A New Semiselective Medium for the Isolation of *Xanthomonas campestris* pv. *campestris* from Crucifer Seeds

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ABSTRACT

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Antibiotics were evaluated for inclusion in a semiselective medium for isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seed. Ampicillin, penicillin G, and tetracycline completely inhibited the growth of *X. c. campestris* B-24 at 6.25 μg/ml or less, whereas carbenicillin, cephalothin, gentamicin, and kanamycin inhibited *X. c. campestris* at 25–80 μg/ml. Bacitracin, neomycin, and streptomycin were least inhibitory with the minimal inhibitory concentrations at >400, 160, and 640 μg/ml, respectively. CS20ABN medium was developed by incorporation of bacitracin, neomycin, and cycloheximide at 100, 40, and 100 μg/ml, respectively, into CS20A. Washings from samples of crucifer seed were assayed for *X. c. campestris* on five media: CS20A, CS20ABN, NSCA, NSCAA, and FS. Saprophytic bacteria were often too numerous to count and overgrew *X. c. campestris* on all media except CS20ABN where 59–100% of recovered colonies were *X. c. campestris*. Colony size of *X. c. campestris* on CS20ABN was two- to threefold or three- to sevenfold larger than that on NSCA and NSCAA or FS, respectively.

Additional keywords: black rot of crucifer, seed assay.

Plating seed washings onto a semiselective medium has been a significant factor for detection of seedborne pathogens (4,5). Semiselective media including nutrient starch cycloheximide agar (NSCA) (3), nutrient starch cycloheximide antibiotic agar (NSCAA) (3), basal starch cycloheximide antibiotic agar (BSCAA) (3), and FS (6) have been widely used for the detection of the causal organism of black rot of cabbage, *Xanthomonas campestris* pv. *campestris*, on and in crucifer seeds. In July 1988, the personnel at the Seed Laboratory of Georgia State Department of Agriculture extended the seed wash time from 3 min to 2 hr. With this procedural change there was an increase in saprophytic bacteria that overgrew *X. c. campestris* on NSCA, NSCAA, and BSCAA. This resulted in no recovery of *X. c. campestris*. A redilution of the seed washings to 10^{-3} and 10^{-4} became necessary in order to recover *X. c. campestris*. An alternative approach to handle seed lots heavily infested with saprophytes was to develop a medium that inhibited the growth of saprophytes without significantly suppressing *X. c. campestris*. This work describes the development of a new semiselective medium and its selectivity in the isolation of *X. c. campestris* from crucifer seeds.

MATERIALS AND METHODS

Antibiotic spectrum of *X. c. campestris* grown in CS20 medium. *X. c. campestris* strain B-24 (ATCC 43304) was used in screening the following 10 antibiotics (Sigma, St. Louis, MO): ampicillin sulfate, bacitracin (59,400 U/g), carbenicillin, cephalothin, gentamicin sulfate, kanamycin sulfate, neomycin sulfate, penicillin G potassium salt, streptomycin sulfate, and tetracycline hydrochloride. Stock solutions were made with sterile distilled water. Eight twofold dilutions were used, starting with the following concentrations: ampicillin, carbenicillin, cephalothin, penicillin G, and tetracycline, 100 μg/ml; gentamicin, kanamycin, neomycin, and streptomycin, 640 μg/ml; and bacitracin, 400 μg/ml. One milliliter of each antibiotic stock solution was added into 499 ml of CS20 agar medium (1,2) when cooled to 45 C. CS20 agar medium consists of deionized distilled water, 1,100 ml; soy peptone (Scott Laboratories, Fiskeville, RI), 2.0 g; Bacto tryptone (Difco, Detroit, MI), 2.0 g; $(NH_4)_2$ HPO_4, 0.8 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.4 g; L-glutamine (Sigma Chemical Co., St. Louis, MO), 6.0 g; L-histidine HCl (Sigma), 1.0 g; dextrose (Fisher), 1.0 g; potato-soluble starch (J. T. Baker Chemical Co., Phillipsburg, NJ), 2.0 g; hemin chloride (Sigma), 0.015 g; phenol red (Sigma), 0.01 g; and Bacto agar (Difco), 12.0 g. The control medium contained no antibiotics. Molten agar was poured to a depth of 2.5–3.0 mm in 100 × 15 mm plastic petri plates. All plates were stored at 4 C and were allowed to equilibrate to room temperature before usage.

A 0.1-ml aliquot of a log-phase cell suspension of *X. c. campestris* B-24 was pipetted onto duplicate agar plates and spread using an alcohol flamed, L-shaped glass rod. After 5 days at 30 C, plates were examined under a binocular microscope at 50×. The minimal inhibitory concentration was defined as the concentration that resulted in no bacterial growth.

Formulation of a new semiselective medium CS20ABN. The CS20ABN medium was prepared by adding stock solutions of bacitracin, neomycin, and cycloheximide into CS20A medium before pouring to obtain a final concentration of bacitracin, neomycin, and cycloheximide at 100, 40, and 100 μg/ml, respectively. CS20A medium consists of all the ingredients included in CS20 medium (1,2) except hemin chloride and phenol red. The amount of potato-soluble starch increases to 10 g in CS20A, as compared to 2 g in CS20 medium. All ingredients were added and dissolved in the order given and autoclaved for 18 min. CS20A broth medium was prepared without agar.

Preparation of NSCA, NSCAA, and FS media. NSCA and NSCAA were prepared as described by Randhawa and Schaad (3), whereas FS was prepared as reported by Yuen et al (6).

Evaluation of CS20ABN medium. Five strains of *X. c. campestris*, originally isolated from commercial crucifer seeds and proved pathogenic to cabbage seedlings, were grown in CS20A broth overnight. The cell suspension of each strain was adjusted to a Klett-Summerson Photoelectric Colorimeter reading of 50. A series of 10-fold dilutions was made to 10^{-8} with CS20A broth. A 0.1-ml aliquot from each of three dilutions, 10^{-6}, 10^{-7}, and 10^{-8}, was pipetted onto triplicate plates of CS20A, CS20ABN, NSCA, and NSCAA. After 3 days' incubation at 30 C, the number of colonies per plate was recorded, and the diameters of 30 colonies on each plate were measured.

Isolation of *X. c. campestris* from crucifer seeds on CS20ABN, NSCA, and NSCAA. Seed assay procedures were as described by Randhawa and Schaad (3), with modifications. A total of

160 g (about 50,000 seeds) from each of 10 seed lots were washed in 250 ml of saline containing Tween-20 (0.1 ml) and benomyl (5 mg) for 2 hr at 25 C, at a constant speed of 115 rpm on a Gyrotory Shaker Model G2 (New Brunswick Scientific). Washings were filtered through four layers of cheesecloth, and the filtrate was centrifuged at a low speed (816 × g for 5 min) to remove debris. The decanted supernatant was centrifuged (10,725 × g for 20 min) to concentrate all microbes. The pellet was washed once with saline and centrifuged at 7,960 × g for 15 min. The final pellet was resuspended in 8 ml of saline and serially diluted to 10^{-1} and 10^{-2}. A 0.1-ml aliquot from both dilutions (10^{-1} and 10^{-2}) was plated on the CS20ABN, NSCA, and NSCAA media. After 48 hr of incubation at 30 C, mucoid colonies surrounded by a starch hydrolysis ring were counted. Two mucoid colonies from each seed lot were transferred onto yeast-extract-dextrose-$CaCO_3$ (YDC) plates (5). If the colony on YDC was mucoid and yellowish color, it was tested for pathogenicity to cabbage (*Brassica oleracea* var. *capitata* L. 'Market Price') seedlings.

Wash times and temperatures. Four crucifer seed lots infected with *X. c. campestris* were assayed as described above, except 80 g of seed were used for each assay. The amount of buffer and saline solutions for the final suspension was reduced to half. Two temperature regimes, 5 C and 25 C (room temperature), were used for seed washing. For each wash temperature, five different seed wash times (3 min; 1, 2, 3, and 4 hr) were tested. A 0.1-ml aliquot of the final wash dilution was pipetted onto triplicate plates of both CS20ABN and FS media. Total number of *X. c. campestris* and saprophytic bacteria were recorded 3–5 days and 5–7 days after plating on CS20ABN and FS media, respectively. Colony size on CS20ABN and FS was determined by measuring the diameter of 10 randomly selected individual colonies for each treatment.

Pathogenicity test. Presumptive strains of *X. c. campestris* were grown in liquid nutrient glucose broth for 18 hr in an incubator shaker at 30 C. The cell suspension was adjusted to an optical density reading of 0.1 at wavelength 540 nm (Gilford 260 Spectrophotometer), and the adjusted suspension was diluted to 1:100. A syringe with a 26-gauge needle was used to inject about 0.1 ml of the cell suspension into the leaf axil of a young (two- to three-leaf) cabbage seedling. Seedlings were placed in the greenhouse and observed for symptoms for 3 wk following inoculation. The presence of black veins surrounded by chlorotic tissue was considered a positive pathogenicity test.

RESULTS

Antibiotic spectrum of *X. c. campestris* strain B-24. The minimal inhibitory concentration of ampicillin, bacitracin, carbenicillin, cephalothin, gentamicin, kanamycin, neomycin, penicillin G, streptomycin, and tetracycline were 6.25, >400, 25, 50, 80, 80, 160, 3.13, 640, and 1.56 μg/ml, respectively. Bacitracin, neomycin, and streptomycin were the least inhibitory to *X. c. campestris* among the 10 antibiotics evaluated.

Evaluation of CS20ABN. Based on colony diameter and number of colonies of five strains of *X. c. campestris*, there were no differences in growth on CS20A and CS20ABN (Table 1). Colony diameters of *X. c. campestris* on both CS20A and CS20ABN were two to three times larger than that on NSCA and NSCAA. Therefore, antibiotics incorporated into CS20A medium were not inhibitory to *X. c. campestris*. Of the 10 commercial seed lots assayed, saprophytes overgrew *X. c. campestris*

TABLE 1. Comparison of colony size and the number of colonies for five strains of *Xanthomonas campestris* pv. *campestris* grown on CS20A, CS20ABN, NSCA, and NSCAA media[w]

Medium	Colony diameter[x] (mm)						Number of colonies per plate[y]					
	1	2	3	4	5	Mean[z]	1	2	3	4	5	Mean
CS20A	4.6	2.9	3.0	3.3	2.6	3.3 a	15.3	10.0	23.3	20.7	19.7	17.8 a
CS20ABN	4.2	2.7	3.5	3.6	3.8	3.6 a	14.3	13.3	15.7	18.7	17.7	15.9 a
NSCA	2.0	0.8	0.9	1.3	2.0	1.4 b	12.0	14.3	17.7	22.3	18.0	16.9 a
NSCAA	2.0	0.8	0.9	1.2	2.2	1.4 b	15.7	12.3	19.7	22.3	21.3	18.3 a

[w] Three plates per medium were inoculated.
[x] Average diameter of 30 colonies.
[y] Average number of colonies on three plates.
[z] Means in a column which are not followed by a common letter are significantly different ($P = 0.05$) by Duncan's multiple range test.

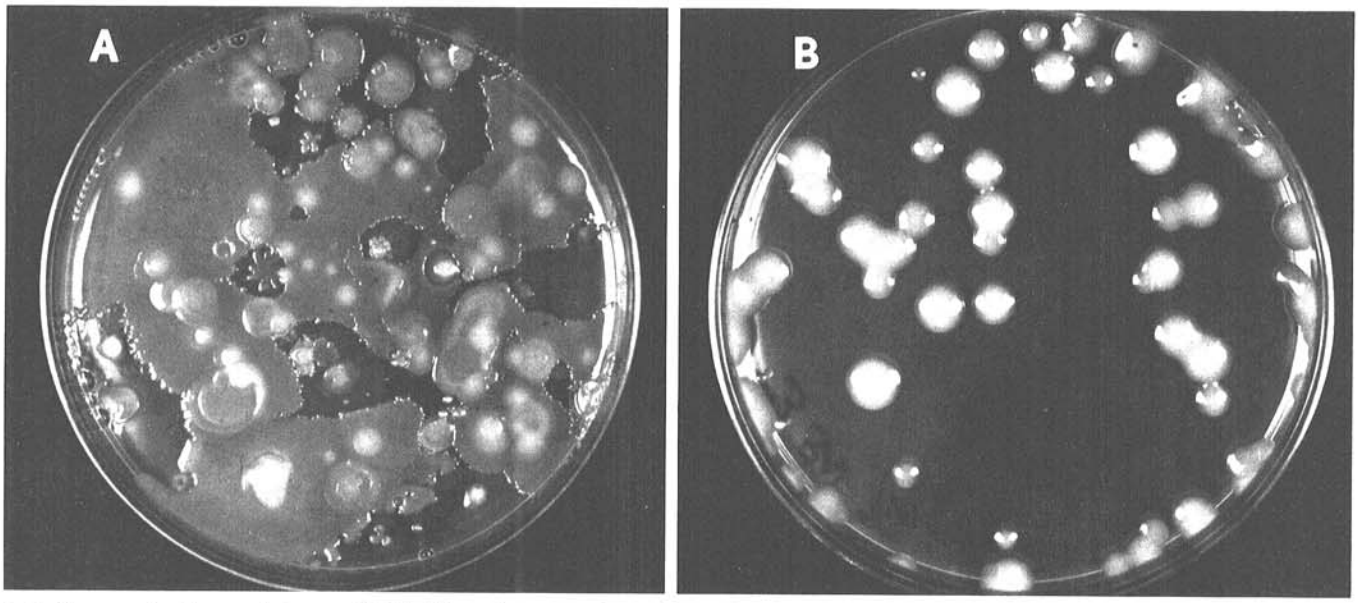

Fig. 1. Overgrowth of saprophytes on CS20A (**A**) and a pure culture of *Xanthmonas campestris* pv. *campestris* recovered on CS20ABN medium (**B**). Plates were incubated for 5 days. Both media were inoculated with a washing of crucifer seed.

on CS20A medium (Fig. 1A), whereas they were inhibited on CS20ABN medium (Fig. 1B). In contrast, 59–100% of the colonies recovered on CS20ABN were *X. c. campestris* (Table 2), which indicates a high specificity of the new medium. A lower frequency of recovery was observed for NSCA and NSCAA (Table 2). Colonies of *X. c. campestris* recovered on CS20ABN were larger than those on NSCA and NSCAA (Fig.2).

Wash times and temperatures. In general, more cells of *X. c. campestris* were recovered on CS20ABN medium than on FS medium, whereas more saprophytes were recovered on FS medium than on CS20ABN medium (Table 3). *X. c. campestris* represented 33–100% of the total bacteria recovered on CS20ABN medium (Table 3). In contrast, *X. c. campestris* represented 6–100% of the total bacteria on FS medium (Table 3). Washing seeds at two different temperatures (25 vs. 5 C) did not result in a significant difference in the recovery of *X. c. campestris* (Table 3). Significant differences were recorded, however, when seeds were washed for different time periods. For example, the number of colonies of *X. c. campestris* recovered from 120-min washings were higher than those from 60-min washings. In turn, few colonies were recovered from 3-min washings. The number of colonies of *X. c. campestris* recovered from 3-hr washings were slightly lower than those from 2-hr washings. In general, a 4-hr washing time gave the highest recovery of *X. c. campestris*.

The colony size of *X. c. campestris* recovered in CS20ABN medium was three to seven times larger than that in FS medium (Fig. 3).

Pathogenicity test. All presumptive strains of *X. c. campestris* were tested for pathogenicity. In most cases, the black veins surrounded by chlorotic tissue appeared in cabbage leaves 4–7 days after inoculation. A total of 31 strains (24%) (Table 4) did not produce symptoms 3 wk after inoculation and were considered nonpathogenic on crucifers.

DISCUSSION

A semiselective medium, CS20ABN, was developed for better detection of *X. c. campestris* on or in crucifer seeds. Saprophytes associated with seed usually overgrew the *X. c. campestris* on NSCA and NSCAA media and interfered with detection of the pathogen. CS20A was an ideal basal medium for the development of this semiselective medium. Through an investigation of the minimal inhibitory concentrations of various antibiotics to *X. c. campestris*, not only could the kinds of antibiotics be selected for the incorporation into basal medium, but the concentrations of the antibiotics could also be determined. For example, we selected bacitracin and neomycin at 100 and 40 μg/ml, respectively, for the medium. The concentration of bacitracin (100 μg/

TABLE 2. Recovery of *Xanthomonas campestris* pv. *campestris* from washings of commercial crucifer seeds on CS20ABN, NSCA, and NSCAA media[u]

	Mean colony-forming units per plate[v]					
	Total bacteria[w]			*Xanthomonas campestris* pv. *campestris*		
Seed lot	CS20ABN	NSCA	NSCAA	CS20ABN	NSCA	NSCAA
1	103	TNC[x]	TNC	103 (100[y])	3 (–[z])	5 (–)
2	18	TNC	TNC	17 (94)	3 (–)	3 (–)
3	97	TNC	TNC	57 (59)	0 (0)	0 (0)
4	86	139	135	86 (100)	49 (35)	52 (19)
5	130	404	267	107 (82)	71 (18)	52 (19)
6	26	52	43	26 (100)	18 (35)	18 (42)
7	60	152	175	60 (100)	47 (31)	52 (30)
8	58	155	152	58 (100)	52 (34)	51 (34)
9	73	295	229	73 (100)	29 (10)	30 (13)
10	433	460	406	432 (100)	233 (51)	234 (58)

[u] Assayed by plating washings from 50,000 g seed samples (3).
[v] Two replications.
[w] Total number of bacteria including both saprophytes and *X. c. campestris*.
[x] TNC = two numerous to count.
[y] Numbers in parentheses represent the percent of *X. c. campestris* from total number of bacteria.
[z] – = not calculated because number of total bacteria were too numerous to count.

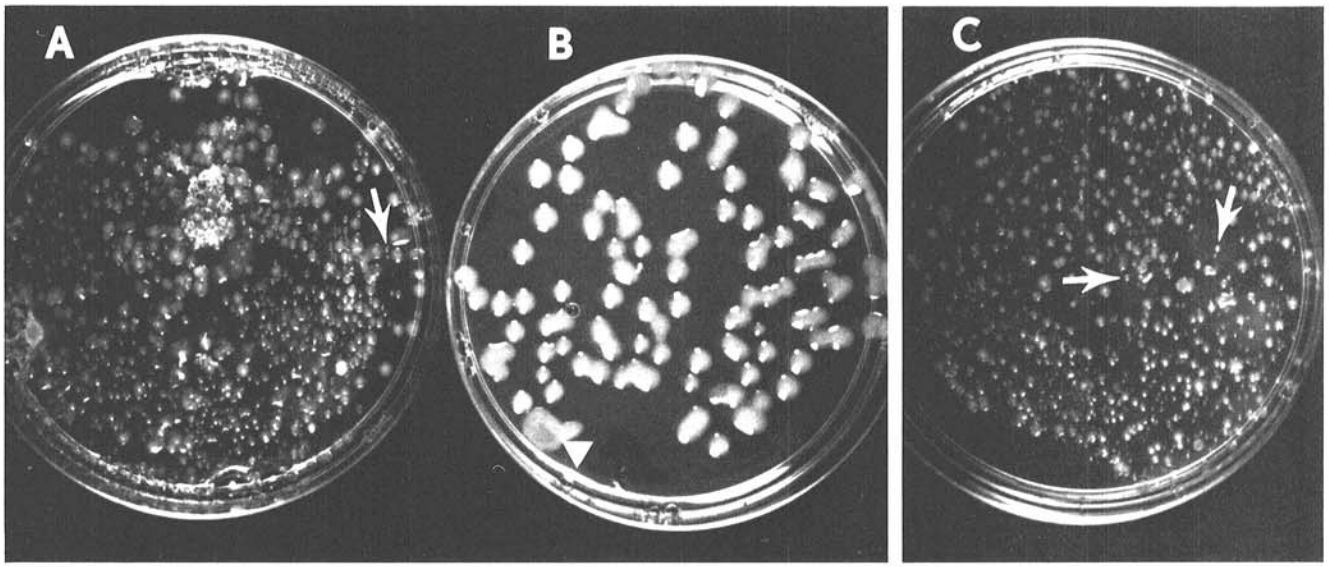

Fig. 2. Colonies (arrows) of *Xanthomonas campestris* pv. *campestris* recovered from washing of crucifer seed on NSCA (**A**), CS20ABN (**B**), and NSCAA (**C**) media. Plates were incubated for 3 days. All colonies on CS20ABN were *Xanthomonas campestris* pv. *campestris* except one (triangle).

TABLE 3. Effects of wash temperatures and times on the isolation of *Xanthomonas campestris* pv. *campestris* from commercial crucifer seed lot on CS20ABN and FS media

Wash temperature (C)	Wash time (min)	Mean colony-forming units ($\times 10^5$) per milliliter[u]							
		Total bacteria[v]				*Xanthomonas campestris* pv. *campestris*			
		Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
CS20ABN									
25	3	3.5	0.9	0.72	3.0	3.0 (86)[w]	0.8 (89)	0.57 (79)	2.0 (67)
25	60	10.2	0.9	0.92	13.0	9.8 (96)	0.8 (87)	0.92 (100)	12.0 (92)
25	120	16.9	3.3	0.34	22.0	16.6 (98)	3.1 (94)	0.32 (94)	20.0 (91)
25	180	14.0	2.3	ND[x]	ND	13.5 (96)	2.2 (96)	ND	ND
25	240	16.5	2.3	0.77	41.0	15.4 (93)	2.1 (91)	0.77 (100)	38.0 (93)
5	3	1.8	0.4	0.38	3.0	1.8 (100)	0.3 (75)	0.36 (95)	1.0 (33)
5	60	10.5	2.3	1.07	17.0	10.4 (99)	2.3 (100)	0.76 (71)	14.0 (82)
5	120	18.7	3.2	0.6	29.0	18.5 (99)	2.9 (91)	0.57 (95)	25.0 (86)
5	180	10.0	1.9	ND	ND	8.8 (88)	1.8 (95)	ND	ND
5	240	27.1	2.4	1.61	42.0	26.7 (99)	2.1 (88)	1.61 (100)	38.0 (90)
FS									
25	3	7.8	1.9	1.37	10.0	2.6 (33)	1.2 (63)	0.32 (23)	2.0 (20)
25	60	17.7	1.0	3.07	74.0	8.5 (48)	0.9 (90)	0.35 (11)	7.0 (9)
25	120	30.2	5.3	1.67	82.0	14.8 (49)	4.5 (85)	0.15 (9)	15.0 (18)
25	180	40.5	3.4	ND	ND	11.8 (29)	2.5 (74)	ND	ND
25	240	39.7	2.4	5.84	215.0	16.7 (42)	1.2 (50)	0.59 (10)	47.0 (22)
5	3	6.5	0.5	0.51	17.0	1.5 (23)	0.5 (100)	0.13 (25)	1.0 (6)
5	60	30.4	2.7	4.29	113.0	6.6 (22)	2.1 (78)	0.27 (6)	10.0 (9)
5	120	39.6	2.4	TNC[y]	190.0	16.7 (42)	2.0 (83)	0.32 (−)[z]	19.0 (10)
5	180	36.0	5.2	ND	ND	13.3 (37)	2.0 (38)	ND	ND
5	240	TNC	6.1	5.72	TNC	22.6 (−)	1.8 (30)	1.09 (19)	29.0 (−)

[u] Mean of three replications.
[v] Total number of bacteria including *X. c. campestris* and saprophytes.
[w] Numbers in parentheses represent the percent of *X. c. campestris* of total bacteria.
[x] ND = not determined.
[y] TNC = too numerous to count.
[z] − = not calculated because number of saprophytic bacteria were too numerous to count.

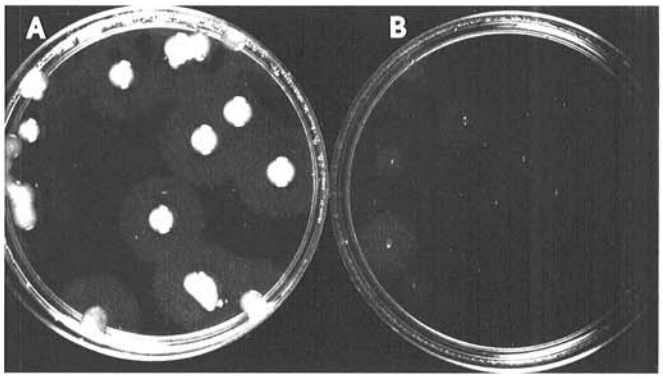

Fig. 3. Colonies (surrounded by starch rings) of *Xanthomonas campestris* pv. *campestris* recovered from washing of crucifer seeds on CS20ABN (**A**) and FS (**B**) media. Plates were incubated for 3 days.

TABLE 4. Comparison of NSCA, NSCAA, and CS20ABN media for recovery of *Xanthomonas campestris* pv. *campestris* from crucifer seedlots

Media	Number of positive seedlots[x,y]	Percent pathogenic isolates
NSCA	68	81 (68)[z]
NSCAA	55	93 (55)
CS20ABN	121	75 (121)

[x] A total of 129 seedlots was tested.
[y] A positive test was based on formation on a starch ring around colony on test media, and formation of a mucoid, yellow colony on YDC medium.
[z] Number in parentheses represents number of isolates tested.

ml) used in CS20ABN was the same as the maximum uninhibitory concentration determined by Randhawa and Schaad (3). They, however, did not use bacitracin in NSCA, NSCAA, and BSCAA (3), because it offered no advantage over nitrofurantoin and vancomycin. A combination of bacitracin and neomycin in CS20A reduced the background saprophytes to less than 20% and, in many cases, to nondetectable levels (Table 2).

It is essential that a medium has a high selectivity for the target bacterium. It is not uncommon that several selected ingredients, usually various antibiotics or amino acids, are incorporated in a basal medium in order to achieve this goal. For example, cycloheximide, nitrofurantoin, and vancomycin are included in NSCAA (3,5) and BSCAA (3,5) media, whereas cycloheximide, D-methionine, pyridoxine HCl, gentamicin, cephalexin, and trimethoprim are included in FS medium (5,6). None of the above ingredients are autoclavable. Individual stock solutions are prepared in distilled water or in a specific organic solvent and sterilized by millipore filtration. All ingredients for CS20ABN medium are autoclavable except cycloheximide, bacitracin, and neomycin sulfate. A stock solution of cycloheximide, a common ingredient in all the mentioned semiselective media for *X. c. campestris*, is prepared by dissolving it in 75% methanol or ethanol. Stock solution of bacitracin and neomycin are easy to prepare because they are both soluble in water. Nitrofurantoin for NSCAA and BSCAA media is dissolved in 50% dimethylformamide, and trimethoprim for FS medium is dissolved in methanol. Both bacitracin and neomycin are water-soluble, which makes CS20ABN medium easier to prepare than the other semiselective media.

A complete seed assay includes the isolation of the target bacterium and a confirmation with a pathogenicity test. The sooner the target bacterium can be isolated, the sooner a pathogenicity test can be performed. With CS20ABN medium, colonies of *X. c. campestris* were 1–2 mm in diameter, surrounded by a zone of starch hydrolysis 2 days after isolation, as compared to 3–5 days on NSCA, NSCAA, and FS media. In general, a zone of starch hydrolysis began to appear in CS20ABN medium 36 hr after isolation. This allowed us to perform pathogenicity tests and hence conclude the entire test and issue certification 2–3 days earlier than with FS medium. Another advantage of CS20ABN medium is that the size of the *X. c. campestris* colonies

was three to seven times larger than that on FS medium, which made the pure culture transfer from assayed plates to YDC much easier.

Conditions for seed washing, such as temperature and length of time, are critical for a satisfactory seed assay. Washing seeds at room temperature (i.e., 25 C) is convenient and provides better penetration of the washing buffer than washing at 5 C. Washing seeds at room temperature also washes out more contaminating saprophytes that overgrow the target bacterium, especially when the number of the target bacterium is very low. Washing seeds at a low temperature would probably avoid proliferation of saprophytes, but it may also decrease the penetration of washing buffer. It is reasonable to assume that the longer the seed washing time, the higher the number of both target and saprophytic bacteria recovered. However, washing seeds for a long period of time is impractical for any seed laboratory that assays 600–700 commercial seed lots annually. This study indicates that washing seeds at room temperature for a period of 2 hr is satisfactory. This procedure differs from that reported by Schaad (5), in which the seeds were washed at 3–5 C for 1.5 hr. Schaad (5) recommended that a more selective medium (such as FS) be used in place of NSCA to compensate for the longer washing time. CS20ABN medium is an ideal option over FS medium for a successful seed assay program.

In Georgia, the State Seed Laboratory included CS20ABN medium in its crucifer seed assay program in August 1988. From 1 August 1988 to 30 April 1990, 129 seed lots were recorded as *X. c. campestris*-positive. Of these seed lots, 68 were recorded as positive on NSCA medium, 55 on NSCAA medium, and 121 on CS20ABN medium (Table 4). CS20ABN medium was two times more selective in detecting *X. c. campestris* from crucifer seeds. There were eight cases where CS20ABN did not detect the bacterium when NSCA and NSCAA did; however, there were 52 seed lots where CS20ABN detected the bacterium and NSCA and NSCAA did not. Of these 52 cases, pathogenicity on cabbage was demonstrated with 37 strains (71%). With the inclusion of CS20ABN medium in the seed assay program, the crucifer seed certification program was strengthened.

LITERATURE CITED

1. Chang, C. J., and Yonce, C. E. 1987. Overwintering of plum leaf scald bacteria in infected trees. Ann. Phytopathol. Soc. Japan 53:345-353.
2. Chang, C. J., and Walker, J. T. 1988. Bacterial leaf scorch of northern red oak: Isolation, cultivation, and pathogenicity of a xylem-limited bacterium. Plant Dis. 72:730-733.
3. Randhawa, P. S., and Schaad, N. W. 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. Phytopathology 74:268-272.
4. Schaad, N. W. 1982. Detection of seedborne bacterial plant pathogens. Plant Dis. 66:885-890.
5. Schaad, N. W. 1989. Detection of *Xanthomonas campestris* pv. *campestris* in crucifers. Pages 68-75 in: Detection of Bacteria in Seed and Other Planting Material. A. W. Saettler, N. W. Schaad, and D. A. Roth, eds. The American Phytopathological Society, St. Paul, MN.
6. Yuen, G. Y., Alvarez, A. M., Benedict, A. A., and Trotter, K. J. 1987. Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris*. Phytopathology 77:366-370.