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USE OF A STARCH AGAR AS SELECTIVE ISOLATION MEDIUM FOR SEED-BORNE STRAINS OF *Xanthomonas campestris* pv. *oryzae*

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The ability of *Xanthomonas oryzae* pv. *oryzae* to digest starch has been used to detect the presence of the pathogen in rice (*Oryzae sativa* L.) seeds with the use of SX agar. Zones of starch hydrolysis appeared around seeds containing pv. *oryzae* 48 h after the seeds were plated onto SX agar. By this method 12 pathogenic strains of pv. *oryzae* were detected and characterised from seeds of 19 cultivars of rice. Starch digesting strains with yellow colonies and tested for their aerobic nature were found to be pv. *oryzae*. This seed-plating procedure was used to monitor the extent of seed infection and to examine the variability among the pathogenic strains of pv. *oryzae* from Southern India.

Bacterial leaf blight (BLB) caused by *Xanthomonas campestris* pv. *oryzae* Dye (1978) is one of the most important diseases of rice in tropical Asia. In India, millions of hectares of rice are severely infected and yield losses vary from 6 to 60% Incidence of pv. *Oryzae* has increased greatly since 1978 and in some parts of India, losses of 60—70% were reported in 1980 (Raina *et al.*, 1982).

Ecological studies on pv. *oryzae* strains relating to their survival in rice seeds, plant parts, irrigation water and soils have limited efficiency because detection of the pathogen is made difficult by the presence of other ubiquitous fast growing yellow saprophytes (Hsieh and Buddenhagen, 1974). An improved method devised for the detection of pv. *oryzae* in rice seed has limited application to a streptomycin resistant strain (Hsieh *et al*, 1974). There no suitable selective medium for pv. *oryzae*. On (1972) observed that the lack of a selective medium rendered the direct detection of pv. *oryzae* in soils, field water, or plant parts very difficult or impossible. Therefore attempts have been made to find better media (Devadath and Kelman, 1980).

In our efforts to characterize the seed-borne strains of pv. *oryzae* from Southern India, SX agar developed originally by schaad and white (1974) for selective isolation of *Xanthomonas campestris* pv. *campestris* was found quite suitable. This report describes the usefulness of SX agar for selective isolation of pv. *oryzae* from rice seed and describes the variability among such strains.

Seeds of 19 cultivars of rice were obtained from Rice Research Station, Ambasamudram (Tamil Nadu Agricultural University). These seeds stored for 5-8 months after harvest were used for isolating pv. *oryzae*. Isolations were made on SX agar (Schaad and white, 1974) (soluble potato starch, 10.0 g; beef extract, 1.0 g; ammonium chloride, 5.0 g; potassium diphosphate, 2.0 g; methyl violet B, 1.0 ml (1% solution

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in 20% ethanol); methyl green, 2.0ml (1% solution), cycloheximide, 0.25 g; agar, 15 g, distilled water 1000 ml; PH 6.8).

Surface sterilized seeds, 150 seeds for each cultivar, were plated individually on SX agar with their abscission ends downwards. After 48-72 h incubation at 30°C, bacterial growth around seeds showing starch hydrolysis zones were identified. These tentatively identified as pv. *oryzae* were purified further on SX and YDC (schaad, 1980) agars. Single colony strains were stored at 4°C on YDC slants (schaad, 1980).

Twelve strains of pv. *oryzae* isolated from rice seeds and characterized further were numbered UBL-XO- 1 to UBL-XO-12. The following tests were performed according to the references in parentheses: Colony colour and type of growth : gelatin liquefaction (Kelman and Dickey, 1980), starch hydrolysis (Gibbs and Skinner, 1966); H_2S production from cysteine HCl and peptone (Dye, 1980) and acid and gas production from carbohydrates,

Pathogenicity tests were made on 35-40 d old greenhouse grown plants of rice cultivars TKM-9 and IR-20. Leaves were inoculated with the leaf clipping technique (Reddy and kauffman. 1972). Cells (Ca. 10^8cells/ml of each strain) obtained from a 48 h-grown culture were used as inoculum. Inoculated plants were maintained under high humidity for first 3 d (24°C) and thereafter on greenhouse benches at 28-30°C. Lesions from infected leaves were measured 10 d after inoculation. Reisolations were made from the lesions on SX agar.

From seeds of 19 cultivars of rice a total of 20 strains of bacteria all having yellow colonies were obtained Out of them 12 were strains of pv. *oryzae* (Table 1) and produced the starch digestion zones on SX agar within 24-48 h after the seeds were plated on the medium. Unlike these, 5 of the remaining strains showed anaerobic growth and were, therefore, eliminated; other 3 strains had rough colonies and were not considered as pv. *oryzae*.

In similar tests, 8 other pathovars of *X campestris* did not produce starch-digestion zones on SX agar.

By the seed plating procedure on SX agar, estimates on the per cent infection of seeds by pv. *oryzae* was found to be quite high (Ca. 80%) in some cultivars even after 5-8 months of storage (Table 1). CO-25 had more than 78% infection. All strains of pv. *oryzae* liquefied gelatin and hydrolysed starch. Some strains (UBL-XO-3, UBL-XO-4 and UBL-XO-5) UBL-XO-10 liquefied faster than others (3 d) while others took longer 7-10d (Table 1). The rapid gelatin liquefiers also hydrolysed starch to a greater extent than others(Table 1). All 12 strains were also pathogenic to plants of rice cultivar TKM-9. Eleven strains induced lesions of more than 4cm long and strain UBL-XO-1(from cv. IR-20) totally wilted the inoculated plants. On reisolation starch-digesting pv. *oryzae* strains were recovered. Plants inoculated with sterile water did not show lesions.

The results reveal that pv. *oryzae* strains, UBL-XO-3, 4, 5 and 10 which have rapid liquefying power and greater starch hydrolysing potential also produ-

Table 1. Characteristics of *Xanthomonas Campestris* pv. *oryzae* strains isolated from rice seeds,

Strain	Rice cultivar and percent infection	Colony, colour and texture	Reaction of pv. *oryzae* strains									
			Gelatin liquefaction (in days)	Starch hydrolysis (diam of zone, in cm)	H_2S production		Acid production					Pathogenicity and length of lesion (cm)
					Cystein HCl	Peptone	Glucose	Lactose	Mannose	Arabinose		
UBL-XO-1	IR-20 (8.0)	YS[a]	+[c] (p)[d]	+ (0.6)	+	+	+	+	+	+		+ wilt
UBL-XO-2	Paiyur 1 (83.7)	YS	+ (7)	+ (3.5)	+	+	+	+	+	+		+ (11.5
UBL-XO-3	ADT-31 (81.7)	YS	+ (3)	+ (3.7)	+	+	+	+	+	+		+ (7.0)
UBL-XO-4	Co.43 (11.3)	YS	+ (3)	+ (3.2)	+	+	+	+	+	+		+ (6.9)
UBL-XO-5	PY-1 (79.8)	YS	+ (3)	+ (4.0)	±	+	+	+	+	+		+ (6.5)
UBL-XO-6	ADT-35 (3.0)	WS[b]	+ (p)	+ (0.9)	—	—	+	—	—	+		+ (6.5)
UBL-XO-7	IR-34 (12.3)	YS	+ (10)	+ (2.4)	+	+	—	—	—	—		+ (6.4)
UBL-XO-8	CO-25 (78.3)	YS	+ (7)	+ (3.5)	+	+	+	+	+	+		+ (5.9)
UBL-XO-9	IR-50 (8.7)	YS	+ (7)	+ (3.2)	+	+	+	+	+	+		+ (5.9)
UBL-XO-10	Ponni (10.0)	YS	+ (3)	+ (1.9)	+	+	+	+	+	+		+ (5.8)
UBL-XO-11	ASD-13 (10.0)	YS	+ (p)	+ (1.7)	+	+	+	+	+	+		+ (5.4)
UBL-XO-12	IET 4786 (11.7)	YS	+ (p)	+ (0.8)	—	+	—	—	—	—		+ (4.3)

[a] YS = Yellow, smooth, [b] WS = White, smooth, [c] Symbol + indicates a positive reaction and — indicates a negative reaction, [d] p = partial reaction

ced longer lesions. This may possibly suggest that there is a correlation between gelatin liquefaction/starch hydrolysis and virulence of pv. *oryzae* strains. A correlation of this kind was found earlier by Mukoo and Isaka (1964). However virulent strains like UBL-XO-1 (which caused complete wilting of seedlings) and UBL-XO-6 seem to suggest otherwise (Shekhawat and srivastava, 1968) as they are partial liquefiers of gelatin and induce only limited hydrolysis of starch.

The results suggest that SX agar is a very useful isolation medium for pv. *oryzae*. With it, direct isolation from seeds (without destroying them) or plant parts and dependable detection of pv. *oryzae* strains are possible. whether it will help to determine if rice seeds carry the pathogen from one crop to other and effectively reinitiate the bacterial leaf blight symptoms needs careful and extensive testing.

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