

Kelman A. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* 44:693-5, 1954. [Plant Pathology Dept., North Carolina State Coll., Raleigh, NC]

The destructive plant pathogen, *Pseudomonas solanacearum*, loses virulence quickly in culture. When suspensions were streaked on a tetrazolium chloride (TZC) medium, avirulent colony types could be readily differentiated by their pigmentation from the virulent wild type. Screening for disease resistance in major crops and basic studies on the pathogen were facilitated by use of the TZC medium. [The SCI® indicates that this paper has been cited in over 150 publications since 1961, making it one of the most-cited papers ever published in this journal.]

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"As a graduate student at North Carolina State University, I was encouraged by my major professor, James H. Jensen, to develop improved assays for screening breeding lines of potato and tomato for resistance to the wilt pathogen, *Pseudomonas solanacearum*. The bacterial wilt disease caused by this bacterium ranks as one of the most widespread and destructive of all the bacterial diseases affecting a range of important crop plants in the world.¹ Improved methods of maintaining virulence in cultures of *P. solanacearum* were essential for this project.

"Early investigators experienced great difficulty in maintaining virulence in cultures of *P. solanacearum*.^{1,2} In my initial experiments, I experienced the same difficulties that had frustrated previous researchers. A variety of media and techniques were tested without much success until I noted a paper by Lederberg³ citing use of tetrazolium chloride (TZC) for detection of biochemical mutants of *Escherichia coli*. It was both exciting and upsetting to observe in initial experiments that small numbers of deep red colonies very different in colony morphology and color from the

wild type could be detected when plates of a tetrazolium medium (TZC) were streaked with suspensions from many of my 'pure' stock cultures. When tested, these colony-type variants were either weakly virulent or avirulent. Shortly thereafter, I had the opportunity in 1953 to work with Armin C. Braun at the Rockefeller Institute for Medical Research in New York City. With his kind assistance, single-cell techniques were employed to insure the purity of clones of the different colony types and to confirm the relationship of specific colony types with virulence. Differences in colony appearance were related to presence or absence of an extracellular polysaccharide.

"The TZC medium coupled with the use of obliquely transmitted light to examine plates made it possible to insure the virulence of cultures essential for large-scale greenhouse and field inoculations of tomato, potato, and other crops. It also provided a helpful tool in basic physiological and biochemical studies on the wilt pathogen concerned with efforts to determine the relationship between colony type and virulence as well as mechanisms of pathogenesis including wilt induction.⁴

"The TZC medium enables one to detect very low numbers of the avirulent types of *P. solanacearum* that would not be evident on standard media. The avirulent types increase rapidly particularly in broth cultures when oxygen becomes limiting, and virulence in the case of this bacterium may be lost in eight to ten days. The virulent strain from solanaceous hosts that loses virulence quickly in broth culture is a unique pseudomonad since it is typically non-motile and cannot compete for O₂ with the highly motile flagellate avirulent form.⁵ The TZC medium also led to the discovery that it was possible to maintain virulence in *P. solanacearum* merely as water suspensions at room temperature in capped test tubes⁶ for over 20 years (Kelman and Hisada, unpublished data). In addition to the research on *P. solanacearum*, the TZC medium has been useful in studies on *Erwinia amylovora* (causal agent of fire blight of pear) and certain other pathogens in which formation of extracellular or capsular polysaccharide is correlated with virulence."

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6. Kelman A & Person L H. Strains of *Pseudomonas solanacearum* differing in pathogenicity to tobacco and peanut. *Phytopathology* 51:158-61, 1961.