Bacteriophage Typing of the "Caulobacter Group"

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The phage types of 77 caulobacters were determined using 26 phages specific for this group of stalked bacteria. A standardized procedure, utilizing routine test dilutions, was used to eliminate an inhibition phenomenon which accounted for 53% of the positive reactions when undiluted phage lysates were tested. Among productive hosts, *Caulobacter* and *Asticcacaulis* phages were genus specific, although this specificity was not demonstrated by the inhibition reactions. However, *Asticcacaulis* ϕ 20S was specific for only 2 of the 15 eccentrically stalked isolates tested. This limited specificity was common among the phages regardless of the cellular morphology of their host, since 7 of the 16 phages having productive hosts had no more than 2 hosts. Without phages that were specific for a broad host range of eccentrically stalked caulobacters, the separation of the genera *Caulobacter* and *Asticcacaulis* was not supported by bacteriophage typing.

Host range was one means used by Schmidt and Stanier (3) to characterize phages active against the stalked bacteria of the "Caulobacter group." The established host ranges were then used in a phage typing scheme for further characterization of the caulobacters (2).

Their 23 phages fell into seven groups on the basis of host range and serological properties. Six of these groups attacked only *Caulobacter* species, and one group attacked only *Asticcacaulis* species. This generic specificity of the phage was presented as a distinct difference between the genera in support of Poindexter's split of the family *Caulobacteraceae* into two genera: *Caulobacter*, for isolates with polar stalks and adhesive material at the distal end of the stalk, and *Asticcacaulis*, for isolates with eccentric stalks and separate polar adhesive material (2). Phage typing was also used to characterize species of *Caulobacter*. Schmidt, Stanier, and Poindexter used high-titer lysates for typing purposes, 10^7 to 10^8 plaque-forming units and 10^6 to 10^8 plaque-forming units, respectively.

The need for utilizing standardized methods for phage typing was initially shown to be essential for obtaining reliable and comparable results by Blair and Williams when phage typing *Staphylococcus* (1). Routine test dilutions (RTDs) of the phages were utilized and defined as the highest dilutions of the phages that just failed to give confluent lysis. High-titer lysates were shown to "inhibit" the growth of many *Staphylococcus* strains, but they failed to produce plaques when diluted. The concentration

of phage sufficient to give near-confluent lysis was between 5×10^4 and 2×10^5 particles per ml. Confluent lysis was produced by a phage concentration between 2 and 10 times greater than that required to produce the RTD. The inhibition phenomenon was not reproducible enough to be useful in phage typing unless, in exceptional cases, these reactions provided the only indication of susceptibility to the phage.

In this investigation, the results of phage typing caulobacters were compared using high-titer lysates and RTDs as the typing methods.

Sixty-eight caulobacters isolated from a wide variety of environmental sources by our laboratory (unpublished data) and nine reference strains supplied by Poindexter were tested. Their laboratory numbers and sources are listed in Table 1. The schemes for the classification of caulobacter types are based primarily upon cellular morphology (2); therefore, the morphology of the caulobacters is listed in Table 2. The subvibrioid-bacteroid was a new morphological type isolated in our laboratory. The isolates were maintained on Poindexter complex medium (PYE) made with distilled water and supplemented with 0.01 mg of riboflavin per ml, and cells for inoculation were used after 24 to 48 h of incubation at 30 C.

The sources of the 26 phages used for typing and their nucleic acid contents are given in Table 3. Nineteen were isolated and characterized by G. H. Szezyk (M.S. thesis, West Virginia Univ., Morgantown, 1967), and each of Schmidt and Stanier's seven phage groups (3) is represented. These authors' original designations were modified to standardize the nomenclature for the present study.

The caulobacters were screened for sensitiv-

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TABLE 1. *Caulobacter* isolates used for bacteriophage typing

<i>Caulobacter</i> isolates	Source	Isolated by
CV-6, 7, 8 ^a	Pond water (W. Va.)	Gerencser
CV-12, 14	Pond water (W. Va.)	Gerencser
CV-17, 18, 20, 37	Creek water (W. Va.)	Gerencser
CV-27	Creek water (W. Va.)	Gerencser
CV-42, 46	Intermittent creek (W. Va.)	Gerencser
CV-54	Soil (W. Va.)	Gerencser
CV-56	Creek water (W. Va.)	Babinchak
CV-70, 71, 72	Creek water (W. Va.)	Babinchak
CV-65, 137, 165, 166, 167, 168	Pond water (W. Va.)	Babinchak
CV-74	Creek water (W. Va.)	Babinchak
CV-76, 113, 114, 115	Sewage (W. Va.)	Babinchak
CV-77	Lake water (Ohio)	Babinchak
CV-79, 80, 81, 83	Lake water (Pa.)	Babinchak
CV-84, 86, 87	Lake water (Pa.)	Babinchak
CV-88, 89, 126	Pond water (Ohio)	Babinchak
CV-90, 91, 92, 93	Lake water (Ohio)	Babinchak
CV-103, 105	Lake water (W. Va.)	Babinchak
CV-107, 108	River water (W. Va.)	Babinchak
CV-117, 118, 119	Rain pool (W. Va.)	Babinchak
CV-129	Spring water (Pa.)	Babinchak
CV-134, 135, 136, 161, 163, 164	Pond water (W. Va.)	Babinchak
CV-139	Bog pool (W. Va.)	Babinchak
CV-140	Creek water (W. Va.)	Babinchak
CV-143, 144, 145	Lake water (W. Va.)	Babinchak
CV-155	Creek water (W. Va.)	Babinchak
CV-156, 157, 158, 159	Pond water (W. Va.)	Babinchak
CV-160	Pond water (W. Va.)	Babinchak
CB-7	Lake water (Calif.)	Poindexter
CB11a, 13	Pond water (Calif.)	Poindexter
CB-15	Pond water (Calif.)	Poindexter
AC-20	Unknown	Poindexter
CB-27	Pond water (Calif.)	Poindexter
AC-48	Pond water (Calif.)	Poindexter
CB-51	Pond water (Calif.)	Poindexter
CB-94	Unknown	Poindexter

^a The notation CV-6, 7, 8, etc., is to be read to mean CV-6, CV-7, CV-8, etc.

TABLE 2. *Caulobacter* cellular morphology

<i>Caulobacter</i> isolates	Morphology ^a
CV-6, 7, 8, 12, 20, 27, 37, 65, 72, 77, 79, 81, 137, 161, 166, CB13, 15, 51 ^b	Vibrioid
CV-17, 18, 42, 46, 70, 76, 80, 83, 103, 156, 157, 165, 167	Limonoid
CV-14, 91	Subvibrioid-bacteroid
CV-56, 105	Subvibrioid-fusiform
CV-54, 84, 87, 89, 90, 92, 93, 107, 108, 126, 113, 114, 115, 155, 159, 160, 163, 164, CB7, 11a, 94	Bacteroid
CV-71, 86, 88, 129, 158, CB27	Fusiform
CV-74, 117, 118, 119, 134, 135, 136, 139, 140, 143, 144, 145, 168, AC20, 48	Bacteroid, eccentric stalk

^a Vibrioid = comma-shaped, curved rod; limonoid = lemon-shaped, plump and rounded; bacteroid = rod-shaped, blunt ends; fusiform = rod-shaped, tapered ends; subvibrioid = mixture of vibrioid and bacteroid or fusiform.

^b The notation CV-6, 7, 8, etc., is to be read to mean CV-6, CV-7, CV-8, etc.

TABLE 3. *Caulobacter* bacteriophages

Phage designations used in this study ^a	Phage designations of Szezyko ^b , and Schmidt and Stanier (3)	Type of nucleic acid ^c
	Szezyko	
φ5B	φRP5	DNA
φ6	φSFM6	DNA
φ10A	φCSP10A	DNA
φ10B	φCSP10B	DNA
φ10C	φCSP10C	DNA
φ10D	φCSP10D	DNA
φ19A	φA ₁ P19A	DNA
φ19B	φA ₁ P19B	DNA
φ19C	φDE19	DNA
φ19D	φLTA19	DNA
φ26	φHC26	DNA
φ36A	φHC36A	DNA
φ36B	φHC36B	DNA
φ36C	φBSCII36A ₁	DNA
φ36D	φBSCII36A ₂	DNA
φ36E	φBSCII36B ₂	DNA
φ43A	φAF43	DNA
φ43B	φBC43	DNA
φ47	φAF47	DNA
	Schmidt and Stanier	
φ3S	φCB3	DNA
φ5S	φCB5	RNA
φ6S	φCB6	DNA
φ8S	φCB8r	RNA
φ13S	φCB13	DNA
φ20S	φAC20	DNA
φ23S	φCB23r	RNA

^a Phages are available from V. F. Gerencser.

^b G. H. Szezyko, M.S. thesis, West Virginia Univ., Morgantown, 1967.

^c DNA, Deoxyribonucleic acid; RNA, ribonucleic acid.

TABLE 4. Reaction patterns of various *Caulobacter* isolates to lytic phages

<i>Caulobacter</i> isolates	Phage patterns
CV-6, 7, 8, 12, 14, 159 ^a	6
CV-17, 18, 37, 72, 86, 165, 167	13S/26/43A/43B/47
CV-20	36C/36D/36E
CV-27	26/36B/36C/36D/36E/43A/43B/47
CV-42, 156, CB15	5S/13S/43A/43B/47
CV-46	5S/26
CV-54, 160	3S/6/6S
CV-65, 103	26/43A/43B/47
CV-70, 77, 81, 137, 157, CB13, 51	5S/13S/26/43A/43B/47
CV-71	6/23S
CV-76	3S/5S/43A/43B/47
CV-79	5S/43A/43B/47
CV-80, 161	5S
CV-83	5S/26/43A/43B/47
CV-91	6S
CV-155	3S/6S
CB11a	3S/6S/8S
AC20, 48	20S
CB27	23S
CV-126	3S

^a The notation CV-6, 7, 8, etc., is to be read to mean CV-6, CV-7, CV-8, etc.

TABLE 5. *Inhibition of Caulobacter isolates by phage lysates*

<i>Caulobacter</i> isolates	Phage lysates causing inhibition
CV-91	$\phi 5B$
CV-42, 74, 76, 91, 113, 114, 115, 117, 118, 119, 126, 129, 134, 135, 158, CB11a, 27, 94 ^a	$\phi 6$
CV-46, 70, 72, 77, 79, 80, 81, 83, 156	$\phi 10A$, 10C, 10D
CV-46, 70, 72, 77, 79, 80, 81, 83	$\phi 10B$
CV-37, 105	$\phi 19A$, 19B, 19C, 19D
CV-20, 42, 56, 79, 105, 156, 161, CB15	$\phi 26$
CV-27	$\phi 36A$
CV-20	$\phi 36B$
CV-6, 7, 8, 12, 14, 20, 46, 56, 80, 105, 115, 160, 161	$\phi 43A$, 43B
CV-7, 8, 12, 14, 20, 46, 56, 105, 160, 161	$\phi 47$
CV-42, 72, 90, 91, 113, 114, 137, 156, 159, CB7, 15, 51	$\phi 3S$
CV-72	$\phi 5S$
CV-42, 72, 76, 77, 81, 83, 90, 108, 113, 114, 115, 126, 137, 156, 157, 159, CB7, 15, 51	$\phi 6S$
CV-6, 7, 8, 12, 14, 20, 27, 46, 56, 65, 76, 79, 80, 83, 103, 105, 161	$\phi 13S$
CV-6, 7, 8, 12, 14, 54, 74, 76, 88, 115, 126, 129, 134, 135, 136, CB11a	$\phi 20S$

^a The notation CV-42, 74, 76, etc., is to be read to mean CV-42, CV-74, CV-76, etc.

ity to the phages by placing a drop of undiluted phage lysate on a PYE overlayer inoculated with 0.1 to 0.2 ml of a bacterial strain. Strains showing sensitivity were then typed in the same manner with seven consecutive decimal dilutions of the active phage lysate. Inhibition was differentiated from phage lysis by the abrupt disappearance of lysate action without plaque formation.

The comparison of the two phage typing methods revealed that the inhibition phenomenon accounted for 53% of the positive reactions observed when high-titer lysates were utilized. The phage patterns of caulobacters determined with RTDs are listed in Table 4. The caulobacters which were inhibited by high-titer lysates, but did not produce plaques with RTDs, are listed with the corresponding active phage lysate in Table 5.

Since Schmidt and Stanier (3) and Poindexter (2) utilized high-titer phage lysates for typing purposes, the inhibition phenomenon would be included in their host ranges. Indeed, two

caulobacters, CB-7 and CB-15, which were reported to be hosts for $\phi 6S$ (3), were inhibited only by the high-titer lysate in this study.

The significance of the inhibition phenomenon on the taxonomic relationship between the two genera, *Caulobacter* and *Asticcacaulis*, needs to be investigated, since 12 polar stalked caulobacters were inhibited by the lysate of *Asticcacaulis* $\phi 20S$ and 6 eccentrically stalked strains were inhibited by the lysate of *Caulobacter* $\phi 6$. This finding differed significantly with the absolute generic specificity of the phage found in the Schmidt and Stanier study (3).

Fifteen of the 16 phages which had productive hosts and 10 phages which had no productive hosts among the 77 caulobacters tested had members of the genus *Caulobacter* as propagating strains. The presence of both ribonucleic acid and deoxyribonucleic acid phage and broad and narrow host ranges among this group of phages would make phage typing a valuable tool for classifying the genus *Caulobacter*. However, to insure reliable and comparable results, the predominance of the inhibition phenomenon established the need for utilizing RTDs and standardized procedures for phage typing caulobacters, as outlined for the phage typing of *Staphylococcus* (1).

A broad spectrum of phages specific for eccentrically stalked caulobacters is needed similar to the phage group specific for *Caulobacter* strains. Only three phages have been reported for eccentrically stalked caulobacters, and these could represent reisolates of the same phage. *Asticcacaulis* $\phi 20S$ represents these phages which were isolated from the same environmental source and were indistinguishable in characterization studies (3). This phage group was specific for 3 of Poindexter's 5 eccentrically stalked strains, but none of the 13 *Asticcacaulis* strains isolated by this laboratory served as host for *Asticcacaulis* $\phi 20S$.

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REPRINT REQUESTS

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