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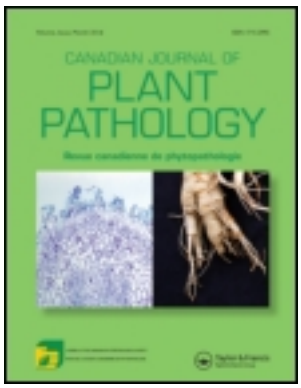

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A Medium for Differentiating Tomato and Pepper Strains of Xanthomonas Campestris pv. Vesicatoria

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A medium for differentiating tomato and pepper strains of *Xanthomonas campestris* pv. *vesicatoria*

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CKTM medium was used to differentiate between pepper and tomato strains of *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot disease of pepper and tomato. On this medium, 2- to 3-day-old colonies of both pepper and tomato strains of *X. c. vesicatoria* were circular with raised morphology, yellow, and surrounded by a clear ring. Minute crystals were formed in the clear ring within 4 days. Strains isolated from pepper remained as described, whereas the tomato strains developed opaque white haloes around the colonies within 2 to 6 days. A total of 60 of 69 strains of *X. c. vesicatoria* from either pepper or tomato were correctly identified to host origin by colony characteristics on this medium.

Sijam, K., C. J. Chang, and **R. D. Gitaitis.** 1992. A medium for differentiating tomato and pepper strains of *Xanthomonas campestris* pv. *vesicatoria*. Can. J. Plant Pathol. 14:182-184.

On a utilisé le milieu de culture CKTM pour distinguer les souches du piment et de la tomate du *Xanthomonas campestris* pv. *vesicatoria*, l'agent responsable de la tache bactérienne du piment et de la tomate. Après 2 ou 3 jours de croissance sur ce milieu, les colonies des souches du piment et de la tomate du *X. c. vesicatoria* étaient régulières, surélevées, de couleur jaune et entourées d'un anneau clair. Des cristaux minuscules furent formés dans l'anneau clair en 4 jours. Les souches isolées du piment demeurèrent ainsi mais les souches isolées de la tomate ont développé des auréoles opaques blanches autour des colonies après de 2 à 6 jours. On a identifié correctement l'hôte d'origine de 60 des 69 souches du piment ou de la tomate de *X. c. vesicatoria* à l'aide des caractéristiques des colonies sur ce milieu de culture.

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Bacterial spot of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) caused by *Xanthomonas campestris* pv. *vesicatoria* (Schaad 1989) is an important disease in most regions where these crops are grown. Yield losses in tomato have been reported (Pohronezny & Volin 1983). Minsavage et al. (1990) demonstrated that three groups of *X. c. vesicatoria* exist based on their pathogenicity on tomato or pepper: strains (XcvT) that infect only tomato, strains (XcvP) that infect only pepper, and strains (XcvPT) that infect both pepper and tomato.

X. c. vesicatoria, like other xanthomonads, can be isolated from host plants by culturing the pathogen on conventional media (Schaad 1989) or on semiselective media. Several semiselective media have been reported for various pathovars of *X. campestris* (Chang et al. 1990, Mulrean & Schroth 1981, Norman & Alvarez 1989, Schaad & Forster 1985, Schaad & White 1974), but only Tween medium is selective for *X. c. vesicatoria* (McGuire et al. 1986). Although the Tween medium can be used to detect the bacterium from soil and plant material, its use in distinguishing pepper and tomato strains is limited because both strains produce minute soapy crystals or a lipolysis zone of variable size. Accordingly, this study addresses the use of a differential medium that distinguishes between pepper and tomato strains of *X. c. vesicatoria*.

A semiselective medium (CKTM) developed for the isolation of *X. c. vesicatoria* from tomato seed was used and prepared as described previously (Sijam et al. 1991).

Bacterial strains were provided by R. D. Gitaitis and S. McCarter of the Department of Plant Pathology, University of Georgia, Athens, GA. Sixty-nine strains of *X. c. vesicatoria* of known origin and two strains of unknown origin, other *X. campestris* pathovars, i.e. *X. c. campestris*, *X. c. phaseoli*, *X. c. raphani*, *X. c. malvacearum*, *X. c. translucens*, and *X. c. incanae* and six pectolytic strains of *Xanthomonas* spp. were streaked onto YDC medium (yeast extract, dextrose, calcium carbonate) and incubated at 30°C for 24 h. Individual colonies were then suspended in sterile tap water, and standardized spectrophotometrically to approximately 1×10^3 colony-forming units (cfu) per mL. Aliquots of 0.1 mL were spread onto duplicate plates of CKTM and Tween media. Plates were covered with plastic bags and incubated at 28°C for 1 week. Test results were sent back to the providers for verification.

Cross inoculation of tomato and pepper strains to pepper and tomato plants, respectively was per-

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formed to determine if colony characteristics differing between the pepper and tomato strains were stable. Four strains each from tomato and pepper were inoculated to both pepper and tomato plants. Suspensions of approximately 1×10^8 cfu per mL of phosphate buffer (0.1 M, pH 7.0) were atomized with a chromatographic sprayer onto leaf surfaces of 4- to 6-wk-old plants. After inoculation, plants were placed in a moist chamber for 48 h, and then incubated on greenhouse benches for 2-4 wk to observe symptom development. When symptoms developed, the bacteria were reisolated onto both CKTM and Tween media and their colony characteristics were noted.

All *X. c. vesicatoria* strains grown on CKTM medium initially developed a clear ring around the colony after 1 to 2 days. After 3 to 4 days minute soapy crystals formed in the clear ring. None of the other *X. campestris* pathovars produced a clear ring, except *X. c. translucens*. In general, crystals that formed in the clear ring of tomato strains were whiter than those formed by pepper strains. After 6 to 10 days, an opaque white halo (Fig. 1A) developed around the yellow colonies of tomato strains, giving a –fried egg' appearance. The clear ring and minute soapy crystals persisted immediately outside the colony. In contrast, pepper strains of *X. c. vesicatoria* did not form the opaque white halo (Fig. 1B) around the colony, but the ring remained clear with minute crystals.

In the "blind" study, where the evaluator did not know the source of the strains, the colony characteristics on CKTM medium were used to identify successfully 60 of 69 (87%) strains of *X. c. vesicatoria* as to their original host, i.e. pepper or tomato, and 100% of the opportunistic pectolytic *Xanthomonas* spp. (6 strains) as being something other than *X. c. vesicatoria* based on the lack of a clear ring. Both unknown strains were not *X. c. vesicatoria* because they did not produce the clear ring.

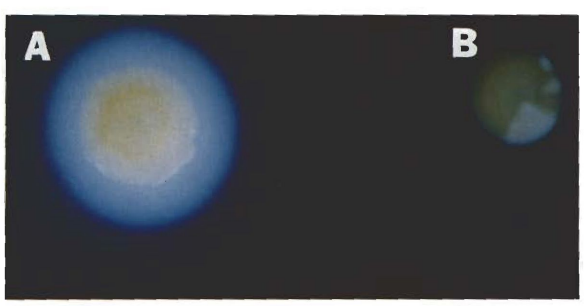

Figure 1. Opaque white halo formation around a colony of **A)** tomato strain but not of **B)** pepper strain of *Xanthomonas campestris* pv. *vesicatoria* on CKTM medium.

Pepper strains inoculated to and reisolated from pepper plants developed typical colony characteristics of the pepper strains, as did pepper strains inoculated to and reisolated from tomato plants. Likewise, when tomato strains were inoculated to either pepper or tomato plants, isolated colonies formed opaque white halos and were identified as tomato strains. The characteristic colony morphology, important for differentiation between tomato and pepper strains, thus was stable.

The characteristic clear ring and white halo formation are useful for differentiating between tomato and pepper strains of *X. c. vesicatoria*, and therefore the isolation from diseased material onto CKTM medium should dramatically improve the ability and speed for making correct diagnoses for certification purposes (Gitaitis et al. 1987). The basis of these reactions, however, is unknown. The addition of Tween 80 to the medium was responsible for clear ring formation, as elimination of Tween 80 resulted in colony growth without formation of the clear ring. In addition, no attempt was made to determine if all races of the pepper strain behaved in the same manner. The predominant pepper race used in these studies was most likely race 2, as most isolates of this race are tolerant of copper. The development of this differentiation medium offers an opportunity for further study of the nature of strains of *X. c. vesicatoria*. For example, the possibility of genetic differences, plasmid involvement, and specific enzyme actions may now be more efficiently explored.

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