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Synthetic and Complex Media for the Rapid Detection of Fluorescence of Phytopathogenic Pseudomonads: Effect of the Carbon Source[1]

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Fluorescence is of diagnostic value for differentiating among species of aerobic pseudomonads (R. Y. Stanier, N. J. Palleroni, and M. Doudoroff, J. Gen. Microbiol. **43**:159, 1966). The standard medium for detecting fluorescence is Medium B (E. O. King, M. K. Ward, and D. E. Raney, J. Lab. Clin. Med. **44**:301, 1954), which supports fluorescent pigment production of most pseudomonads tested (O. Jessen, *Pseudomonas aeruginosa and other green fluorescent pseudomonads, A taxonomic study*, Munksgaard, Copenhagen, 1965; R. Y. Stanier et al., J. Gen. Microbiol. **43**:159, 1966).

Minerals (J. V. King, J. J. R. Campbell, and B. A. Eagles, Can. J. Res. C **26**:514, 1948), amino acids (J. De Ley, Ann. Rev. Microbiol. **18**:17, 1964), and peptones (E. O. King et al., J. Lab. Clin. Med. **44**:301, 1954) affect fluorescence. The effect of carbon sources had not been shown. Although glycerol, glucose, or maltose can be used interchangeably in Medium B for detecting fluorescence of most fluorescent pseudomonads, this report shows that these carbon sources are not equivalent for phytopathogenic pseudomonads.

TABLE 1. *Fluorescence of Pseudomonas species on synthetic and complex agar media: 48-hr incubation*[a]

Species	Medium											
	NA_{Gly}[b]	NA_G	NA_M	NA	N_{Gly}	N_G	N_M	A_G	NBY[c]	B_{Gly}[d]	B_G	B_M
P. phaseolicola (22 strains)	+++	+++	0	0[e]	++-+++	++-+++	0	+-++	+-++	+++	0-+	0
P. syringae (5 strains)	+++	+++	0	0	+++	++-+++	0	+-++	+-++	+++	+-++	+-++
P. tabaci (1 strain)	+++	+++	0	0	+++	++	0	+	++	+++	+-++	0-+
P. fluorescens (3 strains)	+++	+++	+	+	+++	+++	0	+-++	+-++	+++	++	+++
P. aeruginosa (1 strain)	+++	+++	+	+	+++	+++	0	++	++	+++	++	+++

[a] Average of two to four experiments. Growth was good to excellent on all media except N_M, NA, and NA_M.

[b] All species produced blue fluorescent pigment on NA, N, and A media, irrespective of the carbon source.

[c] All species produced blue-green or blue fluorescent pigment.

[d] Medium B of King et al.; blue-green or green fluorescent pigment was produced, irrespective of the carbon source.

[e] Only 14 strains tested.

All synthetic media contained, in grams per liter: $MgSO_4 \cdot 7H_2O$, 0.2; KH_2PO_4, 3.0; Na_2HPO_4, 6.0, and glucose, glycerol, or maltose, 5.0. The sugars and glycerol were autoclaved separately as 10% (w/v or v/v) solutions, and added aseptically. Medium A had L-asparagine (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.5 g per liter, as nitrogen source; medium NA had 0.5 g

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of L-asparagine per liter plus 1 g of NH_4Cl per liter; and medium N had 1 g of NH_4Cl per liter. The subscripts, G, M, or Gly indicate the carbon source as glucose, maltose, or glycerol, respectively.

NBY medium contained, in grams per liter: Nutrient Broth (Difco), 8.0; yeast extract (Difco), 2.0; K_2HPO_4, 2.0; KH_2PO_4, 0.5; glucose, 5.0, and $MgSO_4 \cdot 7H_2O$, 0.25. Glucose (10%, w/v) and $MgSO_4$ (1 M) were autoclaved separately and added aseptically.

Medium B was prepared from the constituents, with glucose, maltose, or glycerol as carbon source. Commercial medium B (Difco Pseudomonas Agar F) was also used (= B_M).

Solid media contained agar (Difco) at 15 g per liter.

Both NBY and Medium B have a considerable amount of blue autofluorescence; media N, NA, and A do not.

Fluorescence under ultraviolet light was determined daily on cultures incubated at 24 to 26 C. For inocula, distilled water suspensions of cultures grown for 24 to 48 hr on NBY, Medium B (Difco), or NA_G were used.

The results in Table 1 show that all tested phytopathogens previously classified as fluorescent, and *Pseudomonas fluorescens* and *P. aeruginosa*, showed intense fluorescence on synthetic agar media with glucose or glycerol as carbon source, and on complex agar media with glycerol. Maltose in synthetic and complex media was poorest for fluorescence of the phytopathogens, but not for *P. fluorescens* and *P. aeruginosa* in complex media. Development of fluorescence on synthetic media with galactose occurred, but was slower than with glycerol or glucose.

Fluorescence, if detectable, generally was seen by 24 hr.

Corynebacterium flaccumfaciens var. *aurantiacum*, 2-A, *Escherichia coli* B, K-12, *Proteus vulgaris* PV-1, *P. solanacearum* K30, and *Xanthomonas phaseoli* XP4, XP104 Sm^R, and XP104W showed no fluorescence on any media. *C. flaccumfaciens* ATCC 6887, *Agrobacterium tumefaciens* AT-1, *E. coli* C, and *X. phaseoli* XP6022, XPS, and K-4 showed weak (+) fluorescence on B_M and B_{Gly}, after a week or longer.

Efforts to use liquid synthetic media for fluorescence studies led to variable results.

R. Y. Stanier et al. (J. Gen. Microbiol. **43**:159, 1966) have shown that maltose is rarely metabolized by fluorescent pseudomonads. This may explain the equivalence of media differing only in maltose (NA and NA_M). Fluorescence on B_M is probably not due to the presence of maltose.

This report shows that fluorescence of phytopathogenic pseudomonads can be detected rapidly and readily on certain complex and synthetic media, but not on the commercial medium for this purpose. Carbon sources affect fluorescence of phytopathogenic pseudomonads, but have little or no effect on fluorescence of *P. fluorescens* and *P. aeruginosa*.

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