Pseudomonas syringae: Rough Colony Type Mutants and Filamentous Cells

J. D. Otta

Assistant Professor of Plant Science, Department of Plant Science, South Dakota State University, Brookings 57006.

Authorized for publication as Journal Series Paper No. 1359.
Accepted for publication 15 October 1975.

ABSTRACT

OTTA, J. D. 1976. Pseudomonas syringae: rough colony type mutants and filamentous cells. Phytopathology 66: 249-252.

Cultures of *Pseudomonas syringae* grown on King's Medium B mutated rapidly to a rough colony type. Filamentous cells were associated with the rough colony type with individual cell lengths ranging from 0.8 to 176 μm. Cells in rough cultures produced rough colony types regardless of cell length. No cells longer than 4.0 μm were observed in any of the smooth colony type cultures. No reversions of rough to smooth occurred when rough cultures were inoculated into sorghum and reisolated after 18 weeks.

Filamentous cell formation by various bacteria when under the effect of sublethal concentrations of different chemicals, antibiotics, nutritional deficiencies, and physical factors is well known (3, 4, 5, 6, 7, 8, 10, 15, 19, 20). However, these filamentous forms generally revert back to their original shape and size when grown under conventional cultural conditions. Reports of viable filamentous mutants among the phytopathogenic bacteria are less frequent. Abnormally long forms of *Erwinia amylovora* (Burrill) Winslow et al., were observed by Billing et al. in England (2) and by Shaffer and Goodman (17) and Vörös and Goodman (18) in the USA. These long-celled strains have varied from virulent (18) to avirulent (17) or were never isolated and properly identified (2).

The appearance of rough (matte) colony types in previously smooth cultures or rough sectors in smooth colonies has been noted in the phytopathogenic pseudomonads (11, 14, 16). Otta and Bain (13) reported a correlation between these rough colony types, filamentous cells, and increased mean cell length for *Pseudomonas syringae* van Hall. This correlation between filamentous cells and rough colony type has also been reported for *E. amylovora* (17) and *P. mori* [Boyer and Lambert] Stevens (16). The studies reported here were designed (i) to examine the relationship between cell length and colony type of *P. syringae,* (ii) to determine the rate of conversion of smooth isolates to rough types on standard laboratory media, and (iii) to determine the stability of the rough form in a host plant.

MATERIALS AND METHODS

Colony type versus cell length.—Fifty-seven cultures of *P. syringae,* 30 smooth and 27 rough, were studied. Eight of the rough cultures were obtained as sectors from smooth colonies and both the parent smooth culture type and the rough mutant type were included in the study. Single-cell isolates of all cultures were used to initiate all studies. All cultures were streaked onto tubes of King's Medium B (MB) (9), and after 24 hours of incubation at room temperature 10 ml of sterile H_2O was added to each tube and allowed to stand for 30 minutes to form a bacterial suspension. Three water mounts of each culture were prepared on slides, dried, and then stained for 2 minutes in a 2% crystal violet solution.

The seventh edition of Bergey's Manual (1) lists the upper cell length limit of *P. syringae* at 3 μm. The assumption was made that cells greater than 5 μm in length were abnormally long. The percentage of cells in a culture longer than 5 μm was determined by counting cells with an eyepiece grid. Approximately six subsamples were selected from slides of each culture until 300 cells had been counted. After the percentage of abnormally long cells had been determined, 100 cells in each culture were measured at random with the total number measured in the normal and abnormal length categories based on the percentages previously determined. Fourteen of the suspensions prepared above, seven each of smooth and rough, were dilution-plated onto MB with developing colonies recorded after 72 hours.

Host plant effect on rough cultures.—Three rough cultures were inoculated into sorghum, *Sorghum vulgare* Pers. var. *subglabrescens* (Steud.) A. F. Hill, (SD503) by vacuum infiltration, placed in a mist chamber overnight and then onto a greenhouse bench. Two weeks after symptom development, diseased leaves were removed and triturated in sterile water. One-milliliter samples of the liquid obtained were dilution plated onto MB. Rough and smooth colony counts were determined after 72 hours. The remaining liquid from the diseased leaves was used to vacuum infiltrate leaves of 2-week-old sorghum. The incubation, isolation, and inoculation procedures were repeated every 2 weeks for 4 months.

Motility.—All cultures were examined for motility with the light microscope by preparing water suspensions of 18-hour-old cultures grown on MB.

Media effect on conversion.—Each culture was grown

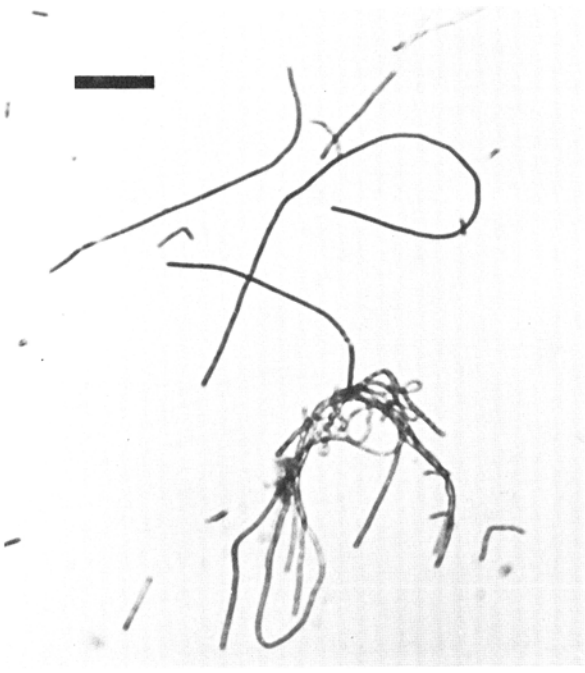

Fig. 1. Typical cells of *Pseudomonas syringae* observed in rough type cultures. Note the abnormally long filamentous cells (bar equals 10 μm).

for 18 hours on MB and was then streaked onto one slant each of MB, nutrient agar (NA), and nutrient agar plus 1 percent dextrose (NDA). The cultures were allowed to grow for 2 days at room temperature and were then placed into refrigerated storage at 7 ± 3 C. The cultures were transferred monthly, with the transfer always being placed on the same medium from which it was taken. One month after initiation of the experiment and every two months thereafter, the bacteria remaining in the tube after transfer were suspended in 9 ml of sterile water. These cultures were then dilution plated onto MB. Three to four days later colony counts were taken to determine the percentage of rough mutants.

RESULTS

Colony type versus cell length.—Abnormally long cells (> 5 μm) were observed in all rough cultures (Fig. 1), but no abnormally long cells were observed in any of the smooth cultures (Table 1, Fig. 2). The mean cell length for all rough cultures was significantly (P = 0.01) greater than the mean of the smooth cultures. Abnormally long cells in individual rough cultures varied from 7% to 85% of the population. Individual cell lengths ranged from 0.8 to 176 μm and 0.8 to 4.0 μm for rough and smooth cultures, respectively. Host of origin had no apparent effect on cell lengths. No reversion of rough colony types to smooth types were observed in the dilution plates of 14 cultures.

Host plant effect on rough cultures.—After 4.5 months in sorghum plants no revertants of rough to smooth were observed. All colony types obtained by reisolation were identical in appearance to the original rough cultures used. Even rough cultures such as S-52, which consisted predominately of normal-length cells (Table 1), did not produce any smooth colony types.

Motility.—All rough and smooth cultures studied were motile; in a few cultures approximately half of the extremely long cells (100 - 175 μm) were not.

Media effect on conversion.—The effects of three

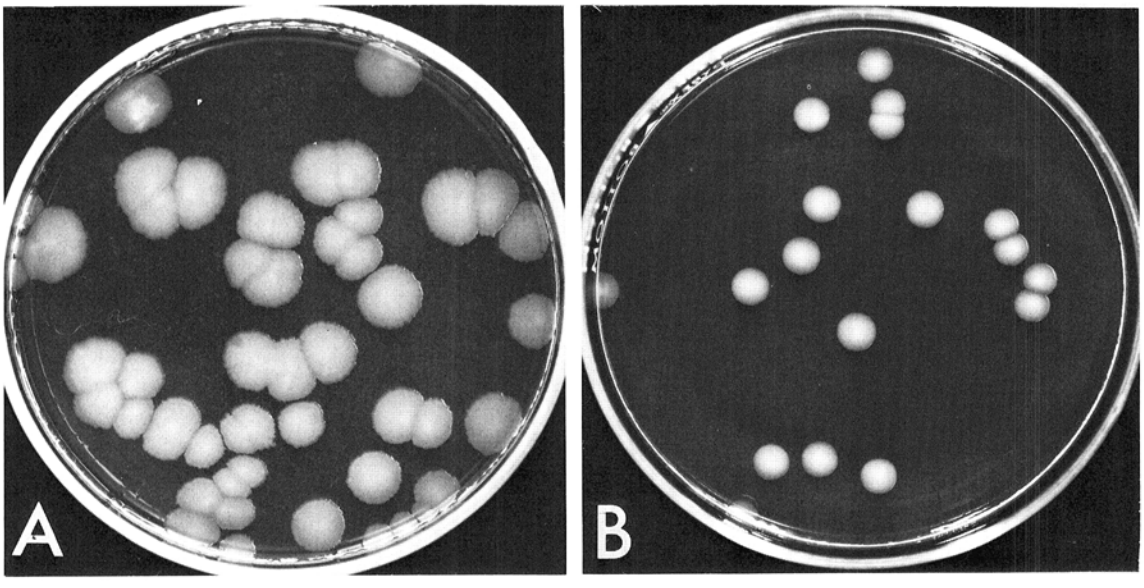

Fig. 2-(A, B). Characteristic rough (A) and smooth (B) colony types of *Pseudomonas syringae* after 1 week on King's Medium B.

TABLE 1. *Pseudomonas syringae* rough and smooth culture cell lengths

Culture type	Host of origin	Culture numbers	Cell lengths (μm)[j] Range	Percent >5 μm	Culture means	Culture type mean
Rough	Lilac	P123[g], 281[a]	0.8 - 140.8	42	4.0 - 32.8	
	Cherry	922[e], 940[e], 99[e], 862a[e]	1.6 - 134.8	58	13.1 - 21.3	
	French prune	GS8[i], 12a[i], GS3[i], TN5[i], 857[e], GS28[i]	1.2 - 146.4	52	8.0 - 17.3	
	Pear	1073[a], 1259[a]	2.4 - 104.0	72	9.2 - 16.4	
	Plum	881[e], Ps251[b]	1.2 - 126.4	34	6.0 - 16.2	
	Sugarbeet	B173[c]	2.8 - 80.0	47	13.6	
	Pepper	Ps173-28[b]	1.6 - 98.0	38	9.5	
	Apple	1304[a], Ps230[b]	1.2 - 60.4	12	5.6 - 6.6	
	Peach	B-5[c]	1.2 - 60.4	10	6.6	
	Almond	30[e]	1.6 - 40.4	18	3.4 - 6.4	
	Apricot	127[e], 130[e]	1.6 - 50.4	12	5.3 - 5.7	
	Okra	1696[b]	2.0 - 108.8	10	5.6	
	Sorghum	S-52[d]	1.6 - 9.2	7	3.1	10.6
Smooth	Plum	881[e]	1.2 - 4.0	0	2.3	
	Tomato	B-238[c]	0.8 - 4.0	0	2.3	
	Unknown	M-2[f]	1.2 - 3.2	0	2.2	
	Pear	Ps179[b], 1259[a], 1073[a], 1083[a]	1.2 - 3.6	0	1.5 - 2.2	
	Pea	B-166[c]	1.2 - 3.6	0	2.1	
	Lilac	1071[a], 524[a]	1.2 - 3.6	0	1.8 - 2.1	
	Apricot	B-154[c], A-3[i], 918[e]	0.8 - 3.6	0	1.9 - 2.0	
	Cherry	940[e]	1.2 - 3.6	0	2.0	
	Pearl millet	1053[a]	0.8 - 3.2	0	1.9	
	French prune	131[i], GS28[i], TN5[i], 2a[i], GS26-56[i]	0.8 - 2.8	0	1.7 - 1.9	
	Peach	B-243[c], B-5[c]	0.8 - 2.8	0	1.7 - 1.9	
	Sugarbeet	PA121[b]	1.2 - 3.6	0	1.9	
	Avocado pear	Ps232[b]	1.2 - 2.4	0	1.9	
	Hawthorn	1296[a]	0.8 - 3.6	0	1.8	
	Guar	Ps259[b]	1.2 - 2.8	0	1.8	
	Bean	HPD[h]	0.8 - 2.8	0	1.7	
	Raspberry	Ps229[b]	0.8 - 2.8	0	1.7	
	Almond	AR6[i]	1.2 - 2.4	0	1.7	
	Okra	1696[a]	1.2 - 3.2	0	1.7	1.9
		LSD ($P = 0.05$)			5.4	2.9
		($P = 0.01$)			7.1	3.8

Original sources of smooth parent cultures:
[a]NCPPB - National Collection of Plant Pathogenic Bacteria, Harpenden, Hertsfordshire, England.
[b]ICPB - International Collection of Phytopathogenic Bacteria, Davis, Calif.
[c]J. E. DeVay, Dep. Plant Pathol., Univ. Calif., Davis.
[d]M. N. Schroth, Dep. Plant Pathol., Univ. Calif., Berkeley.
[e]Harley English, Dep. Plant Pathol., Univ. Calif., Davis.
[f]W. H. Shaffer, Jr., Dep. Plant Pathol., Univ. Missouri, Columbia.
[g]D. W. Dye, Plant Dis. Div., Dep. of Sci. and Ind. Res., Aukland, N.Z.
[h]James Guthrie, Dep. Plant Sci., Univ. Idaho, Moscow.
[i]Isolated by author.
[j]Based on measurement of 100 cells per culture.

media on the rate of conversion from smooth to rough culture types are shown in Figure 3. Rough mutants on MB were detected in two of the three cultures after one month and in the third culture after 3 months. Only two of the cultures on NA and NDA produced rough mutants and then only on the 9th or 13th month of the study (Fig. 3).

DISCUSSION

The correlation between the presence of abnormally long cells and the occurrence of rough colony types in cultures of *P. syringae* suggests a cause and effect relationship. Differences observed between the characteristics of rough types of *P. syringae, P. lachrymans,* and *P. mori* (12, 13, 14, 16) indicate that rough mutants among the phytopathogenic pseudomonads are not of a uniform type. Rough types are only rarely found in nature (12, 14) and the possible ecological disadvantages of lack of motility in *P. mori* rough type (16) and the abnormal cell lengths of *P. mori* and *P. syringae* rough types would appear to be

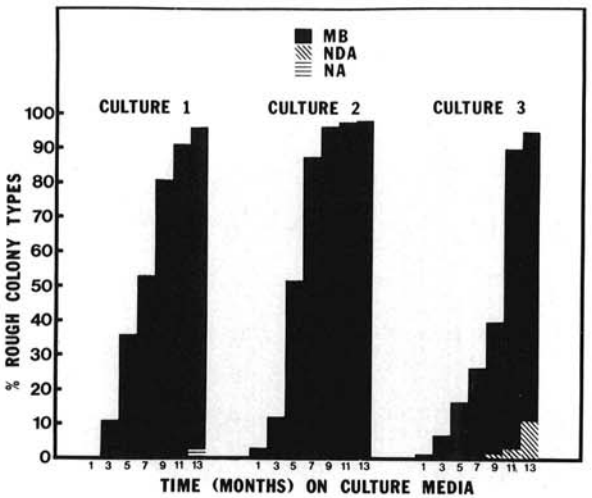

Fig. 3. Occurrence of rough colony types in smooth cultures of *Pseudomonas syringae* on three different media.

reasonable explanations for their absence in natural disease situations, even though they are virulent when artificially introduced into susceptible host tissue.

Rough mutants formed by *P. syringae* on MB are very stable with all viable cells, regardless of length, producing rough colony types. The complete absence of smooth colonies in sorghum reisolations indicates that removing the bacteria from the MB environment for 18 weeks is not enough to induce reversion of the rough mutants. The reason for the rapid appearance of stable rough mutants of *P. syringae* on MB and not on NA nor NDA is not clear at present. However, the high magnesium concentration of MB in relation to the other two media might be a possible factor (19).

It has become apparent over the past seven years that many workers are experiencing difficulty in maintaining *P. syringae* in the smooth form. It has been very common to receive cultures of mixed smooth and rough types from various phytobacteriologists and culture collections. The results of this study demonstrate that a simple microscopic observation of 18- to 24-hour-old cultures of *P. syringae* is often sufficient to detect the presence of rough mutants. The alternative is the more time-consuming dilution plating. It is also apparent that MB should not be utilized as a stock culture medium for *P. syringae* even for short-term storage if the wild-type culture is to be maintained.

LITERATURE CITED

1. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY, 7th Ed. 1957. Williams and Wilkins, Baltimore. 1,094 p.
2. BILLING, E., L. A. E. BAKER, J. E. CROSSE, and C. M. E. GARRETT. 1961. Characteristics of English isolates of Erwinia amylovora (Burrill) Winslow et al. J. Appl. Bact. 24:195-211.
3. DEERING, R. A. 1958. Studies on division inhibition and filament formation of Escherichia coli by ultraviolet light. J. Bact. 76:123-130.
4. FERRONI, G. D., and W. E. INNISS. 1973. Thermally caused filament formation in the psychrophile Bacillus insolitus. Can. J. Microbiol. 19:581-584.
5. FERRONI, G. D., and W. E. INNISS. 1974. Reversal of filament formation in the psychrophile Bacillus insolitus. Can. J. Microbiol. 20:1281-1283.
6. GRULA, E. A. 1960. Cell division in a species of Erwinia. I. Initial observations relating to nutritional dependency. J. Bact. 80:369-374.
7. GRULA, E. A. 1960. Cell division in a species of Erwinia. II. Inhibition of division by d-amino acids. J. Bact. 80:375-385.
8. GRULA, E. A., and M. M. GRULA. 1962. Cell division in a species of Erwinia. III. Reversal of inhibition of cell division caused by d-amino acids, penicillin, and ultraviolet light. J. Bact. 83:981-988.
9. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
10. LOVELESS, L. E., E. SPOERL, and T. H. WEISMAN. 1954. A survey of effects of chemicals on division and growth of yeast and Escherichia coli. J. Bact. 68:637-644.
11. LUCAS, L. T., and R. G. GROGAN. 1969. Serological variation and identification of Pseudomonas lachrymans and other phytopathogenic Pseudomonas nomenspecies. Phytopathology 59:1908-1912.
12. LUCAS, L. T., and R. G. GROGAN. 1969. Pathogenicity and other characteristics of smooth and rough isolates of Pseudomonas lachrymans. Phytopathology 59:1918-1923.
13. OTTA, J. D., and O. M. BAIN. 1971. Relationship of cell length and colony type in Pseudomonas syringae. Phytopathology 61:1025 (Abstr.).
14. OTTA, J. D., and H. ENGLISH. 1971. Serology and pathology of Pseudomonas syringae. Phytopathology 61:443-452.
15. PULVERTAFT, R. S. 1952. The effect of antibiotics on growing cultures of Bacterium coli. J. Pathol. Bact. 64:75-89.
16. SATO, M., and K. TAKAHASHI. 1973. Rough colony type mutant of Pseudomonas mori (Boyer et Lambert) Stevens. Ann. Phytopathol. Soc. Jap. 39:425-428.
17. SHAFFER, W. H., JR., and R. N. GOODMAN. 1962. Progression in vivo, rate of growth in vitro, and resistance to streptomycin, as indices of virulence of Erwinia amylovora. Phytopathology 52:1201-1207.
18. VÖRÖS, J., and R. N. GOODMAN. 1965. Filamentous forms of Erwinia amylovora. Phytopathology 55:876-879.
19. WEBB, M. 1949. The influence of magnesium on cell division. II. The effect of magnesium on the growth and cell division of various bacterial species in complex media. J. Gen. Microbiol. 3:410-417.
20. ZOBELL, C. E., and A. B. COBET. 1964. Filament formation by Escherichia coli at increased hydrostatic pressures. J. Bact. 87:710-719.