

A SEROLOGICAL STUDY OF STRAINS OF *ALCALIGENES RADIOBACTER* AND *PHYTOMONAS TUMEFACIENS* IN THE "M" AND "S" PHASES¹

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The cultural and morphological similarity of *Phytomonas tumefaciens* and *Alcaligenes radiobacter* has been recognized for many years. Many attempts have been made to devise more adequate means of distinguishing cultures of these two forms without resorting to differentiation on the basis of plant infection studies (Conn, Wolfe, and Ford, 1940). To a lesser extent there has been difficulty in distinguishing strains of these two species from certain species of the genus *Rhizobium* (Hofer, 1941). The results obtained in these attempts at laboratory differentiation have served to emphasize the similarity of these forms in laboratory media and to place the final burden of identification upon the ability to infect a susceptible host (Hendrickson, Baldwin, and Riker, 1934).

Inasmuch as the serological studies of Reid, Naghski, Farrell, and Haley (1942) have shown that several plant pathogens characterized as species of *Phytomonas* are in fact transitory adaptations of *Pseudomonas fluorescens*, and the studies of Elrod and Braun (1942) have revealed a similar relationship between certain other forms characterized as *Phytomonas* species and *Pseudomonas aeruginosa*, an examination of *Phytomonas tumefaciens* and *Alcaligenes radiobacter* was undertaken in an effort to determine the relationship of these two similar organisms.

METHODS

Cultures used. The cultures of *Phytomonas tumefaciens* and *Alcaligenes radiobacter* used in this study were obtained from the stock culture collection of the Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, N.Y., through the courtesy of Dr. A. W. Hofer. These strains are ones with which many research workers in the fields of soil bacteriology and plant pathology are acquainted and have been employed by a number of these workers in their investigations (Hendrickson, Baldwin, and Riker, 1934; Hofer, 1941). The following seven strains of *Alcaligenes radiobacter* were used.

R1-1a. Isolated by W. H. Wright of the University of Wisconsin in 1924.

R3. Isolated by E. B. Fred of the University of Wisconsin in 1927.

R3sc1. Single cell isolation from culture R3.

S36. Isolated by Löhnis and Beijerinck in 1904.

S192. Isolated by N. R. Smith of the U. S. D. A. in 1927.

1000. An isolation made by F. Löhnis, date unknown.

ISC. Isolated by R. Hansen of the University of Illinois in 1923.

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The strains of *Phytomonas tumefaciens* used were ScT5fff1 and ScA-1. These are single cell isolates made at the University of Wisconsin. The two strains of *Phytomonas tumefaciens* and six of the seven strains of *Alcaligenes radiobacter* are known as typical of their respective species. Strain S36 of *Alcaligenes radiobacter*, however, has been described by workers in other laboratories as "atypical."

The strains of the two species were tested on tomato plants to check pathogenicity prior to use in this study. The tests were conducted with the organisms in the Dawson "M" phase as received at this laboratory. The strains of *Phytomonas tumefaciens* proved virulent for the tomato plant and the strains of *Alcaligenes radiobacter* proved noninfective.

Media employed. Stock cultures of the organisms in the Dawson "M" phase were held on yeast-extract mannitol mineral-salts agar. In decapsulation attempts yeast-extract mineral-salts broth was used and similar mannitol-free media were employed in the culture of the organisms in the Dawson "S" phase.

Serological techniques. Live antigen suspensions of 48-hour cultures were used in rapid immunization of rabbits. Daily doses of 1.0 ml were given following an initial dose of 0.1 ml and injections continued until approximately 16 ml had been administered intravenously. Titers were determined by the use of standard macroscopic agglutination tests.

Conversion of the Dawson "M" to the Dawson "S" phase of the organism was accomplished by daily transfer in yeast-extract mineral-salts broth containing 10 per cent of the homologous antiserum. The conversion from "M" to "S" phase was observed by daily streaking on solid media, and serological examinations of isolates from the streak plates were made in the case of colonies which exhibited "S" characteristics, according to the method of Dawson and Sia (1931).

Agglutinin absorption tests were performed in accordance with the method of Krumwiede, Cooper, and Provost (1925).

RESULTS

Three of the "typical" strains of *Alcaligenes radiobacter*, R1-1a, R3, and R3sc1, and the two strains of *Phytomonas tumefaciens*, ScT5fff1 and ScA-1, were selected for use as antigens in the "M" phase for animal immunization. The titers obtained with strains R3sc1 and ScT5fff1 are shown in tables 1 and 2, respectively. Titers obtained with antisera from animals immunized with strains R1-1a and R3 were similar to those obtained with R3sc1, and antiserum obtained from an animal immunized with ScA-1 proved similar to that obtained from the use of ScT5fff1, and consequently the results obtained are not shown in tabular form.

As shown in table 1, the six typical strains of *Alcaligenes radiobacter* proved to be similar in antigenic composition in the "M" phase. The atypical strain S36, on the other hand, proved serologically unlike the other six in the mucoid state. The serological examination effected a separation of this strain from the typical strains similar to that made in other laboratories on the basis of cultural tests. It is also to be noted from the data presented in table 1 that the strains of *Phytomonas tumefaciens* employed are antigenically unlike the typical strains of *Alcaligenes radiobacter* in the Dawson "M" phase and no cross agglutination occurred in significant dilutions.

The antiserum prepared with the use of *Phytomonas tumefaciens* ScT5fff1 proved almost equally effective in the agglutination of cells of the other strain of *Phytomonas tumefaciens*, as shown in table 2. As would be expected from the results with antiserum prepared against *Alcaligenes radiobacter* "M" phase cells (table 1), no agglutination of *Alcaligenes radiobacter* cells occurred in the presence of the *Phytomonas tumefaciens* "M" phase antiserum.

Since in each case typical strains within the species proved antigenically similar in the "M" phase, the strains which had been employed as antigens for animal immunization in the "M" phase were selected for further investigation in the

TABLE 1
Agglutination titers of antiserum prepared against *Alcaligenes radiobacter* R3sc1 Dawson "M" phase

CELLS	DILUTION									CON- TROL
	50	100	200	400	800	1,600	3,200	6,400	12,800	
<i>A. radiobacter</i> R1-1a.....	3+	3+	4+	4+	4+	4+	4+	3+	+	-
<i>A. radiobacter</i> R3.....	3+	3+	3+	4+	4+	4+	4+	3+	+	-
<i>A. radiobacter</i> R3sc1.....	3+	3+	4+	4+	4+	4+	4+	2+	+	-
<i>A. radiobacter</i> S36.....	-	-	-	-	-	-	-	-	-	-
<i>A. radiobacter</i> S192.....	2+	2+	4+	4+	3+	2+	2+	+	±	-
<i>A. radiobacter</i> 1000.....	4+	4+	4+	4+	4+	4+	4+	2+	+	-
<i>A. radiobacter</i> ISC.....	2+	2+	3+	4+	4+	4+	3+	2+	+	-
<i>P. tumefaciens</i> ScT5fff1.....	±	-	-	-	-	-	-	-	-	-
<i>P. tumefaciens</i> ScA-1.....	+	-	-	-	-	-	-	-	-	-

TABLE 2
Agglutination titers of antiserum prepared against *Phytomonas tumefaciens* ScT5fff1 Dawson "M" phase

CELLS	DILUTION									CON- TROL
	40	80	160	320	640	1,280	2,560	5,120	10,240	
<i>A. radiobacter</i> R1-1a.....	±	-	-	-	-	-	-	-	-	-
<i>A. radiobacter</i> R3.....	±	-	-	-	-	-	-	-	-	-
<i>A. radiobacter</i> R3sc1.....	±	-	-	-	-	-	-	-	-	-
<i>P. tumefaciens</i> ScT5fff1.....	3+	4+	4+	3+	4+	4+	4+	4+	2+	-
<i>P. tumefaciens</i> ScA-1.....	2+	2+	3+	3+	3+	3+	2+	2+	2+	-

Dawson "S" phase. Removal of the capsule from the cells of *Alcaligenes radiobacter* R3sc1 was first attempted and was accomplished with 19 successive transfers in yeast-extract mineral-salts broth containing 10 per cent homologous antiserum. Decapsulation of *Phytomonas tumefaciens* ScT5fff1 was next attempted and was accomplished with little difficulty in 7 successive transfers. Likewise, the removal of capsular material from cells of *Phytomonas tumefaciens* ScA-1 did not prove difficult.

With much smaller inocula than those employed in previous experiments, cells of *Alcaligenes radiobacter* R1-1a and R3 were freed of capsular material in 12 and

11 successive transfers, respectively. The relative stability of the "M" phase of *Alcaligenes radiobacter* strains as contrasted with the "M" phase of *Phytomonas tumefaciens* strains was obvious in all attempts at conversion.

TABLE 3
Agglutination titers of antiserum prepared against *Alcaligenes radiobacter* R3sc1
Dawson "S" Phase

CELLS	DILUTION									CON- TROL
	40	80	160	320	640	1,280	2,560	5,120	10,240	
<i>A. radiobacter</i> R3sc1 "S"	2+	3+	4+	4+	4+	4+	4+	2+	—	—
<i>A. radiobacter</i> R3sc1 "M"	±	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R1-1a "M"	±	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R3 "M"	±	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScT5fff1 "S"	2+	2+	4+	4+	4+	4+	3+	2+	—	—
<i>P. tumefaciens</i> ScTfff1 "M"	±	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScA-1 "M"	±	—	—	—	—	—	—	—	—	—

TABLE 4
Agglutination titers of antiserum prepared against *Phytomonas tumefaciens* ScT5fff1
Dawson "S" phase

CELLS	DILUTION									CON- TROL
	40	80	160	320	640	1,280	2,560	5,120	10,240	
<i>A. radiobacter</i> R3sc1 "S"	2+	3+	4+	4+	3+	3+	4+	2+	—	—
<i>A. radiobacter</i> R3sc1 "M"	±	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R1-1a "M"	±	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R3 "M"	±	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScT5fff1 "S"	2+	2+	2+	3+	3+	3+	3+	2+	—	—
<i>P. tumefaciens</i> ScT5fff1 "M"	±	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScA1 "M"	±	—	—	—	—	—	—	—	—	—

TABLE 5
Agglutination titers of antiserum prepared against *Alcaligenes radiobacter* R3sc1
Dawson "M" phase

CELLS	DILUTION									CON- TROL
	40	80	160	320	640	1,280	2,560	5,120	10,240	
<i>A. radiobacter</i> R3sc1 "S"	—	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R3sc1 "M"	4+	4+	4+	4+	4+	4+	4+	4+	3+	—
<i>P. tumefaciens</i> ScT5fff1 "S"	—	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScT5fff1 "M"	—	—	—	—	—	—	—	—	—	—

Following conversion of the three strains of *Alcaligenes radiobacter* and the two strains of *Phytomonas tumefaciens* from the "M" to "S" phase, rabbits were rapidly immunized with these cultures in the "S" phase. Agglutination reactions

obtained with antisera prepared with the use of *Alcaligenes radiobacter* R3sc1 and *Phytomonas tumefaciens* ScT5fff1 are shown in tables 3 and 4. The reactions obtained with antisera prepared against the other three strains did not differ from those shown in these tables.

The data presented in tables 3 and 4 show that the antisera prepared against these strains in the "S" phase fail to agglutinate the "M" phase cells of the homologous strains but, on the other hand, indicate a striking serological similarity between the "S" phase cells of the two species.

Tests of "S" phase cells against antisera prepared with "M" phase organisms are shown in tables 5 and 6. It is to be noted that conversion to the "S" phase rendered these cells inagglutinable by antiserum prepared against the homologous strains in the "M" phase.

The antigenic similarity of the "S" phase cells of the two species as shown in tables 3 and 4 was made the subject of further study. The agglutinin absorption technique was employed in an examination of the "S" phase cells and the antisera prepared with the use of such cells. Complete reciprocal agglutinin absorption

TABLE 6
Agglutination titers of antiserum prepared against *Phytomonas tumefaciens* ScT5fff1
Dawson "M" phase

CELLS	DILUTIONS									CON- TROL
	40	80	160	320	640	1,280	2,560	5,120	10,240	
<i>P. tumefaciens</i> ScT5fff1 "S".....	—	—	—	—	—	—	—	—	—	—
<i>P. tumefaciens</i> ScT5fff1 "M".....	3+	3+	4+	4+	3+	4+	4+	4+	2+	—
<i>A. radiobacter</i> R3sc1 "M".....	—	—	—	—	—	—	—	—	—	—
<i>A. radiobacter</i> R3sc1 "S".....	—	—	—	—	—	—	—	—	—	—

was found by the method of Krumwiede, Cooper, and Provost (1925) in the examination of these five strains and respective antisera, indicating that in the "S" phase these strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* are serologically identical.

DISCUSSION

The results obtained in the serological study of typical strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the Dawson "M" phase are in agreement with the findings of Riker, Banfield, Wright, Keitt, and Sagen (1930). These workers likewise found no serological similarity between representatives of these two species in the "M" phase.

A similar apparent lack of serological relationship in the "M" phase was shown by Harris (1940) to exist within the species *Pseudomonas aeruginosa*. Decapsulation of these dissimilar strains of *P. aeruginosa* by Harris, however, yielded "S" phase cells which, by the agglutinin absorption technique, were shown to be antigenically identical. The use of decapsulation and agglutinin absorption techniques in this study have yielded results comparable to those obtained by

Harris in his studies of strains of *Pseudomonas aeruginosa*. Although the strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* used showed no serological similarity in the "M" phase, this difference proved to exist only in the "M" phase, whereas antigenic identity was found to exist in the "S" phase.

These results suggest that the organisms recognized as *Alcaligenes radiobacter* and *Phytomonas tumefaciens* comprise a single species and in their usual "M" phase bear the same relation to each other as do the types of the pneumococcus to one another.

SUMMARY

An investigation has been made of the serological relationship of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* with the use of agglutination and agglutinin absorption techniques employed in the study of typical strains in the Dawson "M" and "S" phases.

It has been found that in the strains under study *Alcaligenes radiobacter* and *Phytomonas tumefaciens* differ serologically in the "M" phase. The same strains are antigenically identical in the "S" phase, as indicated by complete reciprocal agglutinin absorption.

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