DNA Base Composition of Some Phytopathogenic Pseudomonads

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ABSTRACT

A large group of fluorescent phytopathogenic pseudomonads, previously distinguished from fluorescent saprophytes by a more limited nutritional versatility and a negative test for arginine dihydrolase, also exhibit a lower range of values for their DNA base compositions. These pathogens ranged from 57 to 60% G + C, whereas other fluorescent pseudomonads are reported to range from 59-68% G + C. Satellite bands were not observed with buoyant density centrifugation. Phytopathology 60:1863-1864.

Additional key words: Pseudomonas mori, DNA base ratio, taxonomy, buoyant density.

In a previous nutritional study with 189 tests on 150 strains of phytopathogenic pseudomonads, Adansonian analysis yielded a cluster of fluorescent pathogens distinguishable by their lack of many characters common to all other fluorescent species (12). This group of pathogens consisted of more than 30 nomenspecies that induced hypersensitivity in nonhosts, and which were arginine dihydrolase-negative. They were relatively slow-growing fluorescent plant pathogens capable of utilizing 20-43% of the 170 carbon sources as compared to the 40-60% range of the three fluorescent species (saprophytes) recognized by Stanier et al. (14). In this paper we will refer to this group as Group I, as defined by Sands et al. (12), which closely corresponds to Group I of Misaghi & Grogan (10) and Groups I, II, and III of Lelliott et al. (5).

DNA base composition serves as an important criterion when examining relatedness among bacterial species. Bacteria having different DNA base compositions fall into different clusters when analysis of phenotype is carried out by numerical means (7). A study of the DNA base composition of numerically analyzed strains in Group I was therefore undertaken to investigate relationships within the group and to check differences between the pathogens and saprophytes.

Materials, Methods, and Results.—The pathogens listed in Table 1 are representative strains from subgroups a-h of Group I determined by Adansonian analysis. The origin of the strains and their detailed descriptions are published elsewhere (12), with the exception of reference strain COR of *Pseudomonas aeruginosa*. This was obtained from the Institute of Medical and Veterinary Science, Adelaide, Australia. The cells were grown as described by Sands et al. (11). After being washed twice in phosphate buffer, they were stored at −17 C. DNA extraction was carried out by Marmur's method (8) for gram-negative cells, and the DNA was characterized by ultraviolet absorption and deoxypentose and inorganic phosphate analysis. The buoyant density of each DNA preparation was determined in a CsCl density gradient, and compared to a reference DNA from *P. aeruginosa* strain COR which sedimented as a single band with a buoyant density of 1.726 g cm^{-3}. This density and the corresponding base composition of 67% G+C, were determined by comparison with previously studied DNA from *E. coli*, calf thymus, and various *Thiobacillus* species (2). The probable error in base composition as determined by this method is ±1% G+C (1, 13). The DNA of each pathogenic strain studied appeared as a single band in all cases, serving as a check against contamination and as evidence of the absence of minor "Satellite" bands as found in *P. stutzeri* by Mandel (6).

Both Mandel (6) and DeLey (1) have previously reported base ratios of phytopathogenic pseudomonads, and for comparison we included one strain from each of their studies in our work. Using the same density-gradient technique as Mandel, we obtained identical results with *P. putida* strain 53 (59% G+C; buoyant density 1.719 g cm^{-3}). These identical results allow us to make taxonomically accurate comparisons of our data with Mandel's extensive data for saprophytes. DeLey, using thermal denaturation methods, found a base composition of 59.3% G+C for strain 81 of *P. morsprunorum* (N.C.P.P.B. 560) whereas we report 57% G+C for the same strain. DeLey reported for 17 strains in Group I a range of 59.3-61.2% G+C (average 59.8%) whereas we report 57-60% G+C (average 58.3%). These discrepancies in base compositions are probably due to differences in technique. Mandel included only two strains of Group I organisms, *P. phaseolicola* and *P. tabaci*, which contained DNA with 58.9 and 58.2 moles % G+C, respectively. These fall near the center of the range of our data but are below the 59.3-61.2% G+C range DeLey reports for the group.

Discussion.—Although DeLey concluded that the fluorescent phytopathogenic pseudomonads fell within the limits of the genus reported by Mandel (6), we found that the Group I organisms overlap and extend the lower limits of the genus. The phenotypes of Group I pathogens somewhat resemble the phenotypes of *P. fluorescens* biotypes A, B, and G, which have base compositions of 60.5 ± 1.1, 61.3 ± 1.1, and 60.5 ± 1.0 moles % G+C, respectively (6). In our data, the Group I range of 57-60% is only twice the ±1% probable error involved in these measurements, and any speculation concerning subgroup patterns of variation must await a more extensive study. Base composition differences are considered a valid criterion of difference between organisms, but are only null evi-

TABLE 1. DNA base compositions of phytopathogenic fluorescent pseudomonads

Nomenspecies	Strain	Group number	Buoyant density (g cm^{-3})	Base composition (moles % G + C)
Pseudomonas savastanoi	173	1a	1.716	57
P. savastanoi	177	1a	1.717	58
P. phaseolicola	107	1b	1.716	57
P. phaseolicola	109	1b	1.717	58
P. morsprunorum	81	1c	1.716	57
P. mori	85	1c	1.717	58
P. glycinea	71	1b-1c	1.717	58
P. coronafaciens	55	1d	1.718	59
P. helianthi	73	1d-1e	1.719	60
P. syringae	119	1f	1.717	58
P. syringae	120	1f	1.718-9	59-60
P. syringae	127	1f	1.719	60
P. syringae	125	1g	1.718	59
P. cichorii	136	1h	1.717	58

dence for similarity. For this reason, we can use base composition data to support phenotypic differences between Group I pathogens and most biotypes of *P. fluorescens, P. putida,* and *P. aeruginosa,* but we cannot conclude whether Group I subgroups or nomenspecies are valid taxa.

Lanni (4) considered similarity of base composition to be a min requirement for genetic exchange between two organisms. The exceptions to this rule are where the incoming genetic material does not become integrated into the recipient genome or where areas of microhomology do exist (9). Khan & Sen (3) were able to demonstrate high rates of transformation between a number of species of *Pseudomonas,* which presumably have considerable genetic homology despite their slightly different base compositions. Judging from the base composition evidence presented here and from previous nutritional work, the strong possibility exists that a single gene pool integrates all nomen-species in Group I. If such is the case, then the complex problems of virulence and host specificity may be subject to genetic analysis.

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