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Seed Detection of *Xanthomonas campestris* pv. *undulosa* Using a Modification of Wilbrink's Agar Medium[1]

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Abstract

Wilbrink's-boric acid-cephalexin agar medium (WBC) was developed and tested for isolating *Xanthomonas campestris* pv. *undulosa*, the causal agent of bacterial leaf streak of wheat or black chaff, from seed. WBC medium is Wilbrink's medium that has been modified by adding 0.75 g/l of boric acid and 10 mg/l of cephalexin. Compared to the standard Wilbrink's medium, average plating efficiency of 15 strains of *X. c.* pv. *undulosa* isolated from different hosts and locations in Mexico was 100.5% for WBC medium. Recovery of those strains was only 59.7% when 1 mg/l of gentamycin was added to WBC. Recovery was not possible either on WBC amended with 2 mg/l gentamycin or on XTS medium containing 8 mg/l gentamycin.

About 90% of saprophytic bacteria washed from wheat and triticale seed lots harvested in plots infested by bacterial leaf streak were eliminated using WBC, making it easier to identify the pale yellow *X. c.* pv. *undulosa* colonies. Using WBC and washing in saline (30 minutes), 1×10^3 to 6.1×10^6 *X. c.* pv. *undulosa* colony-forming units (CFU) were found per gram of seed, the detection limit being 1×10^3 CFU. WBC agar is a useful alternative for XTS medium because of the toxic effect of gentamycin observed under our conditions.

Introduction

Bacterial leaf streak of wheat (*Triticum aestivum* L.), known as black chaff when on the glumes, is caused by *Xanthomonas campestris* pv. *undulosa* (Smith, Jones & Reddy) Dye 1978 (BRADBURY, 1986). The disease was first described on wheat in 1917 (SMITH, 1917). However, as early as 1893, JACZEWSKI reported evidence of black chaff in China (as cited by BAMBERG, 1936), and in the US the disease was observed in Indiana in 1902 (as cited by SMITH, 1917) and Nebraska (HEALD, 1906).

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On triticale (X *Triticosecale* Wittmack), the disease has been known in Mexico since 1968 (ZILLINSKY & BORLAUG, 1971). Bacterial leaf streak on triticale also has been reported in India (RICHARDSON & WALLER, 1974), the US, Ethiopia (CUNFER & SCOLARI, 1982), Brazil (MOHAN & MEHTA, 1985) and the disease should be considered as a serious potential constraint to the crop.

In recent years, the names *X. translucens* and *X. campestris* pv. *translucens* have been used for any cereal streak pathogen when the pathovar was not determined by its differential host reactions. However, *X. translucens* is not included in the International Society for Plant Pathology list of accepted names of plant pathogenic bacteria (DYE *et al.*, 1980). If the rules and standards of nomenclature are followed as closely as possible the name *X. c.* pv. *translucens* (Jones, Johnson & Reddy) Dye 1978, should be reserved to the barley pathogen originally described by JONES *et al.* in 1917 (BRADBURY, 1986). Strains found on triticale fit descriptions of both pv. *undulosa* and pv. *secalis* (CUNFER & SCOLARI, 1982) appearing in Hagborg's taxonomy (HAGBORG, 1942) and Bergey's manual of systematic bacteriology (BRADBURY, 1984). Research carried out at the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and at the Laboratoire de Phytopathologie, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, have shown that *X. campestris* strains causing bacterial leaf streak on wheat and triticale in Mexico should be identified as *X. campestris* pv. *undulosa* on base of comparison with pathotypes strains in host range studies (MARAITE, personal communication; unpublished data). In view of the above, in this paper the causal agent of bacterial leaf streak is referred to as *X. c.* pv. *undulosa*, except when citing studies that use the name *X. c.* pv. *translucens*.

In 1919, *X. c.* pv. *undulosa* was shown to be seedborne (SMITH *et al.*, 1919) and contaminated seed is considered as an important primary source of inoculum (CUNFER, 1988; FORSTER & SCHAAD, 1988).

XTS semiselective medium (nutrient agar, 0.5% glucose, 8 mg/l gentamycin, 10 mg/l cephalexin, 200 mg/l cycloheximide) was developed to detect the presence of *X. c.* pv. *translucens* on wheat seeds (SCHAAD & FORSTER, 1985) because many strains collected in Idaho grew poorly on KM-1 agar proposed for extracting the pathogen from leaves and soil (KIM *et al.*, 1982). At Kansas State University, XTS medium was modified using two instead of the original 8 mg/l gentamycin to enhance the recovery of the pathogen from wheat seed from Idaho (CLAFLIN & RAMUNDO, 1987).

Repeated assays using XTS medium in Mexico appeared to be ineffective even when isolating *X. c.* pv. *undulosa* from wheat and triticale leaf sample with abundant bacterial exudates. When a seed-washate from samples harvested in contaminated fields was assayed, the few yellow bacteria observed on XTS were not *X. c.* pv. *undulosa*; this was later confirmed

using pathogenicity tests. The objective of this study was to develop a more reliable medium of detecting *X. c.* pv. *undulosa* on seed in Mexico.

Materials and methods

DEVELOPMENT OF WBC (WILBRINK'S-BORIC ACID-CEPHALEXIN) AGAR MEDIUM

In 1987, initial tests were conducted using wheat seed lots harvested in highly infected plots at Toluca, Mexico, at 2640 meters above sea level (masl). *Xanthomonas c.* pv. *undulosa* grows very fast on Wilbrink's non-selective medium (WS) (SANDS *et al.*, 1986), producing a mucoid 1-2 mm diameter colony in about 24-48 hr at 30 °C, and presents a more typical pale yellow color than on nutrient agar medium where *Xanthomonas* can possibly be discriminated from other bacteria after 48-72 hr at 28-30 °C.

In preliminary trials using WS amended with 1.75 g/l boric acid, *X. c.* pv. *undulosa* isolates from Mexico grew abundantly while growth of the most common saprophytes was slowed or totally inhibited. Further testing showed that 0.75 g/l boric acid was more appropriate because *X. c.* pv. *undulosa* colonies had a more homogenous appearance.

For increase of selectivity, the antibiotics gentamycin (8 mg/l) and cephalexin (10 mg/l) were added to Wilbrink's boric acid medium, using the concentration and method proposed for XTS (SCHAAD & FORSTER, 1985). However, no growth was observed on plates inoculated with 0.1 ml of the contaminated seed washate mixtures or concentrated suspensions of *X. c.* pv. *undulosa* CFBP3085 (=CB4 CIMMYT reference strain isolated from triticale collected at Ciudad Obregon, north-west Mexico). Complementary tests with various concentrations of cephalexin and gentamycin showed that CFBP3085 was completely inhibited with 2 mg/l gentamycin; this toxic effect was observed with both available brands of gentamycin (Sigma Chemical Co., St.Louis, MO, USA, or Garamicine G.U., Sheramex, Xochimilco, Mexico). No toxic effect of cycloheximide was observed when the concentration of this compound was raised to 200 mg/l.

The most satisfactory combination appeared to be: WS amended with boric acid (0.75 g/l), cephalexin (10 mg/l) and cycloheximide (75 mg/l), named WBC medium (Wilbrink's-boric acid-cephalexin). To prepare 1 liter WBC, 5 g bactopeptone, 10 g sucrose, 0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$, 0.05 g Na_2SO_3 anhydrous, 15 g agar were dissolved in 850 ml distilled water, autoclaved and cooled to 45 °C. Boric acid (0.75 g) autoclaved separately in 150 ml distilled water was mixed in, after cooling to 45 °C. Then 10 mg cephalexin (1 ml of a 10 mg/ml stock solution in 75 % ethanol) and 75 mg cycloheximide dissolved in 2 ml 75 % ethanol were added to the medium.

WASHING PROCEDURE

Seed samples (10 g, about 300 seeds) were added to 250-ml Erlenmeyer flasks containing 100 ml (considered here as 10^{-1} dilution) of a cold (4 °C) sterile saline (NaCl 0.85%) and Tween 20 0.02% (SCHAAD & DONALDSON, 1980). The flasks were shaken for 30 minutes at room temperature with an Eberbach Corporation (Ann Arbor, Michigan, USA) reciprocating shaker (Cat. No. 6000), at 90 revolutions per minute with 5 cm amplitude. The extraction mixture is adjusted to 10^{-2}, 10^{-3} dilutions or more, if necessary, in phosphate buffered saline (PBS) 0.01 M pH 7.2 (LELLIOTT & STEAD, 1987). One tenth milliliter of each dilution is spread onto WBC plates with an L-shaped glass rod. The plates are incubated at 30 °C for 4-5 days.

This procedure (procedure A) was compared with soaking the seeds at room temperature (procedure B) or at 4 °C (procedure C), each flask being shaken by hand. Comparison of the three procedures was made using 10 g seed samples of wheat genotype Alondra"s" (lot 42) from a highly infected plot at Toluca in 1987; soaking periods ranged from 3 to 60 minutes. Seed germination was studied on a total of 800 grains from several wheat and triticale lots for different seed storage periods up to six months after treatment to determine whether the effect of the saline washing solution is deleterious.

PLATING EFFICIENCY

WBC was tested with 15 strains of *X. c.* pv. *undulosa* for which pathogenicity on wheat and triticale had been confirmed. These strains were isolated from different hosts and areas of Mexico (table 1).

Plating efficiency on WBC (mean recovery) was compared to plating efficiency on nutrient agar plus 0.5% glucose (NAG), XTS, WBC-1 (WBC+5 mg/l cephalexin), WBC-2 (WBC+1 mg/l gentamycin) and WBC-3 (WBC+2 mg/l gentamycin). The cell suspension of each strain was adjusted using a Petroff-Hausser counting chamber to between 300 and 1.5×10^3 cells/ml. One-tenth milliliter was assayed for each strain and each medium in three replicates.

TABLE 1

Origin of Mexican *Xanthomonas campestris* pv. *undulosa* strains used in the study of plating efficiency.

Strain	Location	Elevation (masl)[a]	Host
CB1	Ciudad Obregon	39	*Phalaris sp.*
CB2	Toluca (Atizapan)	2640	Triticale
CFBP3085	Ciudad Obregon	39	Triticale
CB5	Toluca (Atizapan)	2640	Wheat
CB7[b]	Monterrey	800	Barley
CB40	Toluca (CODAGEM)[c]	2640	Wheat
CB44	Toluca (CODAGEM)	2640	Wheat
CB57	Toluca (Atizapan)	2640	Triticale
CB60	Toluca (Atizapan)	2640	Triticale
CB61	Toluca (Atizapan)	2640	Wheat
CB64	Papalotla	2249	Triticale
CB66	El Batan	2249	Triticale
CB67	Ciudad Obregon	39	Wheat
CB69	Patzcuaro	2350	Wheat
CB75	Papalotla	2249	Wheat

a meters above sea level.

b Strain identified as *X. c.* pv. *undulosa* after host range.

c CODAGEM=Centro de organización y Desarollo Agricola en el Estado de México.

REDUCTION OF SEED-ASSOCIATED SAPROPHYTIC BACTERIA AND DETECTION OF *X. C.* PV. *UNDULOSA* ON CONTAMINATED SEEDS

Ten wheat and triticale seed samples from Mexico (table 2), harvested in 1986 and 1987 from bacterial leaf streak infected plots at Toluca and El Batan (2249 masl) were chosen at random. Seed samples (10 g each) were washed using procedure A. The mixture was adjusted to 10^{-3} and 10^{-4} dilution in PBS, and 0.1 ml of each was assayed on two plates of WBC, WS, NAG and WBC-1 media.

TABLE 2

Wheat and triticale seed lots used in detecting *X. campestris* pv. *undulosa* and studying the reduction of seed-associated saprophytic bacteria.

Seed lot	Crop	Genotype	Origin
P9	Wheat	Toluca[a] 1986	PRL"S"
TR8	Wheat	Toluca[b] 1987 plot 8	Alondra"s"
TR37	Wheat	Toluca[b] 1987 plot 37	Alondra"s"
TR53	Wheat	Toluca[b] 1987 plot 53	Alondra"s"
TR73	Wheat	Toluca[b] 1987 plot 73	Alondra"s"
TR82	Wheat	Toluca[b] 1987 plot 82	Alondra"s"
TCL51	Triticale	El Batan 1987	Stier 16-1
TCL75	Triticale	El Batan 1987	Yogui"s"/GRF"S"
TCL95	Triticale	El Batan 1987	S-Roq-D-GI-75-80
TCL118	Triticale	El Batan 1987	Mus"s"/BTA"S"

a Atizapan
b CODAGEM

Colonies of both *X. c.* pv. *undulosa* type and other bacteria were counted in the dilutions assayed. Representative colonies were cloned on WS. Pathogenicity was tested by injecting at 5 cm above the soil level, seedlings of genotypes Seri 82 (wheat) and Mus"s" (triticale) at the five-leaf stage, with a concentrated bacterial suspension of approximately 10^9 CFU/ml. Strain CFBP3085 was inoculated as control. *X. c.* pv. *undulosa* were also tentatively identified by growing on GYCA slants (5 g yeast extract, 5 g glucose, 40 g $CaCO_3$, 15 g agar, 1 liter distilled water). Basic biochemical tests like Gram, Kovac's oxidase, production of acid from glucose, in aerobic and anaerobic conditions, were done following BRADBURY (1970).

The average reduction percentage of saprophytes was calculated for WBC and WBC-1 by using, for each dilution and seed lot assayed, the average number of saprophyte colonies on each medium and the number of saprophyte colonies on WS and NAG. The number of CFU/g seed was calculated using the average number of *X. c.* pv. *undulosa* colonies on WBC for the highest dilution in which the bacterium was detected.

The frequency of individual grains contaminated by *X. c.* pv. *undulosa* was appraised. One hundred grains from genotype Alondra"s" (lot TR73) were washed for 30 minutes in 3 ml of saline, in individual tubes, with the Eberbach shaker speed set to about 180 revolutions per minutes. The washate (0.1 ml) was plated on WBC medium.

Results

WASHING PROCEDURE

On WBC, the diameter of isolated *X. c.* pv. *undulosa* colonies was about 2 mm after four days' incubation at 30 °C. The number of pale yellow colonies of *X. c.* pv. *undulosa* recovered on WBC from genotype Alondra"s" seed lot 42 (table 3) was dependent on the soaking period and washing procedure. The overall average per plate did not increase significantly after 30 minutes of soaking, except in procedure C. However, the number of colonies decreased from flasks 2-6 and 8, after one hour of soaking. It was also observed that in a given procedure the total number of *X. c.* pv. *undulosa* colonies found on WBC may vary for each 10 g seed sample, even among samples coming from the same plot.

TABLE 3

Effect of seed soaking duration and washing procedure on the number of *X. c.* pv. *undulosa* colonies recovered on WBC medium from wheat genotype Alondra"s" seed (lot 42), harvested at Toluca in October 1987. Three flasks with 10 g seed in 100 ml cold sterile saline. Figures for each flask (1 to 9) are the average number of colonies on WBC per three Petri dishes when 10^{-3} dilution is assayed.

Soaking duration (min)	Procedure A				Procedure B				Procedure C			
	flask				flask				flask			
	1	2	3	Mean	4	5	6	Mean	7	8	9	Mean
3	1	2	8	4	2	1	1	1	0	0	8	3
15	5	7	8	7	9	2	5	5	4	2	14	7
30	24	8	10	14	24	3	13	13	8	1	13	7
60	32	4	9	15	0	0	0	0	13	0	29	14

Procedures:

A: Soaking at room temperature using an Eberbach shaker.

B: Soaking at room temperature shaking by hand when seed is added and then every ten minutes.

C: Soaking at 4 °C, shaking by hand when seed is added and then every 10 minutes.

Procedure A with 30 minutes of soaking proved to be adequate because the constant, homogeneous, mechanical shaking made it possible to wash seed samples in a standardized way. No deleterious effects of the saline solution were observed on germination potential of seed stored for up to six months after washing.

PLATING EFFICIENCY

Recovery was quite similar on WS, WBC, and WBC-1, but was strongly reduced on WBC-2 and was not observed on XTS and WBC-3, where apparently gentamycin had some toxic effect on *X. c.* pv. *undulosa* strains used.

TABLE 4

Mean recovery of Mexican *X. c.* pv. *undulosa* strains on WBC, NAG, WBC-1, WBC-2, WBC-3 and XTS media compared to growth on WS medium.

Strains	WBC	NAG	WBC-1	WBC-2	WBC-3	XTS
CB1	[a]94.9	93.6	102.6	64.1	0	0
CB2	121.2	142.4	113.6	95.5	0	0
CFBP3085	111.6	85.5	136.2	100.0	0	0
CB5	125.6	80.8	98.7	103.8	0	0
CB7	80.8	117.7	85.4	39.2	0	0
CB40	94.0	76.1	84.5	19.9	0	0
CB44	88.1	83.3	92.9	40.5	0	0
CB57	100.0	92.9	108.2	54.2	0	0
CB60	112.9	64.5	95.2	33.9	0	0
CB61	97.8	99.7	90.0	79.1	0	0
CB64	100.8	100.8	83.3	45.8	0	0
CB66	85.1	80.9	85.1	23.4	0	0
CB67	92.5	83.7	85.1	82.9	0	0
CB69	99.6	60.5	87.2	37.5	0	0
CB75	103.1	73.8	116.8	75.4	0	0
Average	100.5	87.3	97.7	59.7	0	0

[a] Mean recovery = number of colonies on medium x 100/number of colonies on WS medium. Figures are calculated from the average of colonies counted in three replicates of each agar medium.

Compared to WS (table 4), plating efficiency for WBC ranged from 80.8 to 125.6% (average was 100.5%) and was better than recovery on NAG medium (87.3%). Plating efficiency was very similar on WBC-1 (97.7%), which demonstrated the high tolerance of *X. c.* pv. *undulosa* strains to a 15 mg/l concentration of cephalexin. However, when 1 mg gentamycin was added to the medium, average plating efficiency was only 59.7%.

REDUCTION OF SEED-ASSOCIATED SAPROPHYTIC BACTERIA

Average reduction of saprophytes for the ten seed lots tested was more than 90% and was similar on WBC and WBC-1, when compared to WS or NAG (table 5). *X. c.* pv. *undulosa* colonies were identified on base of their biochemical and pathogenicity characteristics. Strictly aerobic, Gram and Kovac's oxidase negative, they did not reduce nitrate. They were generally easily differentiated on WBC from the often buff-yellow or orange and larger colonies of saprophytic bacteria. For some seed lots, the *X. c.* pv. *undulosa*/saprophyte ratio of counted colonies was reversed, increasing dramatically the percentage of *X. c.* pv. *undulosa*, when mixture from the same dilution tube was assayed on WBC rather than on WS and NAG, particularly for lots TR53 and TR73. This suggests that antagonistic bacteria among the saprophytes reduced the number of *X. c.* pv. *undulosa* colonies on nonspecific media.

TABLE 5

Percent reduction of saprophytes observed by plating washing mixtures of 10 wheat and triticale seed lots on WBC or WBC-1 compared to WS or NAG medium. Average (x) based on the analysis of 10 seed lots and standard deviation (s).

Dilution of the mixture	WBC/WS		WBC-1/WS		WBC/NAG		WBC-1/NAG	
	x	s	x	s	x	s	x	s
10^{-3}	93.0	9.1	94.3	4.2	91.6	7.2	92.5	5.4
10^{-4}	94.0	6.3	94.6	10.3	93.9	6.1	95.5	8.1

RECOVERY OF *X. C.* PV. *UNDULOSA* IN CONTAMINATED SEED LOTS

Xanthomonas c. pv. *undulosa* was more easily recognized on WBC medium than on WS and NAG, where the higher number of saprophytes and their colony appearance, particularly on NAG, increased the risk of misidentification. All pure cultures obtained from single colonies, tentatively identified as *X. c.* pv. *undulosa*, were pathogenic on wheat and triticale. Watersoaked lesions with milky exudates, similar to those induced by CFBP3085 *X. c.* pv. *undulosa* reference strain were observed five days after inoculation. Identification of *X. c.* pv. *undulosa* on WBC was also confirmed by the appearance on GYCA slants. On GYCA, only strains proved to be *X. c.* pv. *undulosa* on base of biochemical and pathogenicity tests, produced a typical pale yellow mucoid mass one to several mm in diameter at the bottom of the tube. No yellow saprophyte associated with wheat and triticale seed has been found to have this characteristic in our conditions.

All the tested seed lots were contaminated, and the average percentage of *X. c.* pv. *undulosa* colonies was always superior on WBC when compared to WS and NAG. Using WBC, CFU/g was from 1×10^3 to 6.1×10^6 (table 6).

When the frequency of contaminated grains was appraised by washing 100 kernels individually and by plating on WBC, *X. c.* pv. *undulosa* colonies were found only on five grains. In one case, 96 CFU were found on the agar medium. The number of CFU was inferior to 10 on the other plates. This result indicates that only a few grains may be responsible for the CFU detected in 10 g of seed.

TABLE 6

CFU/g seed calculated from the average number of *X. c.* pv. *undulosa* colonies counted on WBC medium for the highest dilution in which *X. c.* pv. *undulosa* was detected.

Seed lot	CFU/ g seed
TR8	1.0×10^3
P9	3.0×10^3
TCL51	1.0×10^4
TCL95	3.0×10^4
TCL118	5.0×10^4
TR82	8.0×10^4
TCL75	1.0×10^5
TR73	3.7×10^5
TR37	4.0×10^5
TR53	6.1×10^6

Discussion

WBC is not exclusively selective for *X. c.* pv. *undulosa*, but was determined to be a reliable medium for recovering the bacterium from contaminated wheat and triticale seed lots under our conditions. The reliability of WBC was confirmed in tests carried out on contaminated seed from the US, Brazil and Uruguay (MARAITE, personal communication). Plating efficiency of test strains from different hosts and locations in Mexico approached 100%. *X. c.* pv. *undulosa* was easily recognizable on WBC, and saprophyte reduction was above 90% for the seed lots used.

When antagonistic bacteria among the saprophytes were reduced, the number of *X. c.* pv. *undulosa* detected increased significantly. *X. c.* pv. *undulosa* is very susceptible to antagonistic bacteria. These may be present, even when using WBC medium. In routine seed tests using WBC and 10^{-3} and 10^{-4} dilution at the CIMMYT laboratory, antagonistic bacteria were not observed. However, antagonistic fluorescent pseudomonads were found that induced an inhibition area several millimeters in diameter around *X. c.* pv. *undulosa* colonies, but for a 10^{-2} dilution, when seeds were processed in a blender or when washing leaf samples with old bacterial leaf streak lesions.

XTS medium was not effective in our conditions due to either variation in the antibiotic activity of the two brands of gentamycin available or high sensitivity of *X. c.* pv. *undulosa* populations in Mexico. Negative results have sometimes been obtained when testing bacterial streak-infected wheat seed lots from Oregon using XTS (R.S. KAROW, personal communication). However, when other samples from the same lot from Oregon were sent to the Laboratoire de Phytopathologie in Louvain-la-Neuve (Belgium) for testing, similar quantities of *X. c.* pv. *undulosa* were detected on XTS and WBC. Using the washing procedure A developed at CIMMYT, no difference between the two media was observed but the amount of *X. c.* pv. *undulosa* recovered on both XTS and WBC varied according to the 10 g-seed sub-samples tested (MARAITE, personal communication). This suggests no gentamycin sensitivity of the *X. c.* pv. *undulosa* contaminating the tested seed samples from Oregon. Thus, false negative results may occur if the distribution of contaminated seed is uneven in a seed lot and the size of the tested sample is too small.

At the International Rice Research Institute (IRRI), 75% of *X. c.* pv. *oryzicola* tested were found to be resistant to 10 µg gentamycin (ADHIKARI & MEW, 1985). Gentamycin sensitivity of *X. c.* pv. *phaseoli* has been shown, and assaying new supplies of this antibiotic was recommended (CLAFLIN *et al.*, 1987). *X. c.* pv. *undulosa* sensitivity for gentamycin indicates that seed detection methods may need to be adapted to the conditions where they are used.

Cephalexin is usually considered to be weakly active against gram negative bacteria (FRASER, 1986), and no significant reduction of saprophytes was observed when washates were assayed on WBC-1 containing 15 mg/l cephalexin. Higher concentrations have been used to recover *X. c.* pv. *vesicatoria* from leaves or soil (65 mg/l) (MAC GUIRE *et al.*, 1986) or *Pseudomonas syringae* (80 mg/l) from bean seed (MOHAN & SCHAAD, 1987). In our conditions the use of 10 mg/l cephalexin proved to be satisfactory.

Boric acid concentration used in WBC is higher than in the Tween media proposed for *X. c.* pv. *vesicatoria* (600 mg/l) (MAC GUIRE *et al.*, 1986), but lower than for *P. syringae* from bean seeds (MOHAN & SCHAAD, 1987) or *Clavibacter michiganense* subsp. *michiganense* from tomato seed (1.5 g/l) (FATMI & SCHAAD, 1988). Cycloheximide is commonly used to suppress fungi (BROECK *et al.*, 1984). In this study, fungi contamination was usually not observed with 75 mg/l.

The practical significance of the level of bacteria detected has to be assessed according to environmental conditions because contaminated seed lots do not always produce epidemics. In Idaho, 60% of all spring wheat seed lots have been reported to be infected, but wheat with a low level of contamination with *X. translucens* will not result in field disease (SCHAAD, 1987). Moreover, the correlation between laboratory seed testing and disease expression in the field may depend on the size of the seed sample analyzed, the percentage of contaminated grains and the amount of bacteria per contaminated grain.

Washing the seed for 30 minutes using procedure A was appropriate. Reduction of *X. c.* pv. *undulosa* viable cells observed for a washing duration superior to 30 minutes was probably caused by the antagonistic bacteria released into the washing mixture at the same time as *X. c.* pv. *undulosa*. The uneven distribution of those antagonistic micro-organisms in the seed may explain why the reduction of viable *X. c.* pv. *undulosa* cells was observed in all flasks using procedure B, but not using procedure A. The levels of *X. c.* pv. *undulosa* detected on wheat and triticale lots used in this study are not indicative of infection levels on either crop in the Mexican highlands because seed lots were harvested only in infected plots.

An advantage of the washing method is that seed can be used after testing, if it proves to be free of *X. c.* pv. *undulosa* and is correctly dried. However, since variable levels of *X. c.* pv. *undulosa* were detected in different 10 g seed samples due to the small amount of grains bearing the pathogen, larger quantities should be used when testing commercial seed lots. The technique may also be combined with others as serology. The washing solution itself can be analyzed using for example immunoblot technique or immunofluorescence microscopy with specific monoclonal

antibodies. Combining the washing method and plating on WBC medium with such techniques will make it possible to test a larger number of seed lots.

Until now, the GYCA test has been reliable for *X. c.* pv. *undulosa* diagnosis under Mexican conditions. *Xanthomonas* colonies are also usually mucoid on YDC (10 g yeast extract, 20 g glucose, 20 g calcium carbonate, 15 g agar) (SCHAAD, 1988) which is similar to GYCA. YDC was used for presumptive identification of *X. c.* pv. *translucens* after plating assay on XTS (SCHAAD & FORSTER, 1985). However, since the GYCA procedure is slow (one week), it is generally used only for confirming identification made on WBC with the unaided eye.

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Résumé

Le milieu gélosé Wilbrink's-acide borique-cephalexine (WBC) a été développé et testé pour isoler *Xanthomonas campestris* pv. *undulosa*, l'agent causal de la strie bactérienne du blé ou "black chaff", à partir des semences. Le milieu WBC est une modification du milieu de Wilbrink obtenue en ajoutant 0.75 g/l d'acide borique et 10 mg/l de cephalexine. En utilisant le milieu WBC et en comparant au milieu de Wilbrink standard, on a obtenu, pour 15 souches de *X. c.* pv. *undulosa* isolées de différents hôtes et endroits au Mexique, une efficacité de culture sur boîtes de Petri de 100.5%. On ne retrouva ces souches qu'avec un taux de 59.7% lorsque 1 mg/l de gentamycine était ajouté au milieu WBC. Il fut impossible de les retrouver sur le milieu WBC ammendé avec 2 mg/l de gentamycine ou sur le milieu XTS qui contient 8 mg/l de gentamycine.

Environ 90% des bactéries saprophytes extraites par lavage de lots de semences récoltées dans des parcelles infectées par la strie bactérenne, ont été éliminées en utilisant WBC, rendant plus facile l'identification des colonies jaunes pâles de *X. c.* pv. *undulosa*. En utilisant le milieu WBC et le lavage dans une solution saline (30 minutes), de 1×10^3 à 6.1×10^6 unités de formation de colonie (UFC) de *X. c.* pv. *undulosa* ont été trouvées par gramme de semences, la limite de détection étant 1×10^3 UFC. Le milieu WBC est une alternative utile lorsque le milieu XTS ne peut être employé en raison de l'effet toxique du à la gentamycine, comme c'est le cas dans nos conditions.

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