# Use of Bacteriophage Typing to Distinguish *Propionibacterium acnes* Types I and II

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Strains of serotypes I and II of *Propionibacterium* were compared for phage sensitivity. The two serotypes could be distinguished by using a typing set consisting of 16 bacteriophages at concentrations that demonstrated selective lysis of serotype I or II bacterial strains. Seven phage types were found; three were composed exclusively of serotype I, and four were exclusively composed of serotype II organisms. Generally, serotype I strains were more sensitive to phage lysis than were serotype II strains. No correlation was found between phage type and site of isolation.

*Propionibacterium acnes* is the dominant member of the skin microflora (9). Residing primarily in the deep sebaceous glands (9-11, 13), *P. acnes* can also be isolated from numerous nonsebaceous body sites, including the axilla, arm, rectum, groin, and feet (9). Although primarily known for its role in the cutaneous process of *acne vulgaris*, *P. acnes* is occasionally pathogenic and has been causal in many sorts of infection (20).

Some strains of these organisms have been shown to have remarkable ability to stimulate the immune system, resulting in repression or regression of tumors under suitable conditions (17). The strains used are generally labeled *Corynebacterium parvum*, but most of them are serologically and physiologically identical to *P. acnes* (3).

Recent work by Johnson and Cummins (7) and Marples and McGinley (12) has provided accurate means of identifying the trio of cutaneous propionibacteria, *P. acnes, P. avidum,* and *P. granulosum.* Pulverer and his colleagues have also furthered the identification of the propionibacteria via biotyping (15) and phage typing (8, 16), and Cummins (2) has provided a simplified serotyping method for the species. The purpose of this study was to combine phage typing with serotyping to give a more comprehensive subdivision of strains classified as *P. acnes.*

## MATERIALS AND METHODS

**Bacterial strains.** A total of 180 strains of *P. acnes* was tested. One hundred forty-eight strains were isolated from the skin and from clinical samples and were identified according to the methods of Marples and McGinley (12) and serotyped by the methods of Cummins (2). The following strains belong to the culture collection of the Virginia Polytechnic Institute and State University and were also examined: 3706, 3318, 6572, 0204, 3821, 6632, 0162, 6633, 6626, 6624, 6580, 6623, 6625, 1885, 0400, 4938, and 3211-1. *P. acnes* strains 6919 and 6922 were obtained from the American Type Culture Collection (ATCC), and *P. acnes* 51022, Perk, and 46123 were obtained from the Duhring Laboratories culture collection. An additional five strains each of *P. avidum* and *P. granulosum* were isolated from clinical material and identified according to the above methods (2, 12).

**Culture methods.** Bacterial strains were maintained in prereduced, anaerobically sterilized (PRAS), chopped meat broth (6). Phage-typing and culture-purity tests were performed on brain heart infusion agar (BHIA+) with 0.1% Tween 80, 0.1% dextrose, 0.1% sodium lactate, and 5 ml of a solution containing (per liter): 4 g of $MgSO_4 \cdot 7H_2O$, 0.4 g of $MnSO_4 \cdot 4H_2O$, and 0.4 g of $FeSO_4 \cdot 7H_2O$, acidified with 2 drops of a 10 N solution of $H_2SO_4$. All cultures were incubated at 35°C, with plated media being in jars with GasPak generators (Baltimore Biological Laboratories, Inc.) under 10% hydrogen and 90% $CO_2$.

**Bacteriophage.** Twenty-seven strains of bacteriophage were tested. Phages 2 through 16 were isolated from normal individuals whose nasal cultures exhibited translucent and apparently lysed colonies. Isolation was achieved by spreading the glassily transformed colony upon a lawn plate of a strain of *P. acnes* that had been isolated from the same individual. The plates were incubated for 48 h, and the phage particles were washed from the plate, using peptone yeast glucose (PYG) broth (6), which was then centrifuged and filtered through a 0.45-μm membrane filter (Millipore Corp.) to remove cellular debris. The clear phage suspension was then stored at 4°C for 2 weeks to screen out unstable strains of phage. After storage, the suspensions were inoculated to lawn plates of *P. acnes* ATCC 6919, and individual plaques were cloned. This was thrice repeated in order to purify the phage.

Phages ATCC 29399, B, and G were isolated directly from the noses of acne patients. After sampling, the moist swab was eluted with 1% peptone broth which was filtered as before and then applied to a lawn plate of *P. acnes* ATCC 6919. The same purification procedures as described above were carried out. Phage 174 of Zierdt et al. (19), and phages 26 and 20A of Puhvel were obtained from Gerhard Pulverer (University of Cologne, Cologne, West Germany), as were his phage strains 6981, H-12, 6921, 154, 95, 33, 2741, 6947, and 21. All phages received from Pulverer were propagated on their accompanying host strains; other phages were propagated on ATCC 6919.

**Determination of species specificity.** The ability of phage to adsorb to the related species, *P. avidum* and *P. granulosum,* was tested by applying phage at 1,000 times the routine test dilution (RTD, see phage-typing procedure) and by mixing phage at $10^6$ phage-forming units (PFU) per ml with $10^6$ viable cells of five strains of each species in PYG. After a 15-min incubation period, the broth medium was centrifuged to remove cells, and the number of phage particles remaining in the supernatant fluid was counted by plating suitable dilutions. Plated media were inspected for plaques after a 24-h incubation period.

**Induction of bacteriophage.** Single colony isolates were inoculated to 10 ml of PRAS PYG medium with 0.1% Tween 80 and allowed to grow for 8 h at 35°C, after which 0.1 ml of a 200-µg/ml solution of mitomycin C (Sigma Chemical Co.) was added to the log-phase cultures, yielding a final concentration of 2 µg/ml. The cultures were incubated overnight, centrifuged, and filtered to remove cells and debris; a drop was placed on lawn plates of the host strains, ATCC 6919 and VPI 0162, which were then incubated for 48 h.

**Phage-typing procedure.** The RTD for each phage was determined by titrating it on lawn plates of *P. acnes* 6919 (serotype I) and *P. acnes* 0162 (serotype II). The concentration of each phage that would lyse one strain, but not the other, was selected as RTD. After a preliminary study, the 16 phages with the most potentially useful and interesting host ranges were selected for the typing set. Lytic reactions were recorded as greater than 20 plaques (+), less than 20 plaques (w), and no plaques (−).

**Electron microscopy.** The morphology of phage G, 20A, 33, and 6921 was studied by electron microscopy of negatively stained preparations. Each phage was propagated to a titer of about $10^8$ PFU/ml, using the agar-overlay method of Adams (1). A drop of each phage suspension was then applied to a grid and stained with 2% uranyl acetate. The grids were examined in a Jeol 100B electron microscope at a primary magnification of ×50,000.

## RESULTS

**Carriage of bacteriophage.** Eighty-five strains of *P. acnes* were treated with mitomycin C, and phage were recovered from fifteen strains. This implies a carriage rate of approximately 18%. There was no difference between serotypes I and II in the proportion of strains which liberated phage.

**Species specificity.** All phages were applied at 1,000 × RTD to five strains each of *P. avidum* and *P. granulosum,* and all failed to lyse them at this concentration. Moreover, no diminution in phage titer could be observed when phage suspensions were absorbed with any of the 10 *P. avidum* or *P. granulosum* strains.

**Morphological studies.** All phage examined possessed polyhedral heads and flexible unsheathed tails (Fig. 1). Phages G, 33, and 6921 had heads of approximately 72 nm in diameter and tails of about 196 by 10 nm. Phage 20A appeared to be larger, with a polyhedral head of approximately 90 nm in diameter and a tail which was 175 by 15 nm.

**Phage typing.** From the 27 original phage strains, a typing panel of 16 was chosen on the basis of a preliminary survey of their host spectra. The origins of the 16 phage strains are shown in Table 1. The RTD of the phages in the final typing set (Table 2) reflected the concentration of each phage that would best discriminate between representative strains of *P. acnes* serotypes I and II. A total of 180 strains of *P. acnes* were tested. Without fail, serotype I strains were more sensitive to phage at RTD than were serotype II strains (Fig. 2).

Seven phage lysis patterns were found (Table 3). In recording the patterns of lysis, the positive reactions were initially recorded as either + (more than 20 plaques) or w (fewer than 20 plaques). However, subsequent study of the reaction patterns did not show any distinctive patterns associated specifically with the weaker reactions, and therefore both + and w reactions have been recorded as sensitive in classifying strains as sensitive or resistant in Table 3. Phage types did not cross serotypic boundaries; phage types Ia, Ib, and Ic were composed exclusively of serotype I strains, and phage types IIa, IIb, IIc, and IId contained only serotype II strains. Bacteria of phage type Ia were uniformly sensitive to all phages at RTD and made up 24.5% of serotype I strains. Bacteria of type Ib were sensitive to phage A and resistant to H-12; sensitivity to other phages was variable. This group comprised 50% of the serotype I strains. Type Ic, which was 11% of serotype I strains, was sensitive to phages 2, 16, H-12, 6921, 26, and 20A; it was resistant to phage 2741. Sixteen of the type I strains (14.6%) did not display a groupable pattern of lysis. Serotype II strains comprised 28.9% of the total number of strains tested and could be divided into four phage types. Type IIa, 38.6% of the serotype, was sensitive to phages 95 and 33 and resistant to 2741. Strains in type IIb made up 10% of the serotype and were sensitive to phage G and resistant to 6981, H-12, 95, 33, 26, 2741, and 6949. Strains in
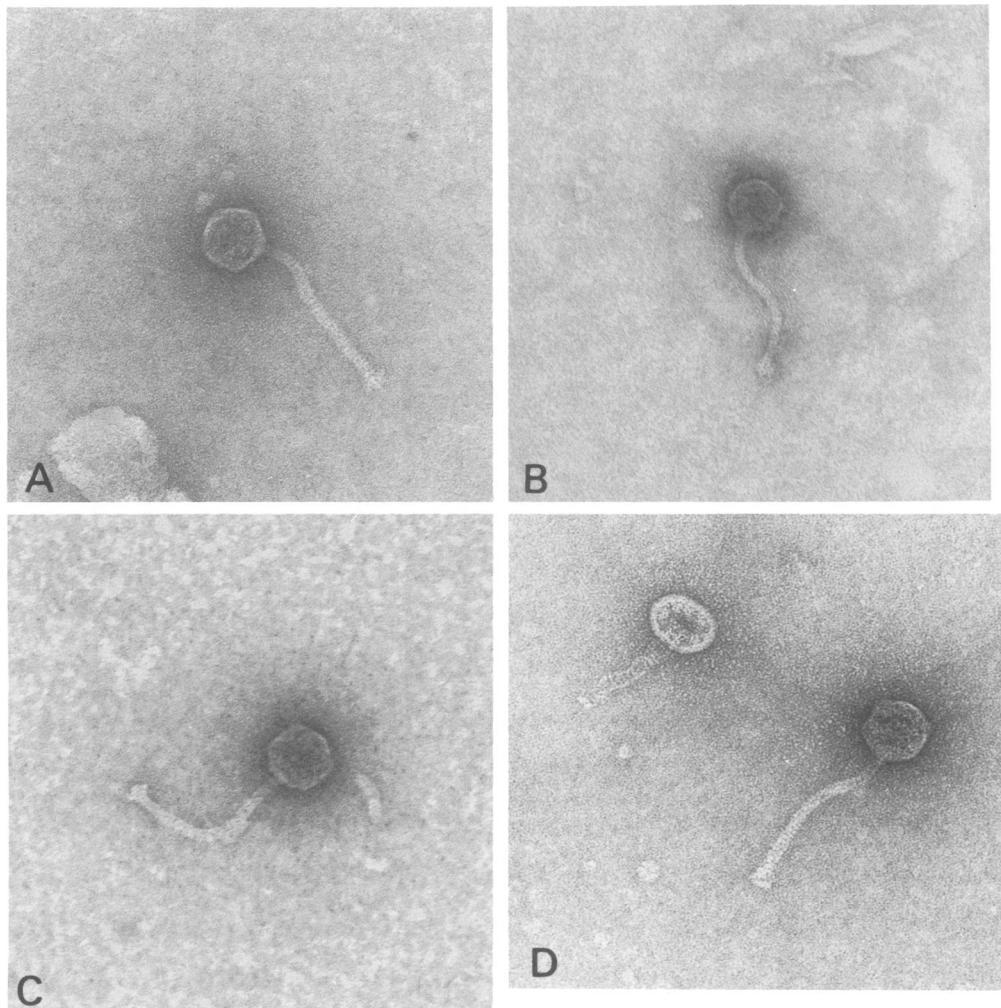
FIG. 1. *Electron micrographs of P. acnes bacteriophages. (A) phage G; (B) phage 16; (C) phage 33; (D) phage 6660. Final magnification, ×125,000.*

type IIc were resistant to all phage types at RTD and made up 40% of the serotype. Type IId contained 7.1% of the serotype II strains. These strains were sensitive to phage 20A and resistant to A, G, 16, H-12, 154, 6949, 21, and 174. Finally, 4.29% of the serotype II strains tested displayed no discernable pattern of lysis and were deemed untypable.

**Correlation of site of isolation of bacteria with phage type.** The specific site of isolation was known for 91 of the freshly isolated bacterial strains (Table 4). The 91 strains were isolated from clinical samples (i.e., blood cultures, wounds, abscesses, cysts, etc.; this group did not include skin samples), and swabs from the forehead, scalp, ear, antecubital fossa, axilla, and groin. Of these sites it was felt that only the

forehead, scalp, and clinical groups contained enough strains to permit meaningful analysis. When the incidence of a particular phage type of bacteria within a site group was compared with the incidence of that phage type in the group of 91 strains, no statistically significant relationships were found. It was noted, however, that there appeared to be a higher proportion of Ib strains in the group of 91 strains than in the entire 180 strains.

## DISCUSSION

The present work was undertaken in the first instance to determine whether there was any relationship between the phage type of *P. acnes* strains and their serotype. In general, *P. acnes* strains of serotype I are more susceptible to

TABLE 1. *Origins of the 16 bacteriophage strains used in typing P. acnes*

| Phage strain | Source | Origin (where known) |
| --- | --- | --- |
| H-12<br>21<br>33<br>95<br>154<br>2741<br>6921<br>6949<br>6981 | Gerhard Pulverer, Institute of Hygiene, University of Cologne, West Germany | Strain H-12 was obtained by spontaneous lysis, other strains by mitomycin induction (see Jong et al., reference 8) |
| 20A<br>26 | Gerhard Pulverer, Cologne, originally from S. M. Puhvel, University of California, Los Angeles | |
| 174 | Gerhard Pulverer, Cologne, originally from Charles Zierdt, National Institutes of Health, Bethesda, Md. | Spontaneous plaques on strain ATCC 11827 (see Zierdt et al., reference 19) |
| A (= 29399B, ATCC)<br>G<br>2<br>16 | Duhring Laboratories, University of Pennsylvania | Filtrates of nasal swabs |

TABLE 2. *RTD*[a]

| Phage | RTD (PFU/ml) |
| --- | --- |
| A | $5.6 \times 10^4$ |
| G | $5.0 \times 10^4$ |
| 2 | $2.4 \times 10^3$ |
| 16 | $5.0 \times 10^3$ |
| 6981 | $4.1 \times 10^2$ |
| H-12 | $5.0 \times 10^3$ |
| 6921 | $1.0 \times 10^3$ |
| 154 | $1.1 \times 10^5$ |
| 95 | $5.6 \times 10^5$ |
| 33 | $5.8 \times 10^4$ |
| 26 | $1.6 \times 10^4$ |
| 2741 | $7.0 \times 10^2$ |
| 6949 | $1.2 \times 10^1$ |
| 20A | $3.0 \times 10^4$ |
| 21 | $3.2 \times 10^2$ |
| 174 | $4.8 \times 10^2$ |

[a] RTD determinations were performed as described in the text.

phage lysis than are serotype II strains (Fig. 2), and, when the results with the whole set of 16 phages are considered, several patterns can be recognized that are distinctive for each serotype. However, we have not found any bacteriophage strains that would unequivocally separate the two serotypes by lysing strains of one serotype only. The reason for the greater phage resistance of type II strains is not known. The increased resistance may possibly reflect a higher rate of lysogenization among these strains, although this was not indicated by our phage-induction studies.

The 18.0% figure for phage carriage was determined by mitomycin C induction. Pulverer (16) and his co-workers compared the efficiency of induction by ultraviolet irradiation, novobiocin, and mitomycin C, and found that only mitomycin C was capable of inducing bacteriophage liberation. Similar results have been found in our laboratories regarding induction by ultraviolet light, bleomycin, and mitomycin C. Abrupt changes in temperature occasionally resulted in phage liberation, but phages induced by this method were also induced by mitomycin C.

Prevot and Thouvenot (14), using 6 different phages isolated from culture filtrates, were able to demonstrate 11 phage types covering 212 strains of anaerobic corynebacteria: one of their types comprised 55% of the strains examined. However, their strains of bacteria were described as belonging to seven different species of anaerobic coryneforms, including *C. avidum, C. parvum, C. anaerobium, C. granulosum, C. liquefaciens, C. pyogenes,* and *C. acnes,* and it is therefore difficult to correlate their results with ours.

Recent work by Jong et al. (8) has produced a phage-typing system for *P. acnes* that bears some resemblance to the system presented here, although they did not attempt to correlate serotype with phage type. Some of the phage strains used by Jong and his colleagues were also used in this study, and limited comparisons may be made. In both systems, a group of *P. acnes* strains was lysed by all phages at RTD.

This group made up 79% of all strains tested by Jong et al., as opposed to 15% in the present series. This great difference may be attributable to differences in the RTD. In this study, RTD was determined so that phage would selectively lyse one serotype but not the other, whereas Jong et al. defined RTD as that concentration of phage which would produce confluent lysis on the propagating strain. The phage concentration

at RTD used by Jong et al. may therefore be much higher than our RTD determinations, thus giving a higher proportion of totally susceptible bacterial strains. A second difference between systems is in the number of strains of bacteria that were totally resistant to phage lysis at RTD. Jong et al. found that 1.7% of their bacterial strains displayed this pattern as opposed to our finding that 15.6% of all strains
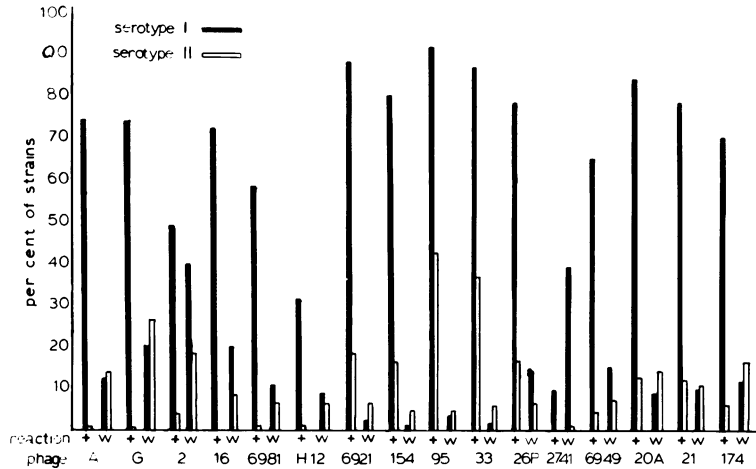


FIG. 2. Histogram showing the degree of lysis of P. acnes types I and II when tested against various phages.

TABLE 3. Characteristics of phage type

| Sero-type of orga-nism | Phage types | No. of strains | % Total strains (types I and II) | % Strains of sero-type | Culture collection strains[a] | Characteristic pattern of lysis | |
|---|---|---|---|---|---|---|---|
| | | | | | | Resistant to: | Sensitive to: |
| I | Ia | 27 | 15 | 24.54 | ATCC 6919 and 6922 VPI 3706 and 6572 | | All phages |
| | Ib | 55 | 30.6 | 50 | UofP 51022 VPI 3318 | H-12 | A |
| | Ic | 12 | 6.67 | 10.9 | | 2741 | 2, 16, H-12, 6921, 26, 20A |
| | Iu | 16 | 8.89 | 14.55 | | Untypable: no distinctive pattern | |
| II | IIa | 27 | 15.0 · | 38.57 | VPI 6583, 0204, 6632, 0162, 6626, 6625, 4938 | 2741 | 95, 33 |
| | IIb | 7 | 3.89 | 10 | VPI 3821 | H-12, 6981, 95, 33, 26, 2741, 6949 | G |
| | IIc | 28 | 15.56 | 40 | UofP 46123 VPI 6633, 6580 1885, 3211-1, 6623 | All phages | |
| | IId | 5 | 2.78 | 7.14 | VPI 0400 | A, G, 16, H-12, 154, 6949, 21, 174 | 20A |
| | IIu | 3 | 1.67 | 4.29 | VPI 6624 | Untypable: no distinctive pattern | |

[a] VPI, Culture collection of Virginia Polytechnic Institute and State University, Anaerobe Laboratory; UofP, Culture collection of Duhring Laboratories, University of Pennsylvania.

TABLE 4. *Distribution of 91 strains of P. acnes of different phage types*

| Isolation site[a] | Phage type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ia | Ib | Ic | Iu | IIa | IIb | IIc | IId |
| Forehead | 1 | 12 | 0 | 0 | 1 | 0 | 2 | 0 |
| Scalp | 4 | 13 | 3 | 0 | 2 | 0 | 3 | 1 |
| Ear | 2 | 4 | 1 | 0 | 0 | 0 | 1 | 2 |
| Antecubital fossa | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| Axilla | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Groin | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lesions | 2 | 17 | 3 | 3 | 3 | 0 | 3 | 0 |

[a] Although the numbers for some sites are very small, it appears that strains of phage type Ib are most common at all sites except the axilla and antecubital fossa.

were resistant to phage. Again, this difference could be explained by higher concentrations of phage used in Jong's study.

Although only 91 strains were analyzed with regard to phage type and site of isolation, our data do allow a tentative conclusion regarding the role of *P. acnes* as a pathogen. In addition to its involvement in the development of *Acne vulgaris*, *P. acnes* has been implicated in many sorts of infection, including endocarditis, septicemia, meningitis, wound infections, and abscesses (20). In the foregoing disease states, a clear-cut case for *P. acnes* involvement was made. However, such clear-cut examples are rare, and it is generally thought that *P. acnes* is essentially nonpathogenic, even though it is frequently isolated from infections in the company of well-established pathogens. Whether the strains of *P. acnes* isolated from mixed infections are more pathogenic than the "average" strain is unknown, but it might be reasonable to assume that such a difference would be reflected in phage type, and that a higher incidence of this phage type would be found in clinical material. The data presented here, however, show no correlation between phage type and clinical isolation rate. This gives support to the contention that *P. acnes* is indeed an infrequent pathogen and, when present in mixed infection, is probably not of clinical significance.

In addition to the description of a bacteriophage-typing system for *P. acnes*, this study also notes the presence of the phenomenon of glassy transformation in *P. acnes*. Glassy transformation is the result of lysis of a whole colony by bacteriophage and has seldom been reported in the literature (4) since Twort's notice of bacteriophage in 1915 (18). Marples (11), in 1974, published a photograph of colonies transformed in this manner, suggesting that the transformation resulted from infection by phage liberated

from other bacteria before the organisms were inoculated to the agar plate. The cause of this apparently rare phenomenon is, as yet, unexplained. It is our feeling that the phage responsible for the colony lysis is probably liberated from an organism within the already formed colony rather than coming from an exogenous source, since a bacterium that had been previously infected by a virulent phage would not be capable of multiplying to form a colony. However, this still does not completely explain the observation, since once colonies have become large enough to see, they are presumably past the point of phage infectability (4).

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