Vegetative Compatibility Groups and Subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*

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ABSTRACT

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Isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the incitant of crown and root rot disease of tomatoes, were characterized by their ability to form heterokaryons with each other by using nitrate nonutilizing (*nit*) mutants. Isolates that formed mutual heterokaryons were placed in the same vegetative compatibility group (VCG). Seven VCGs were found among 218 isolates from Israel, Belgium, Canada, Greece, France, Italy, Japan, and the United States. Two of these VCGs could be divided into two subgroups each. The assignment of isolates to subgroups within a VCG was based on their capacity to form strong heterokaryons with isolates of one subgroup; whereas, their interactions with isolates of the other subgroup were weak and slow. At least one isolate was compatible with two VCGs. Isolates of *F. o. radicis-lycopersici* were not vegetatively compatible with isolates of *F. o. lycopersici*, *melongenae*, and *melonis*. In starch gel electrophoresis, the banding patterns of eight isozymes were identical for all 49 isolates of *F. o. radicis-lycopersici* and 19 isolates of two other formae speciales of *F. oxysporum* that were examined. One of the VCGs of *F. o. radicis-lycopersici* was characterized by a major esterase band that was present in 34 of 36 isolates tested.

Fusarium crown and root rot of tomatoes (*Lycopersicon esculentum* Miller) is caused by *Fusarium oxysporum* (Schlechtend.:Fr.) f. sp. *radicis-lycopersici* Jarvis & Shoemaker. This pathogen is distinct from *F. oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) Snyd. & Hans., the incitant of Fusarium wilt of tomatoes, in the symptoms it causes, epidemiology, and cultivar susceptibility (2,11,12,20). Although *F. o. radicis-lycopersici* is a relatively newly recognized pathogen, it has spread to many tomato-growing areas in the world, including Israel (2,11,15). The origin of *F. o. radicis-lycopersici* is not known nor have physiologic races or other groups been described.

In the absence of a known sexual stage in *F. oxysporum* heterokaryosis may be used as an alternative means of determining genetic relatedness among isolates of this diverse species. Isolates that are able to anastomose and form stable heterokaryons constitute a vegetative compatibility group (VCG) (18) and have been shown to share certain traits such as colony size, isozyme patterns, and virulence, as summarized by Correll et al (4). Since the formation of anastomoses is a prerequisite for potential exchange of genetic material through the parasexual cycle, isolates that are not vegetatively compatible would belong to genetically distinct populations. The principles and the techniques of using nitrate nonutilizing (*nit*) mutants, demonstrated by Puhalla (18) and developed further by Correll et al (4), have been used to study subforma specialis organization of several pathogenic strains of *F. oxysporum*. Certain populations, such as *F. o. vasinfectum* race 3 (14) and *F. o. dianthi* race 2 (13), were found to be uniform and composed of single VCGs. In other pathogens, such as *F. o. melonis* (10) and *F. o. cubense* (17), isolates fell into several VCGs, indicating that each forma specialis contains distinct subpopulations that might contribute to its genetic diversity.

Isozyme analysis, as performed in starch gel electrophoresis, has also been a valuable tool in the study of fungal taxonomy and genetics (e.g., 16,23). For example, isozyme polymorphism was found among isolates of *F. oxysporum* from crucifers, which correlated with formae speciales and VCGs (3).

The purpose of this study was to determine if vegetative compatibility and isozyme electrophoretic patterns can be used as criteria to estimate genetic diversity in the population of *F. o. radicis-lycopersici* in Israel and in seven other countries.

MATERIALS AND METHODS

Media. Potato-dextrose agar (PDA) was used for isolation of *Fusarium* from diseased plants, to maintain cultures, and to grow inoculum for pathogenicity tests in preliminary experiments. PDA was either homemade, according to reference 1, or purchased from Difco (Detroit, MI) or Biolife (Milano, Italy). Czapek-Dox agar (CD), used to grow inoculum for routine pathogenicity tests, consisted of (per 1 L): 30 g of sucrose, 2 g of $NaNO_3$, 1 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, and 15 g of agar. The Fusarium-minimal medium (FMM) was Puhalla's minimal nitrate agar (MM), which is a sucrose-salt medium that contains nitrate as the nitrogen source (18). Nitrite, hypoxanthine, and ammonium media were used for partial phenotypic characterization of *nit* mutants such as *nit*1, *nit*3, or NitM (4). CD and FMM were used as nitrate minimal media to recognize *nit* mutants and for complementation (heterokaryon) tests. Chlorate media, based on FMM or PDA amended with 15 g/L of $KClO_3$, were used to generate *nit* mutants (4,18). The liquid medium, GYP (2% glucose, 0.5% yeast extract, 0.5% peptone), was used to grow mycelia for isozyme electrophoresis.

Pathogens. The following pathogenic strains of *F. oxysporum* were used: f. sp. *radicis-lycopersici* (Table 1); f. sp. *melonis* Snyd. & Hans. (*nit* testers FOM-S and FOM-V); f. sp. *lycopersici* (Sacc.) Snyd. & Hans. (isolates FOL-649 [race 1; ATCC 66045] and FOL-1295 [race 2; ATCC 66047]; *nit* testers FOL-M and FOL-R of

TABLE 1. Isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* listed according to vegetative compatibility groups and subgroups, geographic origin, and esterase phenotype

Isolate	Location (source)[a]	Esterase phenotype[b]
VCG 0090/subgroup I		
0-1090[c]	Canada (Ont) (C)	+
FORL-C63F	Israel	+
FORL-C79C	Israel	+
FORL-C91A	Israel	+
FORL-C401C	Israel	+
FORL-C405B	Israel	+
FORL-C421	Israel	+
FORL-SIC2D[d]	Sicily (B)	+
FORL-DJV78	Crete (D)	+
FORL-19(FA-1)	France (E)	−
FORL-19R	France (F)	−
Group of 10 isolates[e]	Israel	NT
VCG 0090/subgroup II		
FORL-II	Israel	+
FORL-C68G	Israel	+
FORL-C84	Israel	+
FORL-C119	Israel	+
Group of 20 isolates[f]	Israel	+
Group of 26 isolates[g]	Israel	NT
Total: 71 isolates		
VCG 0091/subgroup I		
FORL-I	Israel	−
FORL-CRNK72	Israel	−
FORL-C54	Israel	−
FORL-C68H	Israel	−
FORL-C74B	Israel	−
FORL-C75	Israel	−
FORL-C403	Israel	−
FORL-C459AA	Israel	NT
FORL-GAR1[d]	N. Italy (G)	−
FORL-88-27	France (F)	−
ATCC 60095	Canada (A)	NT
J-36	Canada (Ont) (I)	−
OSU-374	USA (OH) (I)	NT
OSU-422	USA (OH) (I)	NT
TX-FORL-L (84) (ATCC 62941)	USA (TX) (L)	NT
TX-FORL-J (85)	USA (TX) (L)	NT
Group of 16 isolates[h]	Israel	−
Group of 39 isolates[i]	Israel	NT
Group of 3 isolates[j]	N. Italy (G)	NT
Group of 3 isolates[k]	France (F)	NT
Group of 4 isolates[l]	Canada (Ont) (J)	NT
VCG 0091/subgroup II		
FORL-C69E	Israel	−
FORL-C417	Israel	−
FORL-22 (FA-2)	France (E)	−
OSU-471	USA (OH) (I)	NT
HRS-SB082	Canada (J)	NT
01157	Belgium (H)	NT
Group of 3 isolates[m]	Israel	−
Group of 5 isolates[n]	Israel	NT
Total: 95 isolates		
VCG 0092		
FORL-CRNK67	Israel	−
FORL-CRNK78	Israel	−
FORL-C76	Israel	−
FORL-C207	Israel	−
FORL-C423	Israel	−
Group of 6 isolates[o]	Israel	−
Group of 14 isolates[p]	Israel	NT
Total: 25 isolates		
VCG 0093		
FORL-C201	Israel	−
FORL-C202	Israel	−
FORL-C204	Israel	−
Total: 3 isolates		
VCG 0094		
01147	Belgium (H)	−
01148	Belgium (H)	−
01150	Belgium (H)	−
01152	Belgium (H)	−
Group of 11 isolates[q]	Belgium (H)	NT
Total: 15 isolates		
VCG 0095		
HRS-SB086	Canada (Que) (J)	NT
VCG 0096		
FORL-C304B	Israel	NT
FORL-C651	Israel	NT
Group of 3 isolates[r]	Israel	NT
Total: 5 isolates		
Self-incompatible isolates		
01133	Belgium (H)	−
01141	Belgium (H)	NT
J3-212A	Japan (K)	NT

[a] Isolates obtained from: (A) ATCC (1); (B) G. Cartia; (C) J. C. Correll ex J. Puhalla; (D) N. E. Malathrakis and D. J. Vakalounakis (21); (E) K. S. Elias ex C. Alabouvette; (F) INRA-Avignon; (G) A. Garibaldi; (H) A. Vanachter; (I) L. Mihuta-Grimm; (J) W. R. Jarvis; (K) H. Komada; (L) R. D. Martyn.

[b] Presence (+) or absence (−) of a major esterase band in starch gel electrophoresis. NT = not tested.

[c] Isolates 0-1090 used by Puhalla (18), was originally isolate 1/75 (see Materials and Methods).

[d] Name of isolate given by us.

[e] This group includes isolates: FORL-C422A, -C439BF, -C454AC, -C480A, -C655B, -C661, -C682, -C700, -C708, and -C709.

[f] This group includes isolates: FORL-C55, -C89A, -C89B, -C106, -C108A, -C142, -C205C, -C206, -CRNK11D, -C402, -C407, -C409, -C412, -C413, -C415, -C425, -C426, -C427, -C428, and -C433.

[g] This group includes isolates: FORL-C447H, -C459AY, -C460A, -C475, -C606, -C608, -C610, -C617, -C657, -C658, -C660, -C664, -CRH667, -NT676, -NT677, -NT678, -C681, -C683, -C684, -C688, -C690, -C691, -C692, -C696, -C705 and -GS713.

[h] This group includes isolates: FORL-C56Q, -C58M, -C61, -C62F, -C70, -C71, -C82, -C83, -C85A, -C86, -C92, -C97C, -C404, -C430, -C431, and -C432.

[i] This group includes isolates: FORL-C52, -C59, -C104, -C107, -C435A, -C437, -C440B, -C602, -C611, -C612, -C613, -C614, -C615, -C616, -C618, -C619, -C620, -C621, -C626, -C628, -C652, -C653, -C654, -C656, -C659, -C662, -C663, -C665A, -CRH668A, -CRH671, -CRH672, -CRH674, -CRH675, -C680A, -C687, -C693, -C697, -C698, and -C699.

[j] This group includes isolates: FORL-GAR2[d], -GAR3[d], and -GAR4[d].

[k] This group includes isolates: FORL-90-29, -90-75, and -1151.

[l] This group includes isolates: HRS-SB036, -SB048, -SB064, and -SB170.

[m] This group includes isolates: FORL-C418, -C424, and -C434.

[n] This group includes isolates: FORL-CRH669, -CRH670, -C673, -C695, and -C702.

[o] This group includes isolates: FORL-C116, -C203B, -C208, -C209, -C210, and -C211.

[p] This group includes isolates: FORL-C212B, -C213, -C301, -C448, -C470B, -C607, -C609, -C627, -C666, -C685, -C686, -C689, -C694, and -GS712.

[q] This group includes isolates: 01149, 01151, 01156, 01159, 01162, 01163, 01164, 01166, 01167, 01169, and 01170.

[r] This group includes isolates: FORL-C622A, -C623, and -C624A.

VCG 0030); and f. sp. *melongenae* Matuo & Ishigami (*nit* testers FOMG-750/2 and FOMG-750/3).

Isolates of *F. o. radicis-lycopersici* and pathogenicity tests. Isolates of *F. oxysporum* were obtained from tomato plants that showed typical symptoms of crown and root rot disease (11,20). The plants were collected during four winter growing seasons (December–March) from commercial greenhouses and open-field plots in two regions in southern Israel (Besor and Arava). The fungus was isolated by plating plant tissues (surface-disinfested with 1% sodium hypochlorite for 2 min) on PDA and incubating them at 24 C for 5 days. Monoconidial cultures were prepared from most, but not all, of the isolates. Pathogenicity tests of the isolates of *Fusarium* were carried out in the greenhouse, by inoculating each isolate on two replicates of five seedlings of tomato (cv. Rehovot 13). Eighteen days after being sown, seedlings were removed; their roots were washed to remove adhering soil and they were dipped for 2 min in an inoculum suspension (1.5–3.5 $\times 10^6$ conidia per milliliter) prepared by macerating a 7-day-old culture (9-cm plate) with 75 ml of water. After planting, the seedlings were maintained in the greenhouse at 20–24 C for 20 days. Two types of disease symptoms were observed: quick collapse of the seedlings within 7 days, caused by girdling of the crown; and brown lesions on the roots and the crown, observed only after removal of the seedling and thorough rinsing of the roots. Pathogenic isolates caused disease symptoms of either type in 60–100% of the inoculated seedlings, from which typical cultures of *F. oxysporum* were then reisolated. Uninoculated plants, maintained as controls under the same conditions, remained healthy throughout the pathogenicity tests.

An additional 46 isolates of *F. o. radicis-lycopersici* from seven other countries, as listed in Table 1, were also included in this study. Among them was isolate 0-1090, the only isolate of *F. o. radicis-lycopersici* used by Puhalla (18). Isolate 0-1090 was originally isolate 1/75 (ATCC 52429; DAOM 161807) of Jarvis (12) (information obtained from P. E. Nelson, Fusarium Research Center, Penn State University, College Park). Mutants of isolate 0-1090 were used as tester strains of VCG 0090 (18).

Isolation of *nit* mutants and complementation tests. Plates (9-cm-diameter) of chlorate media were inoculated at four points with small mycelial transfers of CD cultures (one isolate per plate) and incubated at 27 C. Fast-growing sectors that emerged from the restricted colonies were transferred to CD or FMM plates (6-cm-diameter), and examined after a 3-day incubation period. Colonies with an expanding thin mycelium were considered *nit* mutants. All *nit* mutants showed wild-type growth on PDA. Complementation between *nit* mutants was tested on CD or FMM plates (6-cm-diameter). Usually, three mutants were inoculated on each plate, in triangle formation, and the plates were incubated at 27 C. Complementation was evident by the formation of a dense aerial wild-type mycelium where two mutants had met and formed a heterokaryon (14). Absence of wild-type growth at the contact zone between two *nit* mutants of the same parent isolate indicated allelic, overlapping, or otherwise noncomplementary mutations, or vegetative self-incompatibility (5,10). On the other hand, absence of wild-type growth at the contact zone of *nit* mutants from different parent isolates indicated either noncomplementarity or inability to form heterokaryons because of a lack of vegetative compatibility. Heterokaryons were usually evident within 5–14 days. When mutants of two different isolates formed a heterokaryon, their parent isolates were assigned to the same VCG.

Isozyme analysis. Mycelia were grown in 50-ml Falcon test tubes that contained 20 ml of GYP liquid medium. After a 2-wk incubation period that included shaking (120 rpm) at 27 C, the mycelium was collected by vacuum filtration with two layers of Whatman No. 1 filter paper. The mycelium was ground in 100 µl of buffer (0.1 M Tris-HCl, pH 7.5, containing 2% reduced glutathione), and the homogenate was absorbed onto 3- × 8-mm Whatman No. 3 MM filter paper wicks. Starch gels (12.4%) were prepared with Tris-citrate buffer (15 mM Tris, 4 mM citrate,

TABLE 2. Formation of complementary heterokaryons between complementary *nit* mutants of isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and NitM testers of subgroups of VCG 0090

Isolate/mutant	Phenotype	NitM testers			
		0-1090/B	0-1090/1	FORL-IID	FORL-IIE
VCG 0090					
FORL-C63F/2	*nit1*	++[a]	++	+	+
FORL-C63F/7	NitM	++	−	+	+
FORL-C421/6	NitM	++	++	+−	−
FORL-C421/7	NitM	++	++	−	−
FORL-C655B/7	*nit1*	++	++	+	++
FORL-C655B/6	NitM	++	++	++	++
FORL-C655B/8	NitM	++	++	+−	+
FORL-C682/2	*nit1*	++	++	+	+
FORL-C682/5	*nit3*	++	++	+	+
FORL-C700/3	*nit1*	++	++	+	+−
FORL-C700/2	NitM	++	++	−	+
FORL-C708/5	*nit1*	++	++	+	+
FORL-C708/3	*nit3*	++	++	+−	+
FORL-SIC2D/7	*nit1*	++	++	+	+
FORL-SIC2D/5	NitM	++	−	+	−
FORL-SIC2D/3	NitM	++	++	−	+
FORL-C447H/6	*nit1*	+	+	++	++
FORL-C447H/5	NitM	+	+	−	++
FORL-C681/41	*nit1*	−	+−	++	++
FORL-C681/42	NitM	−	+	−	++
FORL-C683/1	*nit1*	−	+−	++	++
FORL-C683/2	NitM	−	−	−	++
FORL-C684/4	*nit1*	−	−	++	++
FORL-C684/5	NitM	+−	+−	−	++
FORL-C691/1	*nit1*	+−	−	++	++
FORL-C691/5	NitM	+−	+−	−	++
FORL-C692/81	*nit1*	−	−	++	++
FORL-C692/51	NitM	−	−	−	++
FORL-C692/82	NitM	−	−	++	++
FORL-C705/5	*nit1*	−	+−	++	++
FORL-C705/6	NitM	−	+	−	++

[a] ++ = Wild-type growth after 4–7 days; + = weak or slow growth after 8–20 days; +− = uncertain; − = no growth after 20 days.

pH 8.1) and run at 200 V at 5 C for 6 hr with 0.3 M boric acid buffer (pH 8.2) (23). Each gel was then sliced horizontally into four slices; each slice could then be stained for one of the following enzymes (22): acid phosphatase (ACP), EC 3.1.3.2; alcohol dehydrogenase (ADH), EC 1.1.1.1; adenylate kinase (AK), EC 2.7.4.3; aldolase (ALDO), EC 4.1.2.13; aspartate aminotransferase (AAT), EC 2.6.1.1; isocitric dehydrogenase (IDH), EC 1.1.1.42, phosphoglucoisomerase (PGI), EC 5.3.1.9; phosphoglucomutase (PGM), EC 2.7.5.1; and esterase (EST), EC 3.1.1.2.

RESULTS

Pathogenicity tests. Isolates of *F. oxysporum* were obtained from tomato plants that showed typical symptoms of crown and root rot disease. Results of pathogenicity tests, done with inoculum prepared on PDA, were highly variable and frequently irreproducible. In extreme instances, the proportion of diseased seedlings in repeated inoculation experiments ranged from 0 to 100%. The source of PDA was found to have a crucial effect on the results when inocula that contained $1–4 \times 10^6$ conidia per milliliter were compared. For example, the least pathogenic inoculum was obtained from cultures grown on homemade PDA, more pathogenic inoculum was obtained from cultures grown on Difco PDA, and the most pathogenic inoculum was obtained from cultures grown on Biolife PDA. However, the best mycelial growth of the pathogen in culture was on homemade PDA. In further experiments, highly pathogenic inoculum, which gave reproducible results, was obtained from cultures grown on CD medium. The proportion of diseased seedlings obtained from repeated inoculations with pathogenic isolates was ±20% of the average. Both types of symptoms, quick collapse and brown lesions, were evident; whereas, nonpathogenic isolates had no effect. Therefore, CD medium, which also has the advantage of having a defined composition, was used for growing the inoculum for routine pathogenicity tests. Eighty-eight percent of isolates of *F. oxysporum* obtained from naturally infected plants that showed typical disease symptoms were classified as *F. o. radicis-lycopersici*.

Vegetative compatibility groups. The first two isolates of *F. o. radicis-lycopersici* from Israel, FORL-I and FORL-II, were obtained in 1986. Complementary *nit* mutants were generated from each isolate and paired with FORL testers 0-1090/A (*nit*1) and 0-1090/B (NitM) of Puhalla's VCG 0090 (18). Some mutants of FORL-II produced heterokaryons when paired with tester 0-1090/B, and hence the isolate was placed in VCG 0090. Since the reaction between the mutants of FORL-II and the *nit* testers of 0-1090 was slower and weaker than the reaction between mutants within each isolate, additional mutants were generated from isolate 0-1090 and paired with those of FORL-II and among themselves. With the new mutants the difference between interstrain and intrastrain reactions remained unchanged, but Puhalla's tester 0-1090/A, which showed poor complementation with most mutants, was replaced by mutant 0-1090/1 (NitM) as the second reliable tester of VCG 0090. Complementary *nit* mutants were generated from field isolates obtained during 1988–1990 in Israel, and from isolates obtained from ATCC and scientists of seven other countries (Table 1). Mutants were paired with the testers of VCG 0090, as well as with complementary mutants of FORL-II (FORL-IID and FORL-IIE, both NitM) and among themselves, in order to determine vegetative compatibility. It soon became apparent that complementary mutants of some isolates that were placed in VCG 0090 based on heterokaryosis with testers 0-1090/B and 0-1090/1 reacted weakly,

TABLE 3. Formation of complementary heterokaryons between complementary *nit* mutants of isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and NitM testers of subgroups of VCG 0091

Isolate/mutant	Phenotype	NitM testers			
		FORL-C544	FORL-C758	FORL-C69E3	FA222
VCG 0091					
FORL-C611/12	*nit1*	++[a]	++	−	−
FORL-C611/11	NitM	++	++	+	−
FORL-C628/3	*nit1*	++	++	+	−
FORL-C628/4	NitM	++	++	++	+
FORL-C628/5	NitM	++	++	+	−
FORL-C687/2	*nit1*	++	++	+−	−
FORL-C687/3	NitM	++	−	−	+−
FORL-C693/3	*nit1*	++	++	+−	−
FORL-C693/2	NitM	−	++	−	+−
FORL-C693/1	NitM	++	++	−	+−
FORL-90-29/1	NitM	++	++	−	−
FORL-90-29/2	NitM	++	++	−	−
FORL-1151/5	*nit1*	++	++	−	+
FORL-1151/4	NitM	++	++	+−	−
TX-FORL-L (84)/3	*nit1*	++	++	−	−
TX-FORL-L (84)/7	NitM	++	++	−	−
FORL-C417/1	*nit1*	+−	+−	++	++
FORL-C417/4	NitM	−	+−	−	++
FORL-C417/5	NitM	−	−	++	++
FORL-C434/2	*nit1*	+−	+−	++	++
FORL-C434/3	NitM	−	+	−	++
FORL-C669/4	*nit1*	−	−	++	++
FORL-C669/3	NitM	−	−	−	++
FORL-C673/1	*nit1*	+−	+−	++	++
FORL-C673/2	NitM	+−	−	++	++
FORL-C695/5	*nit1*	−	+−	++	++
FORL-C695/15	NitM	−	−	++	++
FORL-C702/4	*nit1*	+−	−	++	++
FORL-C702/31	NitM	−	−	−	++
FORL-C702/32	NitM	+−	−	++	++
01157/3	*nit1*	+	+	++	++
01157/4	NitM	−	+	−	++
01157/6	NitM	+−	+	++	−

[a] ++ = Wild-type growth after 4–7 days; + = weak or slow growth after 8–20 days; +− = uncertain; − = no growth after 20 days.

slowly, or not at all with the complementary mutants FORL-IID and FORL-IIE of the same VCG. Details of this observation for seven isolates, each represented by two or three complementary mutants, are presented in Tables 2 and 3. For example, the two complementary NitM mutants of isolate FORL-C421 reacted strongly with both testers of 0-1090, but no reaction was observed with the complementary testers of FORL-II. On the other extreme was isolate FORL-C655B, of which two mutants formed strong heterokaryons with both pairs of testers (although the heterokaryotic growth with FORL-II was initially weaker than that observed with 0-1090, it was categorized as strong after seven days). A third mutant gave a weak reaction with FORL-IIE. The other five isolates, which represent the majority of such strains, lie between these two extremes; the mutants of these isolates formed strong heterokaryons with at least one (usually both) of the testers of 0-1090, but weak or slow heterokaryons (Fig. 1) with one or both testers of FORL-II. The opposite was also observed, namely strong heterokaryosis with the testers of FORL-II and weak or no reactions with the testers of 0-1090. Details of such behavior in a second group of seven isolates, each represented by two or three complementary mutants, are also shown in Tables 2 and 3. Whereas all the mutants formed strong heterokaryons with at least one of the testers of FORL-II, only a few of them gave positive but weak reaction with testers of 0-1090. One extreme among these isolates was FORL-C447H, of which two complementary mutants formed weak heterokaryons with both testers of 0-1090. On the other extreme was FORL-C692, of which none of three complementary mutants reacted with the testers of 0-1090. Most of the isolates gave negative or uncertain reactions (Fig. 1), in spite of the use of complementary mutants. Although few mutants (e.g., FORL-655B/6) gave strong heterokaryons with certain testers of both 0-1090 and FORL-II, it became clear that based on the relative intensity of their reaction with these testers, all the isolates of VCG 0090 could be divided into two subgroups: VCG 0090 I, which included isolates whose mutants gave stronger reactions with the testers of 0-1090; and VCG 0090 II, which included isolates whose mutants gave stronger reactions with the testers of FORL-II. To test whether this distinction represented differences between the entire subgroup or only between the two key isolates, 17 random mutants of nine isolates of subgroup I, and 17 random mutants of 14 isolates of subgroup II were paired in different combinations, and heterokaryon formation was recorded for up to 20 days (Table 4). The reactions between mutants within each subgroup could be defined clearly within 5–7 days as either positive (complementary) or negative (noncomplementary). On the other hand, quick positive reactions between mutants of different subgroups were rare; growth of most of the heterokaryons was weak and slow, frequently discontinuous, or it occurred as patches or dots of mycelial tufts (Fig. 1). Furthermore, many of the pairings between subgroups showed inconsistent or uncertain results even after prolonged incubation (Fig. 1; Table 4). The negative, weak, and uncertain reactions were not enhanced by repeated pairings on CD or FMM, or when these media were supplemented with 30 mM $Na_2MoO_4 \cdot 2H_2O$ (7) or activated charcoal (17). With the same random mutants, the two testers of subgroup I identified all the nine isolates of subgroup I, but failed to identify five out of 14 (36%) isolates of subgroup II. Similarly, the two testers of subgroup II identified all 14 isolates of this subgroup, but failed to identify three of nine (33%) isolates of subgroup I. These results indicate that the relationship between isolates 0-1090 and FORL-II manifests the relationship between subgroups I and II, respectively, and that not two but four testers (0-1090/B and 0-1090/1; FORL-IID and FORL-IIE) are required to identify isolates belonging to VCG 0090 in heterokaryon tests (Table 2). Of the 218 isolates of this study, 16 from Israel and five from other countries were placed in subgroup 0090 I, and 50 isolates from Israel were placed in subgroup 0090 II.

The mutants of isolate FORL-I failed to form heterokaryons with the testers of either subgroup of VCG 0090. Therefore, this isolate was placed in a separate VCG, designated VCG 0091 according to the system of Puhalla (18). Two subgroups were also found in VCG 0091, when mutants of isolates from Israel

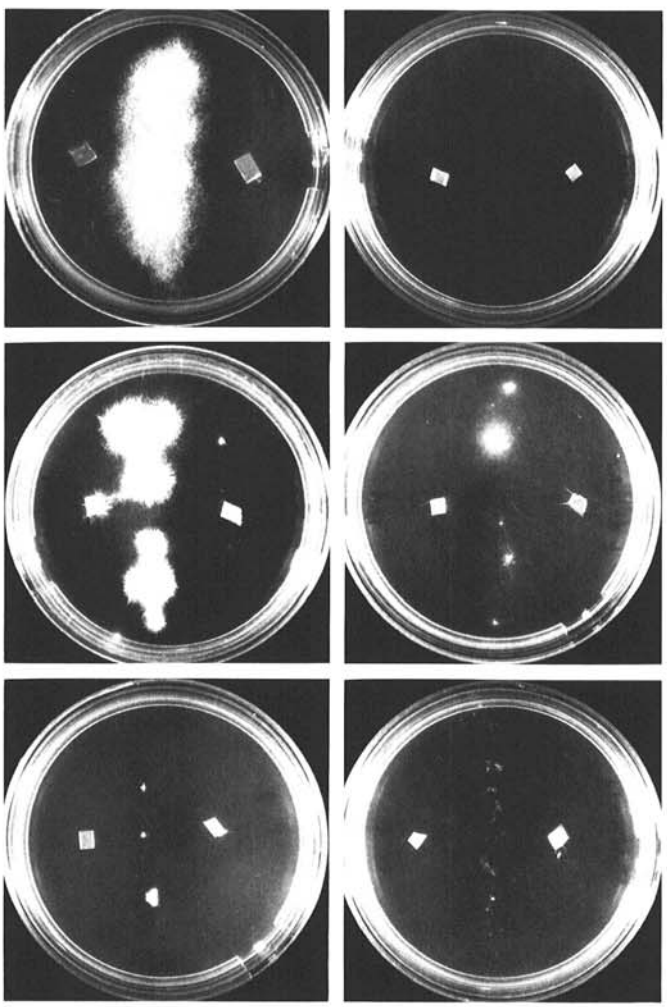

Fig. 1. Types of complementary heterokaryotic growth between *nit* mutants of isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* that belong to the same (upper plates) or different (middle and bottom plates) subgroups of VCG 0091. Strong heterokaryon (top left), no complementation (top right), slow discontinuous heterokaryon (middle left), heterokaryotic mycelial tufts (middle right) and uncertain reactions (bottom) after 20 days (top left: 6 days) of growth on nitrate minimal medium.

TABLE 4. Formation of complementary heterokaryons between random *nit* mutants of the same or different subgroups of VCG 0090 and VCG 0091 of *Fusarium oxysporum* f. sp. *radicis-lycopersici*

VCG	Subgroup combination	Heterokaryons[a]			Total[b]
		+	−	+−	
0090[c]	I + I	22	10	4	36
	II + II	73	13	1	87
	I + II	33	76	17	126
0091[d]	I + I	104	26	1	131
	II + II	7	2	1	10
	I + II	21	107	26	154

[a]Growth at the contact zone between *nit* mutants on nitrate minimal medium: + = complementation (including strong, discontinuous, weak, and slow types of heterokaryotic growth); − = no complementation; +− = uncertain.

[b]Total number of isolate combinations examined (not all possible combinations were tested).

[c]Seventeen random mutants of nine isolates of subgroup I, and 17 random mutants of 14 isolates of subgroup II. Tester strains not included.

[d]Thirty-three random mutants of 31 isolates of subgroup I, and eight random mutants of five isolates of subgroup II. Tester strains not included.

and other countries, which were not placed in VCG 0090, were paired with mutants of isolate FORL-I and among themselves. The testers of VCG 0091 I were FORL-C544 and FORL-C758, and the testers of VCG 0091 II were FORL-C69E3 and FA-222 (all four were NitM) (Table 3); each of them originated from a different parental isolate. The characteristics of heterokaryon formation within and between subgroups of VCG 0091 were similar to those observed in VCG 0090 (Fig. 1; Table 4). With 33 random mutants of 31 isolates of subgroup I, and eight random mutants of five isolates of subgroup II, the two testers of subgroup I identified all the isolates of this subgroup, but failed to identify four of the five (80%) isolates of subgroup II. Similarly, the two testers of subgroup II identified all the isolates of this subgroup, but failed to identify 17 out of 31 (55%) isolates of subgroup I. Subgroup 0091 I included 63 isolates from Israel and 18 isolates from other countries, and subgroup 0091 II included 10 and four, respectively.

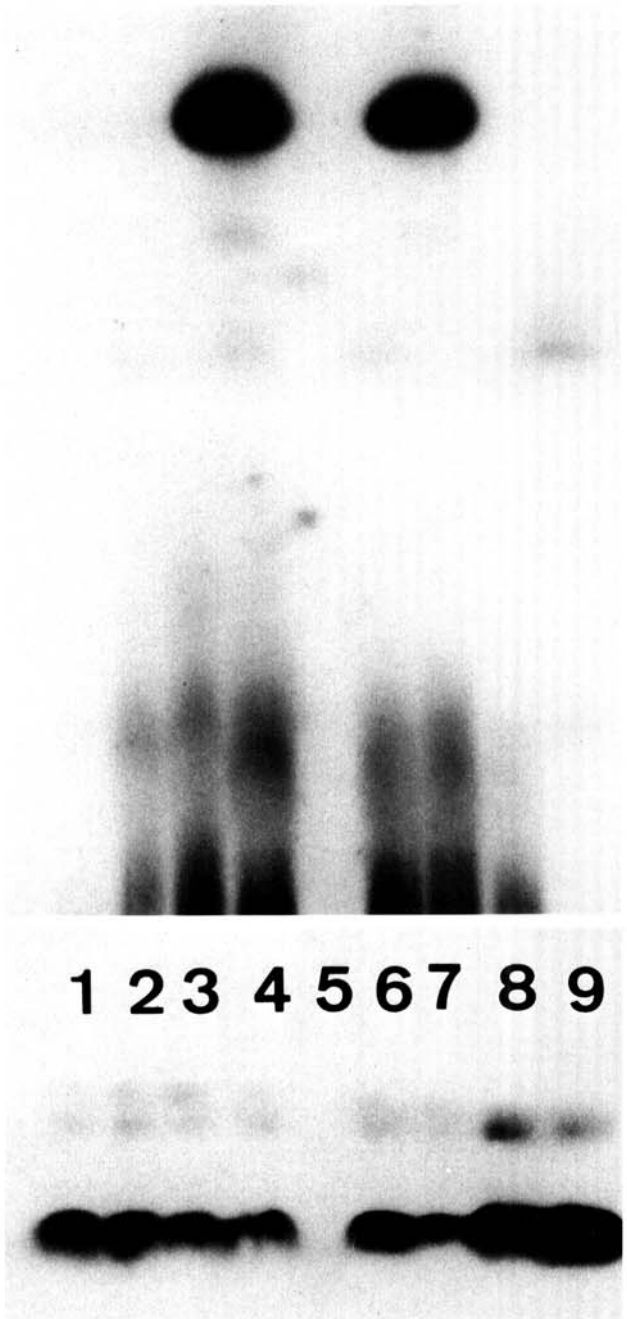

Fig. 2. Electrophoretic phenotypes of isolates of *Fusarium oxysporum* from tomato, revealed by starch gel electrophoresis of mycelial extracts. Each lane represents a different isolate. Lanes 1 and 9: *F. o. lycopersici* (VCG 0030) isolates FOL-649 (race 1) and FOL-1295 (race 2), respectively. Lanes 2 and 6: *F. o. radicis-lycopersici* (VCG 0092) isolates FORL-CRNK67 and FORL-C207, respectively. Lanes 3 and 8: *F. o. radicis-lycopersici* (VCG 0091) isolates FORL-CRNK72 and FORL-C74B, respectively. Lanes 4 and 7: *F. o. radicis-lycopersici* (VCG 0090) isolates FORL-C84 and FORL-C119, respectively. Lane 5: *Fusarium* sp. (non-pathogenic). Lower gel stained for ADH and shows no variation between VCGs and formae speciales. Upper gel stained for esterase and shows a fast-moving major esterase band characteristic of VCG 0090. Migration was from bottom (−) to top (+).

The remaining 33 isolates from Israel were placed in three VCGs. VCG 0092 included 25 isolates and its testers were CRNK-676 and CRNK-678 (both NitM). VCG 0093 included three isolates, all of which came from one site, and VCG 0096 included five isolates. A group of 15 isolates from Belgium were placed in a separate VCG, 0094, with testers 01150-6 and 01152-31. VCG 0095 consisted of one isolate from Canada.

Two isolates from Belgium and one isolate from Japan were characterized as self-incompatible (5); *nit* mutants of each of these isolates failed to form complementary heterokaryons among themselves, in spite of being of different nitrate-reduction phenotypes. Similar strains were described in other formae speciales of *F. oxysporum* (3,6,10).

Examination of the geographic distribution of VCGs of *F. o. radicis-lycopersici* in Israel showed that each of the three major VCGs, as well as the subgroups, contained isolates from both regions where tomato crown rot prevails. Furthermore, on three occasions an initial isolate from a naturally infected plant yielded two monoconidial cultures that were noncompatible with each other and belonged to different VCGs. These pairs of cultures were: FORL-C68G (VCG 0090 II) and FORL-C68H (VCG 0091 I); FORL-C459AY (VCG 0090 II) and FORL-C459AA (VCG 0091 I); and FORL-CRNK72 (VCG 0091 I) and FORL-CRNK67 (VCG 0092). These results show that different VCGs of *F. o. radicis-lycopersici* can coexist in close proximity in nature as has also been observed with *F. o. asparagi* (9).

To determine whether isolates of *F. o. radicis-lycopersici* can belong to more than one VCG, tester strains were paired with *nit* mutants of isolates outside their VCG. No heterokaryon formation was observed in about 430 various intergroup pairings with isolates of VCGs 0091, 0093, 0094, 0095, and 0096, or between them and VCG 0090 and VCG 0092. However, a first indication that an isolate might belong to two VCGs came when *nit* mutants of isolate FORL-19R formed complementary heterokaryons with the testers of both VCG 0090 I and VCG 0092. Three monoconidial cultures were prepared from FORL-19R and complementary *nit* mutants were generated from each of them. Monoconidial cultures prepared from these mutants formed heterokaryons, which showed that the three monoconidial subcultures derived from FORL-19R were compatible with each other. Two such monoconidial complementary mutants formed heterokaryons with the testers of both VCG 0090 I and VCG 0092. Although the heterokaryons formed between this isolate and the *nit* testers of either VCG developed slowly and were weak or discontinuous, compatibility between FORL-19R and *nit* mutant testers from isolates in VCG 0090 and VCG 0092 was evident. Similarly, weak reactions were observed between FORL-19R and 12 other isolates of VCG 0092; whereas, reactions of FORL-19R with six isolates of VCG 0090 I varied from very weak to very strong.

The interaction between VCG 0090 and VCG 0092 was then reexamined to determine if VCG 0092 might be a third subgroup of VCG 0090 rather than a separate VCG. The two testers of VCG 0090 I did not react with one tester of VCG 0092 and gave uncertain or weak reactions with the other tester after prolonged incubation. The testers of VCG 0090 I also gave uncertain and negative reactions with two and 11 random mutants, respectively, of VCG 0092. Four additional mutants of VCG 0090 I, as well as the testers and two additional mutants of VCG 0090 II, did not form complementary heterokaryons with the testers or 13 random mutants of VCG 0092 (the random mutants of VCG 0092 formed complementary heterokaryons in 61% of the

pairings among themselves). Four additional isolates (e.g., FORL-C79C) seemed to be compatible with the testers of both VCG 0090 I and VCG 0092, but the data obtained so far do not justify a change in the present status of VCG 0092 as a separate VCG. No complementary heterokaryons were formed between the testers of the seven VCGs of *F. o. radicis-lycopersici* and tester strains of *F. oxysporum* pathogenic on solanaceous plants (*F. o. lycopersici* VCG 0030; *F. o. melongenae*) and on muskmelon (*F. o. melonis*).

Isozyme polymorphism. After electrophoresis, the gels were sliced and stained for enzyme activity. The banding patterns of enzymes ACP, ADH, AK, ALDO, AAT, IDH, PGI, and PGM were identical for all 49 isolates of *F. o. radicis-lycopersici* examined, as well as for seven isolates of *F. o. lycopersici* and 12 isolates of *F. o. melongenae*. On the other hand, in gels stained for esterase, a major band was revealed that was present only in extracts of isolates of *F. o. radicis-lycopersici* from VCG 0090 (Fig. 2). This major esterase band was present in extracts of all 25 isolates of subgroup 0090 II that were examined and in nine of 11 isolates of subgroup 0090 I. (The isolates of VCG 0090 I that lacked this band were FORL-19[FA-1] and FORL-19R, both from France.) The esterase band was absent from extracts of all 50 *F. o. radicis-lycopersici* isolates of VCGs 0091, 0092, 0093, and 0094 examined (Table 1), as well as from the extracts of isolates of *F. o. lycopersici* and *F. o. melongenae*.

DISCUSSION

Seven vegetative compatibility groups were revealed among 218 isolates of *F. o. radicis-lycopersici* from Israel and seven other countries. Two of these VCGs could be divided into two subgroups each, and at least one isolate was compatible with two VCGs. One of the VCGs (0090) was characterized by a specific major esterase band in starch gel electrophoresis, which was present in 34 of the 36 isolates tested. Studies with other formae speciales of *F. oxysporum* revealed that some of these pathogens consisted of single VCGs (13,14) while others were more diverse and consisted of several VCGs (6,8-10,17). Studies with populations of *F. o. vasinfectum* and *F. o. dianthi* in Israel revealed one VCG for each pathogen (13,14). In contrast, five VCGs of *F. o. radicis-lycopersici* were found in Israel, three of which have been found only in this country. Diversity was also evident among isolates of *F. o. radicis-lycopersici* from France and Canada, where two or three VCGs were found among a small number of isolates tested from each country.

The collection of isolates of this study, although not representative of the world populations of *F. o. radicis-lycopersici*, contained two widespread VCGs (0090 and 0091) and an additional five which, at present, show limited distribution. With such a situation, it is not possible to decide whether the various VCGs originated from a single source or evolved in each site through independent parallel events. It is expected that additional VCGs will be found if more isolates are examined.

F. o. radicis-lycopersici is the first forma specialis of *F. oxysporum* in which subgroups of VCGs have been reported. Although all isolates of *F. o. radicis-lycopersici* that belong to a given VCG were expected to anastomose with each other, their tendency to do so depended on the isolates involved. Table 4 shows that the formation of complementary heterokaryons between random mutants of different subgroups was much lower (14–26%) than between these mutants within subgroups (61–84%). Although some of the negative reactions could be attributable to noncomplementarity of the mutations, the probability of such combinations remained unchanged if the same sets of random mutants were used for pairings within and between subgroups. The differences thus indicated that a high frequency of heterokaryosis occurs between isolates within a subgroup, whereas this process is considerably less frequent and weaker between isolates of different subgroups.

At least one isolate (FORL-19R) was vegetatively compatible with two VCGs (0090 and 0092). Experiments with FORL-19R, as well as with a few similar candidates of VCG 0090 I, indicated that these isolates may differ from other members of their VCG in the relatively slow and weak heterokaryosis reaction with the other strains. Since the strength of a heterokaryon depends not only on compatibility, but also on the biochemical complementation between the mutations involved, many mutants must be compared in order to get combinations that ensure maximal heterokaryotic growth. Until the cross-VCG compatibility phenomenon is studied in more detail, the presence of one or a few such exceptional isolates does not justify the reduction of VCG 0092 to a third subgroup of VCG 0090, because most of the isolates of VCG 0090 and VCG 0092 react as though they belong to separate VCGs. The major esterase band specific for both subgroups of VCG 0090 is another phenotypic manifestation of the close genetic relatedness between the two subgroups of this VCG. However, isolates whose cross-VCG compatibility forms a bridge between VCG 0090 and VCG 0092, point to some degree of genetic relatedness between these two VCGs. From the evolutionary point of view, bridging strains and subgroups may be interpreted as stages in a process of convergence of VCGs or, on the contrary, stages in the divergence and formation of new VCGs. The possibility that subgroups and bridging strains, respectively, exist also within and between VCGs of other formae speciales deserves examination.

F. o. radicis-lycopersici differs from other formae speciales of *F. oxysporum* in the structure of its VCGs and in certain pathogenic traits (2,11,12,20). These differences may reflect its short history in agricultural soils as compared with other formae speciales. Rowe (19) suggested that crown and root rot "could be considered a manmade disease." Our experience with *F. o. radicis-lycopersici* showed that the composition of the medium for inoculum preparation is crucial for the success of seedling inoculation, more than has been experienced with other formae speciales. This sensitivity to the medium composition might explain previous uncertainty regarding the etiology of the crown rot disease (19).

Isozyme analysis of eight enzymes did not reveal differences between *F. o. radicis-lycopersici* and two other pathogens of solanaceous crops (*F. o. lycopersici* and *F. o. melongenae*). This finding is in accordance with the results of Bosland and Williams (3), who found little or no variation in 15 of 18 enzymes examined in strains of *F. oxysporum* pathogenic to crucifers. This uniformity shows that *F. oxysporum* is quite consistent with respect to enzyme profile and therefore not much polymorphism should be expected. However, occasional differences in specific isozymes might be useful in genetic and taxonomic studies.

In the absence of known physiologic races, *F. o. radicis-lycopersici* has been considered a uniform population, especially in breeding programs. This study shows that *F. o. radicis-lycopersici* consists of several distinct subpopulations. It is not known at present whether these subpopulations also differ pathogenically.

LITERATURE CITED

1. ATCC. 1987. Catalogue of Fungi/Yeasts, 17th Edition, p. 415. American Type Culture Collection, Rockville, Maryland.
2. Benhamou, N., Charest, P. M., and Jarvis, W. R. 1989. Biology and host-parasite relations of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Pages 95-105 in: Vascular Wilt Diseases of Plants. E. C. Tjamos and C. H. Beckman, eds. Springer-Verlag, Berlin.
3. Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65:2067-2073.
4. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
5. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*) Mycol. Res. 93:21-27.
6. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396-400.
7. Cove, D. J. 1979. Genetic studies of nitrate assimilation in *Aspergillus*

nidulans. Biol. Rev. 54:291-327.
8. Elias, K. S., and Schneider, R. W. 1988. Isozyme analysis of races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *lycopersici*. (Abstr.) Phytopathology 78:1543.
9. Elmer, W. H., and Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. Phytopathology 79:88-93.
10. Jacobson, D. J., and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. Phytopathology 78:668-672.
11. Jarvis, W. R. 1988. Fusarium crown and root rot of tomatoes. Phytoprotection 69:49-64.
12. Jarvis, W. R., and Shoemaker, R. A. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679-1680.
13. Katan, T., Hadar, E., and Katan, J. 1989. Vegetative compatibility of *Fusarium oxysporum* f. sp. *dianthi* from carnation in Israel. Plant Pathol. 38:376-381.
14. Katan, T., and Katan, J. 1988. Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.
15. Krikun, J., Nachmias, A., Cohn, R., and Lahkim-Tsror, L. 1982. The occurrence of Fusarium crown and root rot of tomato in Israel. Phytoparasitica 10:113-115.
16. Micales, J. A., Bonde, M. R., and Peterson, G. L. 1986. The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon 27:405-449.
17. Ploetz, R. C., and Correll, J. C. 1988. Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. Plant Dis. 72:325-328.
18. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
19. Rowe, R. C. 1980. Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and field-grown tomatoes in North America and Japan. Phytopathology 70:1143-1148.
20. Rowe, R. C., and Farley, J. D. 1981. Strategies for controlling Fusarium crown and root rot in greenhouse tomatoes. Plant Dis. 65:107-112.
21. Vakalounakis, D. J. 1988. The genetic analysis of resistance to Fusarium crown and root rot of tomato. Plant Pathol. 37:71-73.
22. Vallejos, C. E. 1983. Enzyme activity staining. Pages 469-516 in: Isozymes in Plant Genetics and Breeding, Part A. S. D. Tanksley and T. J. Orton, eds. Elsevier Sciences Publishers B.V., Amsterdam.
23. Zamir, D., and Chet, I. 1985. Application of enzyme electrophoresis for the identification of isolates in *Trichoderma harzianum*. Can. J. Microbiol. 31:578-580.