The nonlysogenic status of these colonies was checked by cross-streaking against the indicated phage. Several nonlysogenic colonies were Vi-phage typed in each curing experiment.

The lysogenic status of unirradiated cultures was examined by plating a $10^{-4}$ dilution of the culture destined to be irradiated. One hundred single colonies from the unirradiated plates were cross-streaked against the phage carried by the culture.

Lysogenization. Veal infusion agar plates seeded with nonlysogenic (i.e., cured) cultures were spotted with turbid plaque temperate phages. Resistant growth from lytic areas was streaked out to obtain isolated colonies. Lysogenic colonies were selected by evidence of phage immunity on cross streak plates.

RESULTS

Table 1 shows the changes in Vi-phage type brought about by ultraviolet irradiation and by establishment of lysogenicity. A total of nine Vi-phage types were rendered nonlysogenic by ultraviolet irradiation. It will be noted that three $D_4$ strains, Felix, Craigie, and T4274, were cured and yielded, respectively, $D_4$, $D_5$, and A nonlysogenic Vi-types. Since all three nonlysogenic cultures are converted into type $D_4$ by lysogenization with phage $d_4$, it is evident that at least two precursor Vi-types are possible for type $D_4$. However, it has been found by us that type $D_4$ strains Felix and Craigie are not identical to strain T4274 in sensitivity to Vi-phages. Vi-phages 36 and 40 may be used to demonstrate the difference.

To ascertain differences, other than Vi-phage type, between lysogenic and cured cultures, antibiotic sensitivity, sugar fermentation, and colonial morphology were examined on those cultures listed in Table 1. All lysogenic and cured cultures were sensitive to dihydrostreptomycin, neomycin, polymyxin B, and tetracycline. Arabinose and dulcitol uniformly were not fermented. Xylose was fermented by all cultures with the exception of type 25, untreated and cured. Neither of these cultures fermented the sugar. On veal infusion blood agar the colonial morphology of the paired lysogenic and cured cultures was identical. In summary, there were no differences in antibiotic sensitivity, fermentation characteristics, or morphology noted between the paired lysogenic and cured cultures. Lysogenic immunity as measured by Vi-phage type was the only characteristic shown to be influenced by these temperate phages.

EMB medium proved to be a valuable aid in the detection of nonlysogenic cultures resulting from ultraviolet irradiation of the nine Vi-types listed in Table 1. There was evidence, however, that the usefulness of the medium might be limited. Phages $d_4$, $d_5$, and $d_7$ were inhibited from reacting on type A grown on EMB plates. A substitute for EMB as an indicator of lysis was sought in the hemolytic effect produced by phage lysis of colonies on blood agar (Schiff and Bornstein, 1940). The action of phages $d_1$, $d_4$, and $d_7$ on colonies of type A (presumably the nonlysogenic base of types $D_4$, $D_5$, and $D_9$) resulted in marked hemolytic action. In contrast, colonies of ultraviolet irradiated types $D_4$, $D_5$, and $D_7$ sprayed with phages $d_1$, $d_4$, and $d_7$, respectively, did not become hemolytic. Obviously, no curing occurred and the cultures were immune to phage action.

Lyso-lysogenic Vi-phage types $B_2$, $C_5$, $C_9$, $D_1$, $D_5$, $E_4$, and 26, none of which was cured by ultraviolet irradiation, were plated on veal infusion agar containing streptomycin. One hundred resistant colonies from each culture were cross-streaked against the specific temperate phage possessed by each culture. No nonlysogenic colonies were detected. Thus, the relationship between streptomycin resistance and nonlysogen-
genicity observed by Lederberg and Lederberg (1953) with Escherichia coli strain K12 was not encountered among these eight lysogenic cultures. Likewise, no correlation was found between streptomycin resistance and nonlysogenicity with three cultures capable of being cured by ultraviolet. Streptomycin-resistant colonies of types 29, E7, and E5 retained their lysogenic status.

Trial was made of another method of curing lysogenicity but no success attended its use. This was the method of Bertani (1958) and Groman (1955) which involves superinfection with a virulent mutant of the temperate phage. Although most of the temperate phages of S. typhosa produce clear plaque mutants (Read and Ferguson, 1959), only phage d1, produces a mutant able to lyse the parent culture. Superinfection of type D1 with the virulent mutant of phage d1 did not result in a loss of prophage in the survivor cells.

**DISCUSSION**

The frequency of occurrence of nonlysogenic cells among the survivors of ultraviolet irradiation varied considerably among different Vi-phage types and also in the same type at different times. The highest frequencies were obtained with types 29 and E7. As many as 80% of the survivor colonies were nonlysogenic. In other cultures, the frequencies were between 1 and 10%. The greatest numbers of nonlysogenic colonies were always found on the plates with the fewest survivors.

Very low frequencies affect the success of finding nonlysogenic colonies and probably result in some cultures being falsely classified as not curable. A very low frequency of curing was obtained by Huybers and Jennings (1957) after ultraviolet irradiation of a lysogenic staphylococcus. Only 2 nonlysogenic colonies were found among 2214 survivors.

Anderson (1956) reported that nonlysogenic variants occur spontaneously in old, slant cultures of S. typhosa Vi-types lysogenic with phage 29′ or related phages. The frequency of nonlysogenic cells in our stock cultures of types 29, C3-30, Ds Felix, and E7 was found to be approximately 1% after the cultures had been stored 15 months at 5 C. Spontaneous loss of the lysogenic status yielded the same Vi-phage types (i.e., A, C1, Ds, and E1) as obtained by ultraviolet irradiation.

Although spontaneous loss of lysogenicity was not encountered in unirradiated physiologically young cultures, the presence of nonlysogenic cells in old cultures suggests that the mode of action of ultraviolet may be selective rather than inductive. Bertani (1958) has stated that the action of ultraviolet on inducible cultures may be selective for nonlysogenic cells since they are not subject to lysis. The easily cured types 29 and E7 do not show detectable lysis with graded doses of ultraviolet. Thus, curing of these cultures apparently does not involve selection of a non-inducible fraction.

Irradiation did not select ultraviolet-resistant cells that were nonlysogenic, since the ultraviolet sensitivities of the cured types 29 and E7 were the same as their corresponding lysogenic cultures.

The group serological relationship of temperate phages 25′, 29′, C3-33′, C3-30′, d6, e7, and e9 has been established by Anderson and Felix (1953) and by Ferguson, Juenker, and Ferguson (1955). It is perhaps significant that it has not been possible to cure S. typhosa cultures lysogenic with phages unrelated to this group.

The statistics of distribution of Vi-phage types on a worldwide basis (Nicolle, 1958) show that more than half of all cultures from typhoid fever cases and carriers are comprised of types A1, C1, and E1. It will be noted in Table 1 that these three types are the precursor cultures of several Vi-phage types of restricted Vi-phage sensitivity (A for types Ds T4274, 25, and 29; C1 for types C3-33 and C3-30; E1 for E7 and E9). It is also known that types A, C1, and E1 may be converted to types 26, C8, and E9, respectively, by lysogenization with phage 26′. The possibility is suggested that types A, C1, and E1 may be common precursor bases for many of the Vi-types in the Craigie-Felix typing schema.

None of the nine cultures after curing carried phages detectable on type A. For this reason, and because the cured cultures were converted back to the original lysogenic Vi-phage types by lysogenization with single plaque purified phages, the nine curable cultures are presumably singly lysogenic. Polylysogeny in S. typhosa has been observed by us and will be the subject of a separate communication.

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SUMMARY

Structural formulas of lysogenic Vi-phage types of *Salmonella typhosa*, according to Anderson and Fraser, have been confirmed by isolation of nonlysogenic precursor cultures after ultraviolet irradiation of nine lysogenic Vi-types. Lysogenization of the nine cured cultures with the same temperate phages carried before ultraviolet irradiation established the specific Vi-phage types.

REFERENCES


