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# RAPD PCR-based differentiation of *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans*

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**Abstract**

A RAPD PCR-based method was used to differentiate between isolates of *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans*. Using random primer OP-G11, a single, high intensity band of 820 bp was amplified from DNAs of all *X. c.* pv. *phaseoli* var. *fuscans* isolates, while multiple amplification products of varying sizes were generated from *X. c.* pv. *phaseoli* DNAs. Whereas RAPD PCR differentiation gave an unambiguous result in under 4 h, standard differentiation by recording the production of a brown pigment by *X. c.* pv. *phaseoli* var. *fuscans* isolates took up to 7 days and showed variation both between isolates and between media. The unequivocal nature of the RAPD PCR method was demonstrated when isolate 408, originally classified as *X. c.* pv. *phaseoli* var. *fuscans*, failed to produce the 820 bp band typical of *X. c.* pv. *phaseoli* var. *fuscans* isolates, and after also failing to produce a brown pigment, was re-classified as *X. c.* pv. *phaseoli*.

## Introduction

Common bacterial blight and fuscous blight, caused by *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* respectively, are major world-wide seed-borne diseases of bean (*Phaseolus vulgaris*), causing yield reductions from 10 to 40% in susceptible cultivars (Vidaver, 1993; Wallen and Jackson, 1975). Although commonly regarded as a single pathogen, a variety of techniques, including isoenzyme profiling (El-Sharkawy and Huisingh, 1971), plasmid profiling (Lazo and Gabriel, 1987), restriction fragment length polymorphism (Lazo et al., 1987), DNA-DNA hybridisation (Hildebrand et al., 1990) and amplified DNA polymorphisms (Xue and Goodwin, 1993), have all revealed *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* to be two distinct classes. Caution should thus be taken when considering the relationship between these isolates and, indeed, Goodwin and Sopher (1994) have proposed that they retain distinct taxonomic status.

Pathogenic variation both between and within *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* isolates signifies a potential problem in breeding for resistance to bacterial blight (Ekpo and Saettler, 1976; Maraite, 1989; Schuster and Coyne, 1981). Detailed analysis of such variation is thus a fundamental prerequisite to designing an informed resistance breeding program. Numerous reports have demonstrated that *X. c.* pv. *phaseoli* var. *fuscans* isolates are more pathogenic than *X. c.* pv. *phaseoli* isolates, generally causing greater stem collapse (Allen, 1983; Basu and Wallen, 1967; Leakey, 1973; Ekpo and Saettler, 1976; Rudolph, 1990; Opio et al., 1996). Moreover, a clear differential resistance to these organisms has been recorded (Burkholder and Bullard, 1946). An unambiguous, reliable method is therefore required to distinguish between *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* isolates. Currently, *X. c.* pv.

*Table 1.* Bacterial isolates

| Isolates | Location | Source |
|---|---|---|
| *X. c.* pv. *phaseoli* var. *fuscans* | | |
| 266 | Argentina | V. Verdier |
| 239 (ATCC 19315) | Canada | C. Hale |
| 180 | Colombia | V. Verdier |
| 1065, 1068 | Ethiopia | A.F. Opio |
| CFBP1816 | Greece | C. Bragard |
| 2721, 2722, 3403, 3430, 3485 | New Zealand | C. Hale |
| 1005, 1007, 1010, 1033 | Uganda | A.F. Opio |
| 072 | USA | V. Verdier |
| *X. c.* pv. *phaseoli* | | |
| 123 | Argentina | V. Verdier |
| 095, 306 | Colombia | V. Verdier |
| 418 | Dominican Rep. | V. Verdier |
| 1069b | Ethiopia | A.F. Opio |
| 408 | Guatemala | V. Verdier |
| 9076 | Hungary | C. Hale |
| NCAIMB01523 | Hungary | P. Ott |
| 2725 | New Zealand | C. Hale |
| 364 | Nicaragua | V. Verdier |
| 1038b, 1048 | Uganda | A.F. Opio |

*phaseoli* var. *fuscans* is distinguished from *X. c.* pv. *phaseoli* by its production of a brown pigment on a variety of media, including King's B medium (KBM) (King et al., 1954), modified nutrient broth yeast agar (MNBY) (Vidaver, 1967), yeast dextrose chalk agar (YDCA) or mineral salt casein medium (MSC) (Goodwin and Sopher, 1994). However, both the time from inoculation and extent of such production can vary substantially (Goodwin and Sopher, 1994; and this study). Other biochemical characteristics are not useful in distinguishing between *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* isolates, since these are similar for all pathovars of *X. campestris* (Dye, 1962). This report describes a fast and reliable RAPD PCR-based method for the differentiation of these two groups.

All *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* isolates used in this study are listed in Table 1. Isolates were routinely grown on YDCA at 27 °C and stored at 4 °C for up to 2 weeks. For longer term storage, 0.5 ml of a 16 h LB culture was mixed with 0.5 ml sterile freezing medium (per litre: 12.6 g $K_2HPO_4$, 0.9 g $C_6H_5Na_3O_7.2H_2O$, 0.18 g $MgSO_4.7H_2O$, 1.8 g $(NH_4)_2SO_4$, 3.6 g $KH_2PO_4$, 88.0 g glycerol), frozen in liquid nitrogen, and kept at −80 °C in 0.1 ml aliquots.

*Pigment production*

*X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* isolates were tested for production of brown pigment following growth on three media, YDCA, KBM and MNBY. After inoculation from colonies grown on YDCA at 27 °C for 72 h, each isolate was grown at 27 °C in triplicate on each medium and examined daily from inoculation. As expected, after fifteen days no pigment was observed on any of the plates inoculated with *X. c.* pv. *phaseoli* isolates (results not shown). However, one isolate that had previously been classified as *X. c.* pv. *phaseoli* var. *fuscans*, isolate 408, failed to produce detectable levels of the pigment within the time-frame of this experiment and was accordingly reclassified as *X. c.* pv. *phaseoli* (Table 1). All other *X. c.* pv. *phaseoli* var. *fuscans* isolates produced a brown coloration on triplicate plates of each medium, although the time for this to be detected varied between isolates and between media. All *X. c.* pv. *phaseoli* var. *fuscans* isolates produced a strong brown coloration on KBM medium after 1-2 days, while a less intense coloration was observed on both YDCA and MNBY after 5-6 days and 5-7 days respectively. To clearly observe pigment production on YDCA and MNBY it was necessary to compare plates with an *X. c.* pv. *phaseoli* isolate as a negative control to avoid subjective and often ambiguous readings (Figure 1). African *X. c.* pv. *phaseoli* var. *fuscans* isolates consistently produced pigment one day earlier than other *X. c.* pv. *phaseoli* var. *fuscans* isolates on both KBM and MNBY media (data not shown). This was unlikely to be due to differences in growth rate as, although all isolates grew fastest on MNBY medium, there was little difference in growth rate between isolates on a given medium. Thus such differences are likely to be due to variation in either the level or time of pigment production.

We concluded that the pigment test for distinguishing between *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans*, whilst time-consuming and laborious, also varies according to the medium used. In addition, variation in the length of time for different isolates to produce the brown pigment, together with differences in pigment intensities, could potentially lead to the erroneous classification of *X. c.* pv. *phaseoli* var. *fuscans* isolates as *X. c.* pv. *phaseoli*.