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# IMPROVED SELECTIVE MEDIA FOR ISOLATION OF *TRICHODERMA* SPP. OR *FUSARIUM* SPP.

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Modifications were made to improve the *Trichoderma* selective medium (TSM). TSM supplemented with benomyl was efficient for isolating *Fusarium* spp. from soil; and TSM supplemented with captan was a specific selective medium for *Trichoderma* spp., even in the presence of *Fusarium* in the soil.

*KEY WORDS:* Biocontrol; soilborne plant pathogens; *Trichoderma harzianum; Trichoderma hamatum.*

The effectiveness of a recently developed *Trichoderma* selective medium (TSM) (2) as a tool for isolating *Trichoderma* spp. from the soil was demonstrated in various field experiments (1, 3, 4, 5, 6). However, R. Baker (Colorado State University, Fort Collins, CO, U.S.A.) and A.W. Doornik (Bulb Research Centre, Lisse, The Netherlands) reported (personal communications) that *Fusarium* spp. could also grow on this medium when used with soils containing a high *Fusarium* spp. population. The purpose of this work was to develop new selective media suitable for isolating either *Fusarium* spp. or *Trichoderma* spp., which would obviate interference between the two fungi.

TSM (2), which was the basic medium used in this work, consisted of the following components (g/liter distilled water): $MgSO_4.7H_2O$, 0.2; $K_2HPO_4$ 0.9; KCl, 0.15; $NH_4NO_3$, 1.0; glucose, 3.0; chloramphenicol (Chloromycetin, Sigma Chemical Co., U.S.A.), 0.25; fenaminosulf [sodium 4-dimethylaminobenzenesulphonate; Lesan (formerly Dexon) 60% w.p., Bayer AG, W. Germany], 0.3; quintozene (pentachloronitrobenzene; Terraclor 75% w.p., Olin Corporation, U.S.A.), 0.2; rose bengal (tetrachlorotetraiodofluorescein, BDH Chemicals Ltd., England), 0.15; agar (Difco Laboratories, U.S.A.), 20. The fungicides benomyl [methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate; Benlate 50% w.p., E.I. du Pont de Nemours and Co., Inc., U.S.A.] and captan [*N*-(trichlorome-thylthio)-cyclohex-4-ene-1,2-dicarboximide; Merpan 50% w.p., Makhteshim, Israel] were added to TSM, at 2 and 20 mg/l, respectively, after autoclaving. These media were compared with a selective quantitative agar (SQA) medium for *Fusarium* spp. (7,8,9), which contains (g/l distilled water): $KH_2PO_4$, 1; $MgSO_4.7H_2O$, 0.5; peptone (Difco Laboratories), 15; chloramphenicol (Chloromycetin), 0.25; quintozene (pentachloronitrobenzene; Terraclor 75% w.p.), 10; agar, 20; and 1 ml of 50% lactic acid (BDH Chemicals Ltd.) added after autoclaving the medium.

The ability of several *Fusarium oxysporum* (A, B, C, SF) and *Trichoderma* (*harzianum*, 203; *hamatum*, 43; ST) isolates to germinate and grow on the variations of TSM and on SQA was tested as described by Elad *et al.* (2) (Table 1). Among the test fungi there was one *Fusarium* (SF) and one *Trichoderma* (ST) isolate, both taken from soil dilutions on SQA (Table 1). *Fusarium* spp. germinability on TSM, SQA, or

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TABLE 1: GERMINATION AND GROWTH OF *FUSARIUM* AND *TRICHODERMA* SPP. ON A *TRICHODERMA*-SELECTIVE MEDIUM (TSM) ALONE OR SUPPLEMENTED WITH CAPTAN (TSMC) OR BENOMYL (TSMB), OR ON A *FUSARIUM*-SELECTIVE QUANTITATIVE AGAR (SQA) (Data are averages of three experiments)

| Medium | *Fusarium oxysporum* isolates[c] | | | | *Trichoderma* spp. | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | SF[d] | *harzianum* (203) | *hamatum* (43) | ST[d] |
| | Colony-forming units (number per plate ± S.E.)[e] | | | | | | |
| TSM | 45.5±5.8 | 24.3±4.7 | 40.6±4.5 | 28.6±4.8 | 35.2±4.4 | 45.5±5.5 | 64.4±10.2 |
| TSMC[a] | 6.1±2.3 | 7.5±2.5 | 16.4±2.7 | 12.4±5.9 | 33.1±4.7 | 43.4±5.6 | 58.3±6.4 |
| TSMB[b] | 49.2±6.5 | 25.2±3.7 | 47.4±3.8 | 30.7±6.4 | 0 | 0 | 0 |
| SQA | 45.9±6.4 | 17.4±3.6 | 42.4±5.6 | 24.5±3.5 | 21.2±3.4 | 22.4±4.4 | 24.2±5.6 |
| | Average colony diameter (mm ± S.E.) | | | | | | |
| TSM | 3.4±0.4 | 4.3±0.5 | 4.2±0.5 | 3.3±0.5 | 10.5±1.4 | 7.0±0.8 | 8.0±1.0 |
| TSMC | 1.5±0.2 | 1.1±0.2 | 1.7±0.2 | 0.8±0.2 | 3.9±0.4 | 3.5±0.7 | 5.4±0.7 |
| TSMB | 1.7±0.2 | 2.1±0.3 | 2.2±0.4 | 2.3±0.3 | 0 | 0 | 0 |
| SQA | 9.5±1.0 | 10.2±0.9 | 10.5±1.5 | 11.4±1.4 | 15.0±0.16 | 12.5±1.5 | 14.6±1.6 |
| | Average linear growth (mm/day ± S.E.) | | | | | | |
| TSM | 1.98±0.32 | 1.85±0.25 | 2.10±0.25 | 1.48±0.42 | 3.50±1.20 | 4.11±2.05 | 6.12±1.44 |
| TSMC | 0.58±0.12 | 1.06±0.22 | 0.99±0.05 | 1.04±0.06 | 4.63±0.87 | 3.93±0.42 | 4.48±0.32 |
| TSMB | 1.79±0.45 | 1.59±0.15 | 1.57±0.16 | 1.54±0.32 | 1.14±0.32 | 0.99±0.35 | 0.14±0.05 |
| SQA | 2.79±0.23 | 2.69±0.48 | 3.88±0.55 | 3.58±0.28 | 14.04±1.08 | 15.91±1.20 | 10.67±1.55 |

[a]TSM supplemented with 20 μg/ml captan (50%).

[b]TSM supplemented with 2 μg/ml benomyl (50%).

[c]*Fusarium* isolates tested in this study were: A, *F. oxysporum* Schlecht f. sp. *lycopersici* (Sacc.) Snyd. & Hanss.; B. *F. oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Snyd. & Hanss.; and C, *F. oxysporum* Schlecht f. sp. *melonis* (Leach & Currence) Snyd. & Hanss. All fungi were isolated from infected plants.

[d]Both fungi were isolated from naturally infested soil, on SQA.

[e]Spore suspensions (60 in 0.1 ml $H_2 0$ per plate0 of each isolate were dispersed on the appropriate plates by a glass rod.

TSM with benomyl (TSMB) was similar, but on TSM supplemented with captan (TSMC) 56.7-86.6% fewer colonies were formed. On TSM and TSMC an almost equal number of *Trichoderma* spp. colonies was formed, but none appeared on TSMB.

The size of *Fusarium* spp. colonies grown on TSMC, TSMB and TSM was 84-93%, 79-82% and 58-71%, respectively, smaller than those grown on SQA. *Trichoderma* spp. colonies formed on TSMC were 32-63% smaller than those formed on TSM.

The average linear growth of the Fusaria on TSMC, TSMB and TSM was 61-80%, 36-61% and 29-59%, respectively, less than their growth on SQA. The linear growth of *Trichoderma* spp. isolates on TSMC and TSM did not differ statistically. The effect of adding benomyl to TSM (*i.e.*, TSMB) was a drastic inhibition of *Trichoderma* spp. growth.

The small size of *Fusarium* spp. colonies formed on TSMB may reduce competition between colonies growing on this medium. Thus, it enables the formation of a larger number of colonies per plate, which facilitates counts and reduces statistical variability. This new medium, TSMB, is more efficient than SQA in preventing the growth of *Trichoderma* and other microbial contaminations.

*Trichoderma* spp. and *Fusarium* spp. may exist together in the same ecosystem. We therefore tested the efficiency of the two selective media in enabling separate isolation and total counts of each fungus. Soil samples were tested

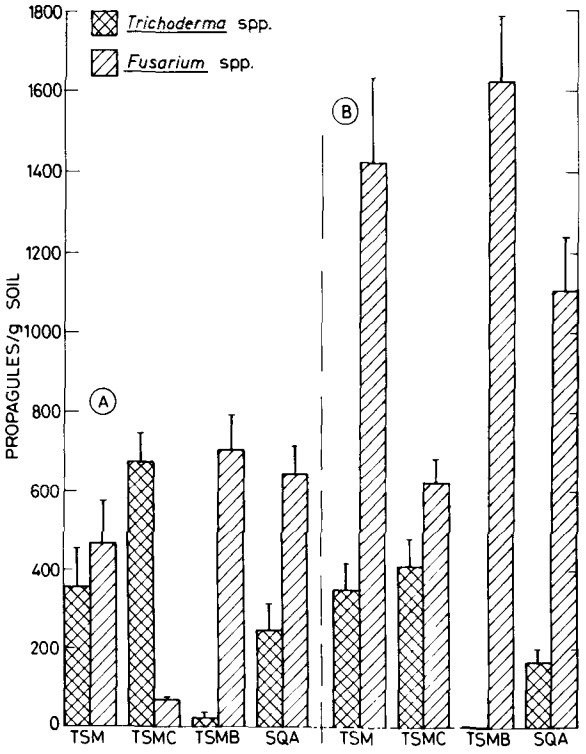


*Fig. 1.* Soil population counts of *Trichoderma* spp. and *Fusarium* spp. on agar plates of the following selective media: *Trichoderma* selective medium (TSM); TSM supplemented with captan (TSMC); TSM supplemented with benomyl (TSMB); and a selective quantitative agar medium for *Fusarium* (SQA). Serial dilutions were carried out with rendzina (A) and loessial sand (B) soils.

and the results obtained from two soils (rendzina and loessial sand) show that TSMC and TSMB improved counts of the respective fungi when both were present in soil from the same field (Fig. 1).

The results revealed that the addition of fungicides to TSM improves the basic selective medium. The new TSMC and TSMB are rather specific selective media, TSMC for isolating *Trichoderma* spp. (*e.g. T. harzianum* and *T. hamatum*), and TSMB for *Fusarium oxysporum*. These media can be useful tools in studying the ecology of the two fungi in mixed ecosystems.

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