# Selective Isolation of *Xanthomonas campestris* pv. *campestris* from Crucifer Seeds

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## ABSTRACT

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Saprophytic and antagonistic bacteria associated with crucifer seeds often interfere with the isolation of *Xanthomonas campestris* pv. *campestris* (hereafter abbreviated to *X. campestris*) on general plating media such as nutrient starch cycloheximide agar (NSCA). The number of colonies of *X. campestris* recovered from various crucifer seed lots often depends upon the number of saprophytic and antagonistic bacteria present on NSCA plates. Up to 50% fewer colonies of *X. campestris* were recovered when antagonistic bacteria were present in the seed washings. Adding nitrofurantoin and vancomycin, respectively, at 10 and 0.5 $\mu$g/ml to NSCA (=NSCAA) and 2 and 0.1 $\mu$g/ml to basal starch cycloheximide agar (=BSCAA) significantly reduced the development of saprophytic and antagonistic bacteria recovered from washings of crucifer seeds. On NSCAA, 20 strains of *X. campestris* had plating efficiencies of 0.87 or more compared to 1.00 for NSCA. On BSCAA, 15 strains had plating efficiencies of 0.85 or greater, whereas those of five additional strains ranged from 0.54 to 0.72. On NSCAA or BSCAA, *X. campestris* was recovered from undiluted or 1:10 dilutions of seed washings, whereas on NSCA, dilutions of 1:100 were needed to reduce saprophytic and/or antagonistic bacteria. Twelve of 102 commercial seed lots assayed during a 2 mo period on NSCA, NSCAA, and BSCAA yielded *X. campestris*. In six of these seed lots *X. campestris* was not detected by using NSCA alone. *X. campestris* was detected in four lots on all three media, in four lots on BSCAA alone, and in one lot on NSCAA. Because of a zero tolerance for black rot and the natural variation in the bacterial flora of crucifer seeds, all three media should be used for assaying seeds for *X. campestris*.

*Additional key words:* antagonism, *Brassica* spp., detection of seedborne bacteria, selective media.

*Xanthomonas campestris* pv. *campestris* (hereafter abbreviated to *X. campestris*), the causal organism of black rot of crucifers, survives primarily as a seedborne pathogen (1,3,15,16). A very low level of seed infestation (three in 10,000) can give rise to high disease incidence in the field (9). Various methods are available for detecting this pathogen in seeds, such as observing symptoms of black rot on seedlings developed on water agar plates (13) or in soil (12), plating disinfested seeds (8) onto a selective medium (10), or plating washings of seeds onto a nonselective differential medium (6).

A starch-based semiselective medium for xanthomonads (SX) agar (10) has proven useful for isolation of *X. campestris* from soils (11), but it is unsatisfactory for isolating the pathogen from seed surfaces. A differential nutrient starch cycloheximide agar (NSCA) appears to be the most useful medium

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available for detecting *X. campestris* in seed washings (6). However, results of assaying over 800 commercial seed lots during the last 2 yr have shown certain lots to be heavily infested with saprophytic bacteria. As a result, washings of some seed lots must be diluted to $10^{-2}$ or more to isolate colonies of *X. campestris* on NSCA. In addition, many seed lots contain high populations of bacteria which are antagonistic to *X. campestris* on NSCA (7). Thus, with certain seed lots, NSCA may be an ineffective assay medium.

This work was initiated to develop a medium specifically for recovering *X. campestris* from seeds. Our aim was to develop a medium that would reduce total numbers of saprophytic bacteria and eliminate bacteria antagonistic to *X. campestris* without significantly suppressing different strains of *X. campestris.*

## MATERIALS AND METHODS

**Bacterial strains.** *X. campestris* strain B-24 was the primary strain used for developing the semiselective media (Table 1). Bacteria antagonistic to *X. campestris* were isolated by washing 4 gm of seeds (~1,000 seeds) from each of several different commercial crucifer (collard, cabbage, cauliflower, and broccoli) seed lots in 10 ml of 0.85% NaCl (saline) for 1–3 min at room temperature. The washings were diluted to $10^{-2}$, and 0.1 ml was pipetted onto triplicate plates of NSCA containing 23 g of nutrient agar per liter (instead of the 10 g incorrectly reported in the original reference [6]) and spread with an ethanol flamed L-shaped glass rod. After 48 hr at 30 C, the resulting colonies were oversprayed, by using a previously sterilized chromatographic sprayer, with a suspension of B-24 containing $10^8$ colony-forming units (CFU) per milliliter and incubated for 36 hr at 30 C. Colonies that prevented growth of B-24 were considered antagonistic and were isolated by repeated streaking on NSCA.

**Effect of antagonists on recovery of *X. campestris.*** In a preliminary test, 0.1 ml of a suspension containing ~10 or 300 CFU of B-24 was spread onto triplicate plates of NSCA as described above. After 10 min, a sterile toothpick dipped once into a suspension of antagonist (~$10^8$ CFU/ml) was gently touched to the agar surface at 16 equidistant sites. Control plates were inoculated either with B-24 only or with a nonantagonistic bacterium. For the low level of B-24 (10 CFU), the total number of colonies per plate was recorded; for the high level (300 CFU) the diameter of the zone of inhibition was recorded.

To simulate the very low numbers of *X. campestris* and of an antagonist that might be expected in a natural situation, 50 μl each of suspensions of B-24 containing ~1 CFU and of antagonist strain RC-3 containing ~1 or 10 CFU were pipetted onto NSCA plates and spread as described above. Similarly, to determine the effect of antagonists on the recovery of *X. campestris* from naturally infected seeds, 50 μl of a suspension of antagonist containing ~10 CFU (determined by plating separately) was plated onto NSCA together with 50 μl of washings from the infected seed lot. As a control, sterile saline was substituted for the antagonist suspension. Each treatment was replicated eight times.

**Development of semiselective agar media.** Several recommended growth factors (14) and nitrogen sources were tested in a modified basal starch cycloheximide agar medium (10) containing the following per liter: 10 g soluble potato starch (J. T. Baker Chemical Co., Phillipsburg, NJ 08865), 3.0 g $K_2HPO_4$, 1.0 g $KH_2PO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.0 mg methyl green (0.2 ml of 1% aqueous solution), 200 mg cycloheximide (8 ml of 2.5% solution in 12.5% methanol), and 15 g Bacto agar. Recovery of 14 strains (B-1, B-2, B-4, B-6, B-24, B-30, B-36, B-53, B-67, B-70, B-75, B-85, B-90, and B-99) of *X. campestris* was compared. Plates of basal medium containing the different test substances and plates of NSCA were seeded with 0.1 ml of a log-phase cell suspension of *X. campestris* prepared from liquid medium 523 (2) as described (10). Dilutions that resulted in 20–200 CFU per plate of NSCA were compared.

Sixteen chemicals (antibiotics and nalidixic acid) were initially screened by using impregnated paper disks (17) to determine their toxicity to *X. campestris* strain B-24. Difco paper sensitivity disks (0.1-ml capacity) soaked in test chemicals were placed on NSCA and after 1 hr were oversprayed with a suspension of strain B-24 (~$10^8$ CFU/ml). After 36 hr at 30 C, the maximum concentration of test chemical that did not inhibit strain B-24 was determined. This concentration was tested against bacterial flora of crucifer seeds. Bacterial flora from six different seed lots were washed from 4 gm of seeds in 10 ml of saline. Chemically impregnated paper disks were placed onto NSCA and sprayed with the seed washings. Inhibition of total or component bacterial flora around the disks was recorded after incubation. The chemicals were also tested against *X. campestris* strain B-24 by broth dilution (17). The concentrations of test chemicals or their combinations were prepared in 50 ml of sterile liquid medium 523 in 250-ml sidearm flasks and tested in duplicate. Each flask was seeded with a 0.1 ml of a log-phase suspension (containing ~$10^6$ CFU) of B-24. After incubation for 24 hr at 30 C on a New Brunswick controlled environment incubator shaker at 100 rpm, turbidity was determined with a Klett-Summerson colorimeter (green filter) (10).

Selected antiobiotics, singly and in combination, were finally tested in NSCA and the basal starch medium to obtain maximum inhibition of seed-associated saprophytic bacteria without reducing the recovery of strain B-24.

**Evaluation of semiselective media.** Test media were compared for recovering 20 strains of *X. campestris*, inhibiting the growth of normal saprophytic bacterial flora of seven crucifer seed lots, detecting *X. campestris* in four naturally infected seed lots, and detecting *X. campestris* in 102 previously untested commercial seed lots. Inoculum of the 20 strains of *X. campestris* (Table 1) was prepared and tested as described above. Seed washings from samples of ~10,000 seeds were sampled as described (6). Results were compared with those on NSCA, a medium for maximum recovery of *X. campestris* (6). Colonies that were recovered from seeds and resembled *X. campestris* were cloned and identified by streaking onto yeast extract-dextrose-$CaCO_3$ agar (5) and by immunofluorescence microscopy (4). Representative colonies were tested for pathogenicity on cabbage seedlings as described (11).

## RESULTS

**Effect of antagonists on recovery of *X. campestris.*** Of 12 antagonistic bacterial strains isolated from crucifer seeds, seven strains significantly reduced the number of colonies of *X. campestris* when the latter was present in low numbers (Table 2, Fig. 1). When colonies of *X. campestris* were numerous (~300 per

TABLE 1. Strains of *Xanthomonas campestris* pv. *campestris* investigated in selective isolation tests

| Strain | Host | Source[a] | Origin |
|---|---|---|---|
| B-1 | *Brassica oleracea* var. *gemmifera* (brussels sprout) | 1 | California |
| B-2 | *B. oleracea* var. *gongilodes* (kohlrabi) | 2 | Georgia |
| B-4 | *B. oleracea* var. *italica* (broccoli) | 2 | Georgia |
| B-6 | *B. oleracea* var. *acephala* (collard) | 2 | Georgia |
| B-24 | *B. oleracea* var. *italica* | 3 | Oregon |
| B-30 | *B. rapa* (rape) | 4 | Germany |
| B-36 | *B. oleracea* var. *botrytis* (cauliflower seed) | 2 | Japan |
| B-53 | *B. oleracea* var. *botrytis* (seed) | 2 | Japan |
| B-65 | *B. oleracea* var. *capitata* (cabbage) | 2 | Brazil |
| B-67 | *B. oleracea* var. *botrytis* | 2 | Georgia |
| B-70 | *B. oleracea* var. *capitata* (seed) | 2 | Japan |
| B-75 | *B. oleracea* var. *capitata* (seed) | 2 | Japan |
| B-85 | *Lepidium virginicum* (weed) | 2 | Georgia |
| B-89 | *Raphanus sativus* (weed) | 2 | California |
| B-90 | *B. campestris* (weed) | 2 | California |
| B-98 | *Cardaria pubescens* (weed seed) | 2 | California |
| B-99 | *C. pubescens* (seed) | 2 | California |
| B-115 | *B. oleracea* var. *capitata* (seed) | 2 | California |
| B-116 | *B. oleracea* var. *italica* (seed) | 2 | California |
| B-447 | *B. oleracea* var. *capitata* | 5 | Netherlands |

[a]Sources: 1 = R. G. Grogan, University of California, Davis (received as strain BBS); 2 = N. W. Schaad, University of Idaho, Moscow; 3 = L. W. Moore, Oregon State University, Corvallis; 4 = K. Rudolph, Germany (received as strain K-2); 5 = J. Van Vruggink, The Netherlands.

plate), antagonist strains RC-3, -8, -10, and -12 produced inhibition zones 5–8 mm in diameter, whereas the remaining strains resulted in zones ≤2 mm in diameter. When numbers of colonies of *X. campestris* per plate of NSCA were low (≤1–2 per plate), the presence of two colonies of RC-3 did not decrease detection of *X. campestris*, whereas the presence of 14 colonies of RC-3 did reduce the number of plates containing colonies of that bacterium (Table 3). Furthermore, the addition of 14 colonies of RC-3 per plate resulted in the failure to recover *X. campestris* from washings of a naturally infected crucifer seed lot, while three colonies of the pathogen were detected among the eight control plates without the antagonist.

**Development of semiselective agar media.** The BSCA medium containing $NH_4Cl$ as a nitrogen source failed to support the growth of two strains (B-1 and B-30) of *X. campestris*. The addition or omission of different growth factors (14) such as methionine and glutamic acid at 1 μg/ml, nicotinic acid at 1 μg/ml, or inorganic salts such as $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, $H_3BO_3$, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$ each at 1 μg/ml and KI, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$ each at 0.01 μg/ml also failed to improve their growth. Only with the replacement of $NH_4Cl$ with glycine did all strains grow. The final composition of our basal medium designated as BSCA contained the following (per liter): 10 g soluble potato starch, 0.2 g glycine, 1 g $K_2HPO_4$, 1 g $KH_2PO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 2 mg methyl green, (0.2 ml of 1% aqueous solution), 200 mg cycloheximide, and 15 g agar.

Many of the 16 chemicals tested were inhibitory to *X. campestris* at 0.1 μg/ml or less in broth dilution test (Table 4). Only vancomycin, bacitracin, and nitrofurantoin at 1.0, 10.0, and 25.0 μg/ml, respectively, suppressed the growth of seed-associated antagonistic and saprophytic bacteria without inhibiting *X. campestris*. Since bacitracin offered no advantage over the other two antibiotics, it was eliminated from further testing.

The optimum concentrations of vancomycin and nitrofurantoin for selectively recovering *X. campestris* depended upon the composition of media used. In the end, two semiselective media were developed: nutrient starch cycloheximide antibiotic agar (NSCAA) and basal starch cycloheximide antibiotic agar (BSCAA). NSCAA was made by adding (per liter) 10 mg nitrofurantoin (2 ml of 5 mg/ml solution in 50% dimethylformamide) and 0.5 mg vancomycin (2 ml of 0.25 mg/ml Millipore-filtered [0.2 μm maximum pore size] aqueous solution) to NSCA. BSCAA was made by adding (per liter) 2 mg nitrofurantoin and 0.1 mg vancomycin to BSCA. Antibiotics were added after the media were autoclaved and cooled to 50 C. The media were poured into plastic petri plates. The pH of both media was unadjusted. Plates of both semiselective media could be stored at 20 C in plastic bags for at least 2 mo without losing their effectiveness.

**Evaluation of semiselective media.** Bacteria in washings from six of seven commercial seed lots were significantly reduced on NSCAA. On BSCAA the reduction was more prominent and varied from 83–100% relative to that on NSCA (Table 5). Plating efficiencies (colonies per plate on test medium ÷ colonies per plate on NSCA) of the 20 strains of *X. campestris* on NSCAA ranged from 0.87 to 1.16 and were not significantly different ($P = 0.05$, according to Duncan's multiple range test) relative to a plating efficiency of 1.00 for every strain on NSCA. Colonies of *X. campestris* became visible both on NSCA and NSCAA after 2–3 days at 30 C as 1- to 2-mm-diameter yellow, shiny, mucoid colonies surrounded by a 2- to 4-mm-wide zone of starch hydrolysis. On BSCAA, plating efficiencies of 18 strains ranged from 0.63 to 1.13 and were not significantly different. However, plating efficiencies of two strains, B-2 and B-4, were significantly lower (0.54 and 0.64, respectively) on BSCAA. *X. campestris* became visible on BSCAA in 3–5 days, depending upon the strain, appearing as 1- to 2-mm-

TABLE 2. Effects of antagonist bacteria isolated from crucifer seeds on recovery of *Xanthomonas campestris* pv. *campestris* on nutrient starch cycloheximide agar (NSCA)[a]

| Antagonist strain | Colonies of *X. campestris* (no. per plate)[b] |
|---|---|
| None, check | 8.5 w |
| RC-9 | 8.8 w |
| RC-16 | 7.3 wx |
| RC-13 | 6.5 wx |
| RC-17 | 6.5 wx |
| RC-15 | 6.0 wx |
| RC-11 | 5.0 xy |
| RC-7 | 4.8 xy |
| RC-4 | 2.3 yz |
| RC-12 | 1.3 z |
| RC-10 | 1.0 z |
| RC-3 | 0.0 z |
| RC-8 | 0.0 z |

[a]Ten minutes after spreading 0.1 ml of a solution containing 6–11 colony forming units of *X. campestris* onto plates of NSCA, antagonistic bacteria were spot-inoculated at 16 equidistant locations on each plate.
[b]Average of four replications. Numbers followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Effect of antagonistic bacterial strain RC-3 on the recovery of relatively low numbers of *Xanthomonas campestris* pv. *campestris* strain B-24 on nutrient starch cycloheximide agar (NSCA)[a]

| Mean colonies of RC-3 per plate | Mean colonies of B-24 recovered per plate | Plates containing B-24 (no.) |
|---|---|---|
| 0, control | 1.5 | 6 |
| 2.1 | 1.6 | 6 |
| 13.6 | 0.4 | 3 |

[a]Fifty microliters each of suspensions of B-24 and antagonist strain RC-3 from suitable dilutions in sterile saline was pipetted onto each of eight replicate plates of NSCA and spread with an ethanol-flamed glass rod.

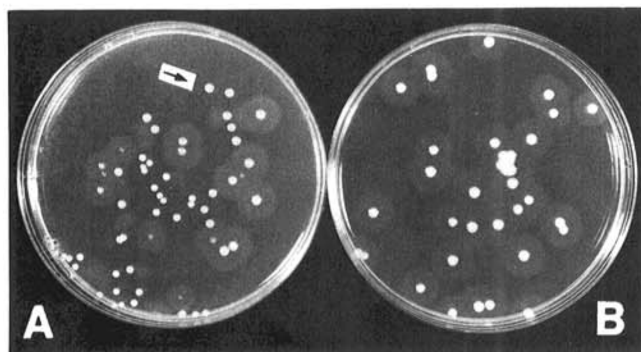


**Fig. 1.** Effect of antagonist strain RC-3 (arrow) on the recovery of *Xanthomonas campestris* pv. *campestris* strain B-24 (colonies surrounded by zone of starch hydrolysis) on nutrient starch cycloheximide agar: **A,** strain RC-3 and B-24 plated together, note the inhibition of B-24 near colonies of RC-3; **B,** B-24 plated alone (control).

TABLE 4. Inhibition of *Xanthomonas campestris* pv. *campestris* strain B-24 by antibiotics and nalidixic acid

| Antibiotic[a] | Maximum uninhibitory concentration (μg/ml): Paper disk | Broth dilution |
|---|---|---|
| Ampicillin, chloramphenicol, erythromycin, gentamycin, kanamycin, penicillin, polymyxin B, streptomycin, and tetracycline | 1.0 | <0.1 |
| Carbenicillin, cephalothin, nalidixic acid, and vancomycin | 10.0 | 1.0 |
| Bacitracin | 100.0 | 10.0 |
| Tyrothricin | 100.0 | 5.0 |
| Nitrofurantoin | 250.0 | 25.0 |

[a]Nalidixic acid, tyrothricin and nitrofurantoin were dissolved in 0.1 M KOH (10 mg/ml), ethanol (28 mg/ml) and 50% *N-N* dimethyl formamide, respectively. All others were dissolved in distilled water. All except nitrofurantoin were sterilized by Millipore filtration (0.2 μm).

diameter transparent colonies surrounded by a 2- to 4-mm-wide zone of starch hydrolysis. After 4–6 days, the colonies were 3–5 mm in diameter, translucent, mucoid, entire, colorless to slightly green, and surrounded by starch digestion zones 6- to 10-mm wide.

**Comparative recovery of *X. campestris* from naturally infested seeds.** The number of colonies of *X. campestris* recovered from naturally infected seed lots depended upon the number of saprophytic bacteria present (Table 6, Figs. 2 and 3). In one seed lot, saprophytic bacteria were so numerous that *X. campestris* was recovered only on the semiselective media. In many instances, plating of diluted seed washings did not reveal large differences between the efficacies of different media and NSCA was as good as semiselective media.

**Assay of commercial seed lots.** Twelve of 102 seed lots assayed during a 2-mo period were found to contain *X. campestris*. Four seed lots were positive on all three media, four on BSCAA alone, one on NSCA alone, one on NSCAA alone, one on NSCA and NSCAA, and one on NSCAA and BSCAA.

## DISCUSSION

Although agar media have been used for some time to isolate seedborne bacteria, this is the first report that antagonistic bacteria infesting seeds can reduce the recovery of a seedborne pathogen. Results of Schaad and Donaldson (7) show that from 0.6 to 5.0% of crucifer seeds yield antagonists. This may explain why plating seeds on NSCA and/or SX agar sometimes fails to detect *X. campestris*. As few as 14 colonies of antagonistic bacteria per plate can reduce the recovery of *X. campestris* present in relatively small numbers in seed washings. Observations during the past 2 yr of assaying >800 commercial crucifer seed lots on NSCA show that colonies of *X. campestris* are almost always found at dilutions of $10^{-2}$ and the number of colonies per plate are usually less than five (N. W. Schaad, *unpublished*). Furthermore, only one of four plates is normally positive. This need to dilute to $10^{-2}$ suggests that *X. campestris* is being inhibited or masked by other bacteria.

The most common antagonists found on crucifer seeds are spore-forming *Bacillus* spp. that can survive for long periods on the surface of crucifer seeds (7). Such antagonists often spread along the agar surface, especially when it is not completely dry, and thus extend their inhibitory effect. Both semiselective media described herein inhibit all the antagonist bacteria we have isolated from crucifer seeds. Furthermore, these media significantly reduce the normal nonpathogenic bacterial flora found in washings from crucifer seeds. This reduction permits improved distinction of *X. campestris*. Bacteria from undiluted seed washings of some seed lots develop as very tiny colonies on BSCAA; however, such colonies do not interfere with the growth and counting of colonies of *X. campestris*.

All of our efforts to enrich the pathogen in seed washings containing antibiotics for various lengths of time resulted in failure to recover the pathogen. The contaminants always overgrew the pathogen.

Few other starch-hydrolyzing bacteria from washings of crucifer seeds grew on the semiselective media; however, colonies similar to *X. campestris* were observed in one seed lot tested. The bacterium produced yellow colonies on YDC similar to *X. campestris*, but was not pathogenic on cabbage. All colonies positive on the semiselective media should, therefore, be purified by streaking onto YDC and be checked for pathogenicity. Because infected commercial seed lots were not always detected on NSCA or either of the two semiselective media alone, all three media should be used for maximum recovery of *X. campestris* from crucifer seeds.

TABLE 5. Bacterial colonies from seven commercial crucifer seed lots recovered on nutrient starch cycloheximide agar (NSCA), nutrient starch cycloheximide antibiotic agar (NSCAA), and basal starch cycloheximide antibiotic agar (BSCAA)[a]

| | Mean colonies per plate[b] from seed lot: | | | | | | |
|---|---|---|---|---|---|---|---|
| Media | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| NSCA | 57 x | 192 x | 208 x | 123 x | 145 x | 119 x | 81 x |
| NSCAA | 32 y | 90 y | 23 y | 0 y | 119 x | 15 y | 2 y |
| BSCAA | 10 z | 28 z | 1 z | 0 y | 5 y | 4 z | 1 y |

[a] Washings of ~10,000 seeds from each lot were collected in 2 ml saline, serially diluted and assayed as described (5). The dilution that yielded 50 to 200 colonies per plate of NSCA was used for comparison.
[b] Average of four replications. Numbers within columns followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 6. Recovery of *Xanthomonas campestris* pv. *campestris* from known naturally infected seed lots on nutrient starch cycloheximide agar (NSCA), nutrient starch cycloheximide antibiotic agar (NSCAA) and basal starch cycloheximide antibiotic agar (BSCAA)[a]

| | | Mean colonies per plate[b] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Seed bacteria | | | *X. campestris* | | |
| Dilution | Seed lot | NSCA | NSCAA | BSCAA | NSCA | NSCAA | BSCAA |
| $10^{0}$ | 1 | TNC | 63.8 | 50.6 | 12.8 | 119.8 | 73.8 |
| | 2 | TNC | 154.0 | 51.0 | 0.0 | 24.8 | 23.6 |
| | 3 | TNC | 24.8 | 1.3 | 125.3 | 287.0 | TNC |
| | 4 | TNC | TNC | 14.8 | 1.0 | 7.5 | 34.0 |
| $10^{-2}$ | 1 | 11.0 | 0.3 | 0.3 | 2.5 | 2.5 | 2.3 |
| | 2 | 12.0 | 1.8 | 1.5 | 0.3 | 0.3 | 1.0 |
| | 3 | 3.8 | 0.0 | 0.0 | 2.8 | 4.5 | 4.8 |
| | 4 | 47.0 | 26.3 | 0.5 | 0.8 | 2.3 | 2.3 |

[a] Assayed by agar plating of washings from ~10,000 seeds (5).
[b] Four replications. TNC = too numerous to count.

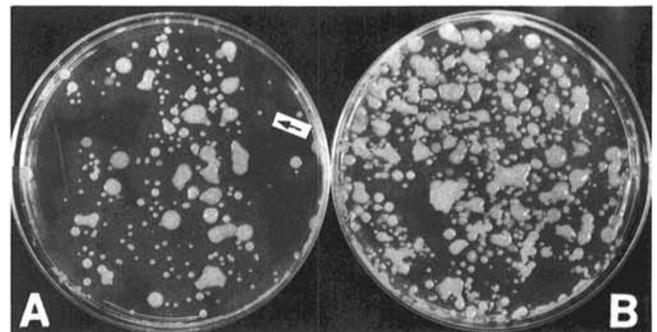


**Fig. 2.** Comparison between: **A,** Nutrient starch cycloheximide antibiotic agar (NSCAA) and **B,** nutrient starch cycloheximide agar (NSCA) in recovery of *Xanthomonas campestris* pv. *campestris* from a naturally infected seed lot. Washings of ~10,000 seeds were concentrated by centrifugation, suspended in 2.0 ml of saline diluted 1:10 and plated at 0.1 ml per plate. Arrow points to a typical colony of *X. campestris* pv. *campestris* surrounded by a zone of starch hydrolysis.

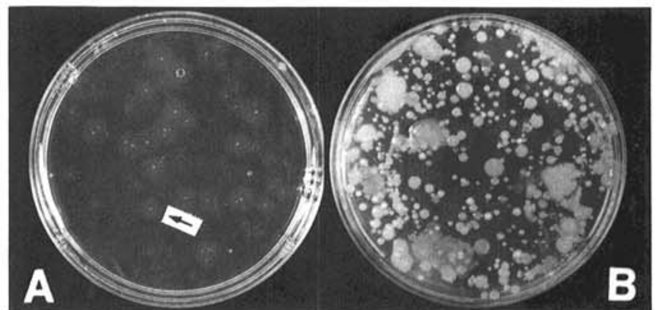


**Fig. 3.** Comparison between: **A,** Basal starch cycloheximide antibiotic agar (BSCAA) and **B,** nutrient starch cycloheximide agar (NSCA) in recovery of *Xanthomonas campestris* pv. *campestris* from a naturally infected seed lot. Washings of ~10,000 seeds were concentrated by centrifugation, suspended in 2.0 ml of saline and plated at 0.1 ml per plate. Arrow points to a typical colony of *X. campestris* pv. *campestris* surrounded by a zone of starch hydrolysis.

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