An Improved Method for Detecting the Presence of Xanthomonas oryzae in Rice Seed

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ABSTRACT

A streptomycin-resistant mutant of *Xanthomonas oryzae* was developed for studying pathogen ecology. An efficient method was developed to assay many rice seeds for this mutant by touching seed abscission surfaces to a streptomycin agar medium. This is the first unequivocal method of assaying large numbers of rice seed for *X. oryzae,* but it is limited to a streptomycin-resistant strain. Although the pathogen was detected from approximately 15% of seed obtained from inoculated panicles, this neither proves nor disproves that effective seed transmission occurs naturally.

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Bacterial blight (caused by *Xanthomonas oryzae*) has become a major rice disease in Asia recently with widespread cultivation of nitrogen-responsive dwarf varieties. Conflicting reports exist on seed transmission of the pathogen (1, 2, 3, 4, 6). We consider past techniques to be equivocal in determining whether seeds (spikelets) were carrying the pathogen and inadequate for proving actual transmission and disease re-establishment in the subsequent crop. Large amounts of seed are transported within, between, and from countries in Asia where the pathogen exists. It is important to determine whether rice seed carries the pathogen from crop to crop, resulting in disease re-initiation in new seasons or regions.

Direct plating of seed on agar has been used in most studies (2, 6), a method we found unsatisfactory because of ubiquitous fast-growing yellow saprophytes confusable with the pathogen. Phage titer increase, used in Japan (7), is valid only if the phage is species-specific and strain-nonspecific; moreover, its efficiency is variable. Only one report has appeared (1) on streaking from crushed seed onto agar; a few colonies of *X. oryzae* were recovered among many saprophytes. All of these methods destroy the seed. Lack of a selective medium for *X. oryzae,* a very slow-growing organism, makes studies of its presence in mixtures difficult and untrustworthy.

Detailed studies of *X. oryzae* ecology have been hampered by the difficulty of detecting the bacterium from nature. A streptomycin-resistant mutant (VNS) was selected from isolate VN by streaking heavily on Wakimoto's medium (8) supplemented with 250 ppm streptomycin sulfate. Colonies which appeared were restreaked serially on plates containing 500, 1,000, 2,000, etc., up to 20,000 ppm streptomycin. VNS, resistant to more than 20,000 ppm streptomycin, was identical with its parent isolate in virulence and in 30 physiological and biochemical characters. A suspension of 10^8/ml was injected into 'IR841' rice stems below the uppermost node of plants at early flowering. Partially wilted panicles, harvested 4 wk after inoculation, were checked microscopically for bacterial streaming. Streaming was observed from panicle axes, sometimes also from secondary branches and pedicels. *X. oryzae* was isolated from all panicle parts showing streaming. Isolations were made by streaking onto Wakimoto's medium, supplemented with 10,000 ppm streptomycin sulfate and 100 ppm nystatin (WSN), from a sterile water drop in which a panicle part had been immersed 5 sec. Suspected *X. oryzae* colonies were checked for pathogenicity on 'TN 1' rice variety using the clipping method of inoculation (5).

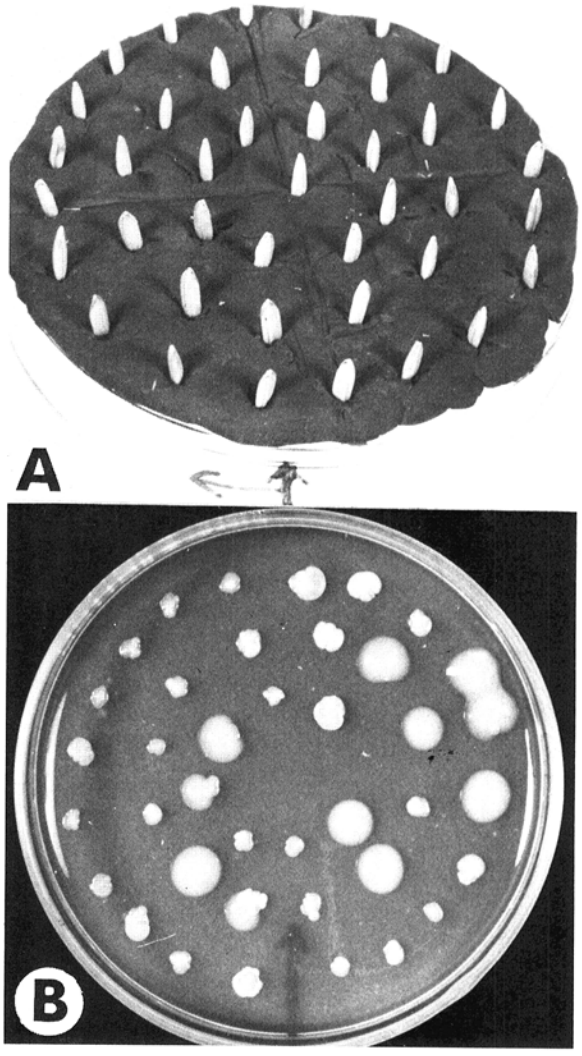

Fig. 1. A) Rice seeds arranged over a petri plate area on 'Star' typewriter type-cleaner, with abscission surfaces upward, in preparation for plating. **B)** Bacterial colonies present on Wakimoto's (WSN) agar (supplemented with streptomycin and nystatin) 10 days after touching plate to seeds. Only the large mucoid colonies are *Xanthomonas oryzae*. Small rough colonies are of streptomycin-resistant unidentified saprophytes.

Bacteria were also isolated from seeds obtained from pedicels showing streaming at the pedicel abscission surface. Seeds were cut with scissors into 1-mm pieces and immersed in a water drop 1 min before streaking on WSN. Of 54 seeds tested, streptomycin-resistant *X. oryzae* was isolated from 51 (94%). Thus, if streaming was observed from the pedicel abscission surface, the attached spikelet was most likely invaded. Although this method could detect bacteria in the seeds, a maximum of only 500 pedicels could be examined and tested per day. Since an abscission surface occurs on both pedicel and spikelet base, streaming from seed abscission surface was tested. Streaming was observed from some seeds immersed in water drops and *X. oryzae* was isolated. On dissection, most oozing was from lemma and palea bases, spikelet parts not attached to the true seed.

It was then found that simply touching seed abscission surfaces to WSN plates for 3-5 sec resulted in consistent growth of streptomycin-resistant *X. oryzae* from infested seeds. Efficiency of this method was tested individually on 200 seeds by comparing recovery with isolation following grinding of the seed. Of 52 seeds showing positive results in the tissue grinding isolation method, 51 (98%) showed positive results by the abscission surface method. Likewise, of 148 seeds showing negative results by the tissue grinding method, none was positive by the abscission surface method.

Efficiency was improved by implanting 40 to 50 seeds, abscission surface exposed, on a piece of 'Star' typewriter type-cleaner (Eberhard Faber Pencil Co.) over a petri plate (Fig. 1-A). Then a plate of WSN medium was touched to those seeds for 5 sec. Colonies of saprophytic bacteria appeared in 2-3 days. Colonies of *X. oryzae* appeared 4-5 days later, and readily grew from saprophytic colonies already present. *X. oryzae* colonies became large and mucoid, and were easily differentiated from saprophytic bacterial colonies which remained small and rough (Fig. 1-B). If the medium was not supplemented with streptomycin, no *X. oryzae* colonies appeared.

By using this method thousands of seeds were tested in a day. Inoculated panicles were kept in an air-conditioned room at 28 C, and 3,705 seeds were examined 3 wk after harvest. *X. oryzae* was obtained from 646 seeds (17%). Five weeks after harvest 3,939 seeds were tested; 499 seeds yielded the pathogen (13%).

The abscission surface test method, used with a streptomycin-resistant strain of *X. oryzae,* enables, for the first time, unequivocal, efficient, direct testing of large numbers of seed for *X. oryzae*. We consider that without the use of this improved method, or an as yet undeveloped selective medium which eliminates yellow saprophytes, reports of seed transmission are to be considered suspect. Our results on the presence of the pathogen at the seed abscission surface and in spikelet parts at moderate frequency following artificial inoculation of the panicle does not prove or disprove effective seed transmission under natural conditions. Since the method does not destroy the seed, experiments can be developed using known infested seeds to study pathogen ecology and transmission under natural seed and plant-handling systems.

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