

## A simple technique for detecting *Xanthomonas oryzae* in rice seeds

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### Summary

A simple technique has been developed for detecting *Xanthomonas oryzae* in and/or on rice seeds. The seeds are germinated by the paper towel method and the seedlings examined under the microscope for bacterial streaming. A positive correlation exists between the bacterial streaming from the germinated seedlings and the successful inoculation of healthy seedlings by the bacterial ooze. The method is recommended for routine testing of seeds for the detection of the pathogen. The presence of viable pathogen has been demonstrated from infected seeds stored at room temperature (15°-38°C) up to 11 months after harvest.

### Résumé

*Une technique simple pour la détection de Xanthomonas oryzae chez les semences de riz*

Une technique simple a été mise au point pour la détection de *Xanthomonas oryzae* à l'intérieur et/ou à la surface des semences de riz. Les semences sont mises à germer par la méthode sur papier et les plantules sont examinées au microscope pour la présence de suintement bactérien. Il existe une corrélation positive entre le suintement bactérien des plantules germées et la réussite de l'inoculation de plantules saines par l'inoculum bactérien. La méthode est recommandée en analyse sanitaire de routine des semences pour cet agent pathogène. On a démontré la présence de l'agent pathogène à l'état vivant sur des semences infectées conservées à la température du laboratoire (15 à 38°C) encore 11 mois après la récolte.

### Zusammenfassung

*Eine einfache Technik zur Erkennung von Xanthomonas oryzae an Reissamen*

Zur Erkennung von *Xanthomonas oryzae* an und/oder in Reissamen wurde eine einfache Technik entwickelt. Die Samen werden mittels der Papierstreifenmethode angekeimt und die Keimlinge unter dem Mikroskop auf die Bakterienströmung untersucht. Es besteht eine positive Korrelation zwischen der Bakterienströmung von gekeimten Pflänzchen und der erfolgreichen Inokulation gesunder Pflänzchen durch Bakterienbrühe. Die Methode wird für die Routineuntersuchung von Samen zur Erkennung des Erregers empfohlen. Die Anwesenheit des lebensfähigen Erregers wurde an infizierten Samen, welche bei Raumtemperaturen (15°-38°C) bis zu 11 Monaten nach der Ernte gelagert wurden, demonstriert.

## Introduction

Contradictory reports exist in the literature regarding the role of seed in transmitting *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson, the causal organism of bacterial leaf blight of rice (*Oryza sativa* L.). Mizukami (1961) detected the pathogen in seed stored until the next crop season but did not consider it to be an important source because the pathogen rapidly decreased in June. Fang, Lie and Chu (1956) considered seed as a source of infection in temperate regions. In India, Srivastava and Rao (1964) recovered the pathogen from seeds and concluded that seed is the major source of disease transmission. Chakravarti and Rangarajan (1967) isolated a virulent strain of the pathogen from seed. Chattopadhyay and Mukherjee (1971) confirmed seed transmission of the pathogen. Studies at Hyderabad and the International Rice Research Institute, Philippines indicate that the pathogen can survive on and/or in the seed for only a few weeks at the high temperatures prevailing in the tropics (Kauffman, 1969; Eamchit and Ou, 1970). The controversy is possibly due to lack of a precise method for demonstrating the presence of the pathogen on or in the seed. Several methods have been used to demonstrate the seed transmission of the bacterium. These include direct plating of seed on agar (Srivastava and Rao, 1964; Chakravarti and Rangarajan, 1967; Chattopadhyay and Mukherjee, 1971), phage titer increase (Wakimoto, 1954) and isolating the pathogen from crushed seed by streaking on agar plate (Buddenhagen, 1969). None of these methods gives satisfactory results because in direct plating of seed the growth of the bacterium is masked by fast-growing saprophytic bacterial colonies resembling the pathogen. The phage titer increase method is valid only if the phage is specific to species and not to strains of the pathogen. An improved method of detecting the presence of the bacterium in seed has been suggested by Hsieh, Buddenhagen and Kauffman (1974) but by this method only streptomycin-resistant *X. oryzae* can be detected. A method which the authors believe to be better is described in this paper.

## Materials and methods

The seed samples were collected in the last week of October 1973 from a plot of the cultivar 'Taichung native 1' severely infected by bacterial leaf blight. It was stored at room temperature (15°–38°C) after drying in the sun in cloth bags, a condition in which most of the farmers store their seeds. Two hundred seeds were drawn from the sample and tested for germination by the paper towel method (International Seed Testing Association, 1966). Paper towels (45 cm × 28 cm) were soaked in tap water and seeds were equally spaced between two of them. The towels were rolled and incubated for five days in an upright position at 30° ± 2°C in a plastic tray. There were four replications with 50 seeds in each. The tray was covered with polythene sheet to maintain high humidity. Small pieces of coleoptile, leaf sheath and leaf from germinated seedlings were examined under the microscope for bacterial streaming from the cut end. The pieces showing bacterial ooze were crushed by needle in a few drops

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of sterile distilled water on the microscope slide itself and the suspension was used to inoculate six-week-old seedlings of 'Taichung native 1' grown in pots by the 'clip' method (Reddy and Kauffman, 1972). The inoculated seedlings were covered for 24 hours with a polythene bag to maintain high humidity. Typical blight symptoms were observed after 48 to 72 hours. The pathogen was isolated from the seedlings showing bacterial streaming as well as from the inoculated leaves.

### Results and discussion

Seeds germinated by the paper towel method gave two types of seedlings: healthy seedlings and pale seedlings with poor growth, light to dark brown coleoptiles and discoloured sheaths. Typical blight symptoms were observed after 48 to 72 hours when bacterial ooze was used as inoculum on susceptible seedlings by the clip method.

Maximum percentage of seed germination was recorded in March and minimum in July and September. In November, i.e. immediately after harvest, 21% of seedlings exhibited bacterial streaming falling to 11% in May and June, the period when the seeds are sown in the nursery for transplanting. Six percent of seedlings exhibited the bacterial streaming even in the month of September, i.e. after 11 months of storage. The inoculations by the clip method of healthy seedlings with bacterial ooze were successful in more than 90% of cases, indicating a positive correlation between the bacterial streaming and successful inoculation of seedlings (table 1).

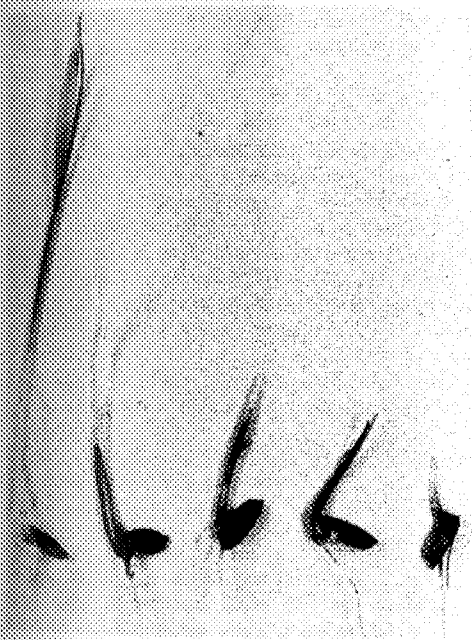


Fig. 1. Seedlings of the same age. Extreme left healthy and the rest show poor growth and discoloured coleoptile.

Table 1. Percentage of rice seed germination, seedlings exhibiting bacterial streaming and numbers of seedlings inoculated with bacterial ooze/showing symptoms of BLB.

Month 1973-74	No. of seeds tested	Germination (%)	Seedlings exhibiting bacterial streaming (%)	No. of seedlings	
				inoculated	showing symptoms
Nov.	200	92	21	42	40
Dec.	200	93	18	36	35
Jan.	200	93	20	40	35
Feb.	200	95	20	40	36
March	200	96	17	40	30
April	200	94	12	24	23
May	200	92	11	22	20
June	200	91	11	22	19
July	200	90	9	18	17
Aug.	200	91	8	16	15
Sept.	200	90	6	12	10



Fig. 2. Bacterial leaf blight symptom produced on leaf tip after inoculation with bacterial suspension from seedlings.

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No. of seedlings showing symptoms (%)	No. of seedlings	
	inoculated	showing symptoms
42	40	
36	35	
40	35	
40	36	
40	30	
24	23	
22	20	
22	19	
18	17	
16	15	
12	10	

Fig. 2. Bacterial leaf blight symptom produced on leaf tip after inoculation with bacterial suspension from seedlings.

#### DETECTION OF XANTHOMONAS ORYZAE

The presence of viable *X. oryzae* cells in seed stored for 11 months at room temperature (15°–38°C) indicates that the pathogen can survive in and/or on seed till the next season. Earlier workers were not able to demonstrate the presence of the pathogen for such a long period because the initial viable population of the bacterium is reduced with time and it becomes difficult to isolate it in the absence of a selective medium. In the method now described even if the number of viable cells in seed has decreased during storage, the pathogen can be detected easily. There are other advantages over the method described by Hsieh *et al.* (1974). It is simple to conduct, does not require any special isolation technique and can detect all the strains of the pathogen. Large numbers of seed samples can be tested and the results become available in a week.

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