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Enniatin Production by *Fusarium* Strains and Its Effect on Potato Tuber Tissue

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Several *Fusarium* strains produce the cyclohexadepsipeptide enniatin, a host-nonspecific phytotoxin. Enniatins are synthesized by the 347-kDa multifunctional enzyme enniatin synthetase. In the present study, 36 *Fusarium* strains derived from a wide range of host plants were characterized with respect to enniatin production in different media. Thirteen of these strains produced enniatins on one or more of these media. To determine whether enniatin production affected virulence, an assay on potato tuber tissue was performed. Seven enniatin-producing and 16 nonproducing strains induced necrosis of potato tuber tissue, so that enniatin synthesis is not essential for the infection of potato tuber tissue. The application of a mixture of enniatins to slices of potato tuber, however, caused necrosis of the tissue. Therefore, enniatin production by the enniatin-synthesizing strains may affect their pathogenicity. The enniatin synthetase gene (*esyn1*) of *Fusarium scirpi* ETH 1536 was used as a probe to determine if similar sequences were present in the strains examined. In Southern blot analyses, DNA sequences hybridizing with the *esyn1* probe were present in all but two of the strains examined. In some cases, enniatin-nonproducing strains had the same hybridization pattern as enniatin producers.

Many *Fusarium* species are serious plant pathogens, causing symptoms such as necrotic lesions, rot, and wilt (4). Several *Fusarium* strains produce the low-molecular-weight phytotoxin enniatin, a host-nonspecific toxin. Enniatins are N-methylated cyclic hexadepsipeptides with phytotoxic, antibiotic, and insecticidal activities (10, 11, 13), probably related to their ionophoric properties (27). They consist of alternating residues of D-2-hydroxyisovaleric acid and a branched-chain *N*-methyl-L-amino acid linked by peptide and ester bonds (Fig. 1).

The biosynthesis of the enniatins is well understood and follows a nonribosomal thiotemplate mechanism catalyzed by the 347-kDa multifunctional enzyme enniatin synthetase (2, 23, 28, 29). This polypeptide has all the catalytic functions necessary for synthesizing enniatin from D-2-hydroxyisovaleric acid; the branched-chain L-amino acid, *S*-adenosylmethionine; and ATP. Enniatin synthetases purified from *Fusarium scirpi* ETH 1536, *F. sambucinum* BBA 63933, and *F. lateritium* BBA 65090 exhibit distinct substrate amino acid specificities and have been characterized as isomultienzymes (24). Recently, the enniatin synthetase gene (*esyn1*) from *F. scirpi* ETH 1536, which encodes an open reading frame of 9,393 bp, has been isolated, sequenced, and characterized by heterologous expression (14, 15).

The mechanism of action and role of enniatins in plant pathogenesis are poorly understood. A contribution of enniatins to the phytotoxic properties of fusaria was first assumed a decade after their discovery in 1950. Gäumann et al. (11) reported a synergistic phytotoxic effect of enniatins A and B on the water uptake by cut tomato sprouts in vitro. Symptoms of toxicity included wilting and loss of turgor followed by necrosis of leaves. Enniatins also were reported to reduce the growth of germinating wheat seeds (5). Hershenhorn et al. (17) characterized *F. avenaceum* as a pathogen of spotted knapweed (*Centaurea maculosa*). Two phytotoxins, enniatin B and acetamido-butenolide, were isolated from this strain. These phytotoxins acted synergistically to cause necrotic lesions on leaves of different plant species. Germination of soybean, barley, and wheat was completely inhibited and germination of spotted knapweed was greatly reduced after inoculation of seeds with hyphae of *F. avenaceum*, while the seeds of rice and squash were resistant to infection. Until now, there has been no further information concerning enniatin production and plant-pathogenic properties of *Fusarium* species.

The goal of the present study was to investigate the relationships among enniatin production, detection of enniatin synthetase gene homologs, and toxicity of a wide variety of *Fusarium* strains. Toxicity was analyzed by assaying the virulence on potato tuber tissue because enniatins induced necrosis of the tissue in vitro. Enniatin synthetase gene homologs were detected by Southern blot analysis with a hybridization probe of the *esyn1* gene of *F. scirpi.*

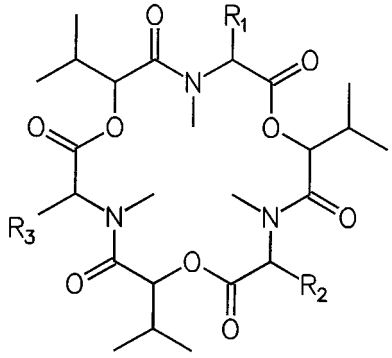

FIG. 1. General structure of enniatin homologs. For enniatin A, R_1, R_2, and R_3 are $-CH(CH_3)CH_2CH_3$; for enniatin B, R_1, R_2, and R_3 are $-CH(CH_3)_2$; for enniatin A_1, R_1 and R_2 are $-CH(CH_3)CH_2CH_3$ and R_3 is $-CH(CH_3)_2$; for enniatin B_1, R_1 is $-CH(CH_3)CH_2CH_3$ and R_2 and R_3 are $-CH(CH_3)_2$.

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TABLE 1. Enniatin production of *Fusarium* strains and relative virulence on potato tuber tissue

Species[a]	Strain[a]	Source	Phytopathogenic properties[b]	Enniatin production (μg/ml)[c]	Relative virulence on potato tuber tissue[d]
F. sambucinum	BBA 62397	*Solanum tuberosum*	NK[e]	75‡	100
	BBA 63933	*Triticum* sp.	NK	350**	4
	NRRL 13500	*Solanum tuberosum*	Potato pathogen	ND[f]	100
	NRRL 13503	*Solanum tuberosum*	Potato pathogen	ND	45
	R-583	*Polygonum* sp.	Potato pathogen	ND	54
	R-5390	*Solanum tuberosum*	Potato pathogen	ND	47
	R-7570	Soil	Potato pathogen	ND	40
	R-5455	*Zea mays*	Potato pathogen	ND	10
	R-6380	*Solanum tuberosum*	Potato pathogen	ND	22
	R-6112	Soil	NK	ND	25
	R-7843	*Dianthus caryophyllus*	NK	ND	20
	R-5690	Soil	NK	ND	12
	R-2633	*Solanum tuberosum*	NK	ND	14
	R-6354	*Zea mays*	NK	ND	15
F. acuminatum	BBA 62148	*Lupinus angustifolius*	Brown discoloring of stem base	65**	5
F. arthrosporioides	BBA 64134	*Agrostis* sp. (seeds)	NK	135‡	55
F. avenaceum	BBA 64338	*Hordeum* sp. (seeds)	NK	180†	45
	BBA 62168	*Brassica oleracea*	Necrotic lesions of stem base	70‡	12
F. compactum	BBA 65671	*Gossypium*	NK	ND	10
F. crookwellense	BBA 64297	*Triticum* sp. (stem base)	Damage of stem base	ND	23
F. ensiforme	BBA 64683	*Ipomoea batatas*	Potato pathogen	ND	250
F. equiseti	BBA 64814	*Secale cereale*	NK	ND	0
F. inflexum	BBA 63203	*Vicia faba*	Wilt	ND	0
F. lateritium	BBA 65090	*Triticum* sp. (stem base)	NK	400‡	15
F. merismoides	BBA 64329	*Secale cereale* (stem base)	NK	500‡	14
F. oxysporum f. sp. *pisi*	BBA 62057	*Pisum sativum*	Wilt	90*	0
F. oxysporum f. sp. *lycopersici*	BBA 62060	*Lycopersicon* sp.	Wilt	ND	5
F. oxysporum f. sp. *lupini*	BBA 62334	*Lupinus albus*	Wilt	40*	0
F. oxysporum f. sp. *batatas*	BBA 64952	*Ipomoea batate*	NK	90*	0
F. oxysporum var. *redolens*	BBA 62390	*Dianthus caryophyllus*	Wilt	ND	14
F. proliferatum	BBA 63625	*Dracaena deremensis*	Necrotic leaf spots	ND	14
F. scirpi	ETH 1536	Soil	Fruit rot, necrotic damage of grain stems	80‡	38
F. solani	BBA 64953	*Ipomoea batatas*	Root and stem rot	ND	87
F. solani f. sp. *pisi*	BBA 62420	*Pisum sativum*	Rot of stem base	ND	5
F. tricinctum	BBA 62446	*Trifolium pratense*	Root rot	90*	4
F. udum	BBA 62451	*Cajanus cajan*	Wilt	ND	0

[a] The fungal strains were obtained from the *Fusarium* collection of the Biologische Bundesanstalt Berlin (H. Nirenberg). K. M. Weltring, Universität Münster, provided *F. sambucinum* NRRL 13500 to R-6354. *F. scirpi* ETH 1536 was from the Eidgenössische Hochschule Zürich.

[b] As described by the suppliers of the strains. The pathogenic properties of *F. sambucinum* NRRL 13500 to R-6354 are given in reference 7.

[c] Enniatin production (micrograms per milliliter). Highest enniatin production in liquid FCM (*), solid FCM (**), solid FDM (†) and solid FDM containing fructose (‡) is indicated.

[d] Mean of the relative virulences from 12 single values obtained from three independent tests. The 2 most deviating values (i.e., the highest and the lowest) of the 12 values obtained for each strain were not considered in the calculation. Standard deviations of means were in the range of 10 to 20%. The virulence of the strains was compared with that of the enniatin producer *F. sambucinum* BBA 62397 (which was set at 100%, corresponding to 2.6 g of decomposed potato tissue per slice).

[e] NK, not known.

[f] ND, not detected at a detection limit of 15 μg/ml.

MATERIALS AND METHODS

Cultivation. *Fusarium* strains were cultivated in FDM (containing [per liter] 25 g of sucrose, 4.25 g of $NaNO_3$, 5 g of NaCl, 2.5 g of $MgSO_4 \cdot 7H_2O$, 1.36 g of KH_2PO_4, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.0029 g of $ZnSO_4 \cdot 7H_2O$) and FCM (3% molasses, 1% corn steep liquor) as described previously (18). FDM-fructose contained 1.25% (wt/vol) fructose instead of sucrose. For submerged cultures, 100 ml of medium in a 500-ml Erlenmeyer flask was inoculated with approximately 5×10^7 spores and incubated at 26°C with shaking at 120 rpm. Cultivation on solid agar medium was carried out at 26°C. After 6 days of incubation, fungal cultures were extracted with chloroform and enniatin was determined spectrophotometrically as described by Audhya and Russell (1).

DNA isolation. Nuclear DNA was purified from 200 mg of lyophilized mycelium (grown for 68 h in FDM) by the method of Möller et al. (19).

Southern blotting. Gel electrophoresis, Southern blotting, and hybridization were performed by standard methods (25). *Sal*I (GIBCO BRL, Eggenstein, Germany)-restricted chromosomal DNA was separated on 0.8% agarose gels in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer at 20 V overnight, and after transfer via a capillary blot in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), it was cross-linked to nylon membrane (Hybond-N; Amersham, Braunschweig, Germany) with UV light. A DNA probe was produced by labeling an internal 7.1-kb *Sal*I fragment from *esyn1* with [α-^{32}P]dCTP to a specific activity of approximately 10^8 cpm/25 ng (Random Primers DNA Labeling System; GIBCO BRL). Blots were hybridized in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–5× Denhardt's solution–0.1% (wt/vol) sodium dodecyl sulfate [SDS] at 65°C overnight and washed at moderate stringency (2× SSPE plus 0.1% SDS at 50°C).

In vitro effect of enniatins on potato tuber tissue. New potatoes (*Solanum tuberosum* cv. Sieglinde) were surface sterilized for 5 min in 1% sodium hypochlorite and washed three times in sterile water. After being dried, the potatoes were cut into slices approximately 6 mm thick and placed on Whatman 3MM paper, soaked with water, in petri dishes. On the center of the potato slices, 20-μl volumes of fusafungin dilutions (0.25 to 5 mg of a mixture of enniatins A, A_1, B, and B_1 per ml in a ratio of 5:15:35:45; a gift from A. Lohmann) in 20% ethanol were applied. After 6 days of incubation at 26°C, petri dishes with potato tuber tissue were weighed before and after removal of the necrotic tissue.

Virulence assay. The virulence of the fungal strains to potato tuber tissue was determined by a slight modification of the method described by Desjardins et al. (8). Slices of potato tuber tissue, prepared as described above, were each infected with a slice (10 mm in diameter, mycelial side down) of a 6-day-old *Fusarium* culture grown on FCM agar and incubated for 6 days at 25°C in the dark.

Virulence was determined by weighing the petri dishes before and after removal of the decomposed tissue. Values were obtained from three independent tests with four potato tuber slices per test for each strain. The two most deviating values for each strain were not considered in the calculation. Standard deviations of means were in the range of 10 to 20%.

RESULTS

Enniatin production by *Fusarium* strains. The 36 *Fusarium* strains characterized in this study were selected to represent a broad range of natural host plants and belong to 9 of the 12 *Fusarium* sections described by Nelson et al. (20). Twelve *F. sambucinum* isolates listed in Table 1 (NRRL 13500 to R-6354) were chosen because the virulence of these strains on potato tubers and their tolerance of the potato phytoalexins lubimin and rishitin (7, 8) were already known.

All *Fusarium* strains were tested for enniatin production in a complex medium (FCM) and two minimal media with different carbon sources (FDM [containing sucrose] and FDM-fructose) in liquid culture and on solid media. With the spectrophotometric assay applied (1), all the different types of enniatins at concentrations of >15 μg/ml of medium are detectable. Thirteen strains, belonging to seven of the nine sections of Nelson et al. (20) represented in this study, were able to produce enniatins with yields varying widely depending on the media used (Table 1). *F. sambucinum* BBA 63933, *F. lateritium* BBA 65090, and *F. merismoides* BBA 64329 produced large amounts of enniatins at concentrations of 350, 400, and 500 μg/ml, respectively. *F. avenaceum* BBA 64338 and *F. arthrosporioides* BBA 64134 produced 180 and 135 μg of enniatin per ml, respectively, whereas enniatin amounts less than 100 μg/ml were detected in eight strains. This is the first report of enniatin production by *F. merismoides*, which is assigned to the section *Eupionnotes* by Nelson et al. (20). *F. merismoides* produced predominantly enniatin B as proved by high-pressure liquid chromatography (HPLC) analysis (22). In general, enniatin production was higher on solid medium than in liquid culture. Cultivation in FDM-fructose resulted in higher enniatin production by some strains compared with growth in FDM (containing sucrose). For demonstrating enniatin production in the infected potato tubers, 1-day-old cultures of *Fusarium* strains were used for infection. After further incubation for 6 days, enniatins were recovered from tissue infected with *F. merismoides*, *F. lateritum*, *F. sambucinum* BBA 63933 and BBA 62397, *F. scirpi*, *F. avenaceum* BBA 64338, and *F. arthrosporioides* in the range of 500 μg/g (*F. merismoides*) to 45 μg/g (*F. arthrosporioides*) of necrotic tissue. Enniatins could not be recovered from tissues infected with enniatin nonproducers and avirulent low producers (enniatin production in culture, <90 μg/ml; relative virulence, <10%) such as *F. tricinctum.*

In vitro effect of enniatins on potato tuber tissue. Fusafungin, a mixture of enniatins A, A_1, B, and B_1 in a ratio of 5:15:35:45, was tested on potato tuber tissue by application of 5, 10, 20, 50, and 100 μg per slice. Approximately 30 min after application, a brown discoloration of the tissue occurred at levels of 10 μg of enniatin per slice or higher. After 6 days of incubation, necrotic lesions of the tissue were visible, depending on the amount of enniatin applied (Fig. 2). At low concentrations (5 to 20 μg per slice), only the superficial tissue was affected, whereas the necrotic damage increased at enniatin concentrations of 50 and 100 μg per slice. The amount of enniatin which caused necrosis of potato tuber tissue was similar to that recovered from the *Fusarium*-infected tissues (60 to 200 μg) and to that which resulted in reduced growth of wheat kernels during germination (5). On spotted knapweed, the minimum amount of enniatin B that caused lesions of wounded leaves was 25 μg (17).

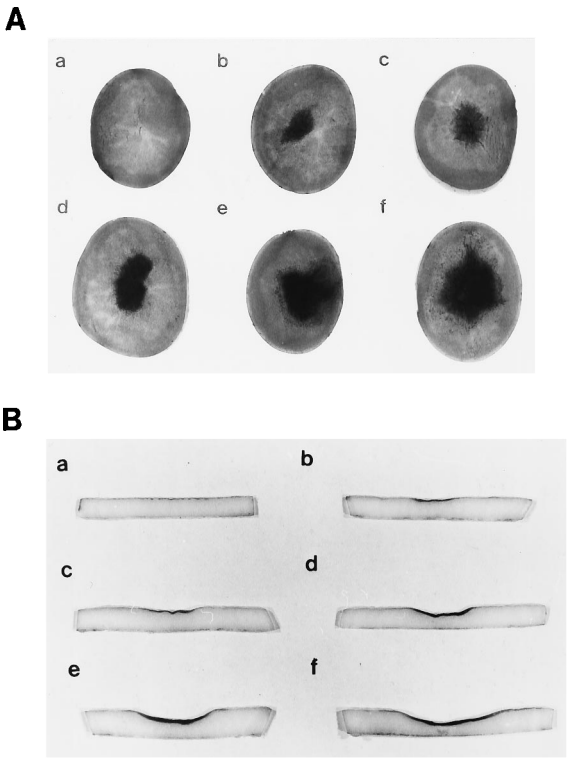

FIG. 2. In vitro effect of enniatin on potato tuber tissue viewed from above (A) and in section (B). (a) 20% ethanol as control; (b) 5 μg of fusafungin per slice; (c) 10 μg; (d) 20 μg; (e) 50 μg; (f) 100 μg.

Identification of *esyn1* homologs. A 7.1-kb fragment of *esyn1* from *F. scirpi* ETH 1536 (bp 1818 to 8920 of *esyn1*; accession number Z18755 in the EMBL nucleotide sequence database) was used as probe for Southern blots of genomic DNA (Fig. 3). DNA hybridizing with the *esyn1* fragment was detected in 34 strains examined after the blots were washed at moderate stringency, but the restriction pattern was not constant. Only *F. equiseti* BBA 64814 (lane 18) and *F. crookwellense* BBA 64297 (lane 34) did not show any hybridization, and *F. ensiforme* BBA 64683 Wollenweber and Reinking (12) (lane 26) and *F. solani* BBA 64953 (lane 28) showed only background levels. In general, the level of enniatin synthesis correlated with the signal intensity; i.e., enniatin producers had a stronger hybridization signal than did nonproducers. The DNA of *F. arthrosporioides* (lane 29) and the two strains of *F. avenaceum* (BBA 64338 [lane 17] and BBA 62163 [lane 31]), all enniatin-producing strains, gave a strong hybridization signal of the same molecular weight as that in *F. scirpi.*

The enniatin-producing *F. sambucinum* BBA 63933 (lane 11) and BBA 62397 (lane 25) had hybridization patterns that were different from that of the *F. scirpi* control. The DNAs of the enniatin-nonproducing strains of *F. sambucinum* (lanes 1 to 8) showed a hybridization pattern similar to each other and to that of the enniatin producer *F. sambucinum* BBA 62397 (lane 25), whereas the hybridization of *F. sambucinum* R-583 (lane 12), R-6112 (lane 14), and R-6380 (lane 15) appeared to be very weak.

Hybridization of the DNA of the enniatin-nonproducing strains to the *esyn1* fragment of *F. scirpi* was not detected under high-stringency conditions (0.1× SSPE plus 0.1% SDS at 65°C

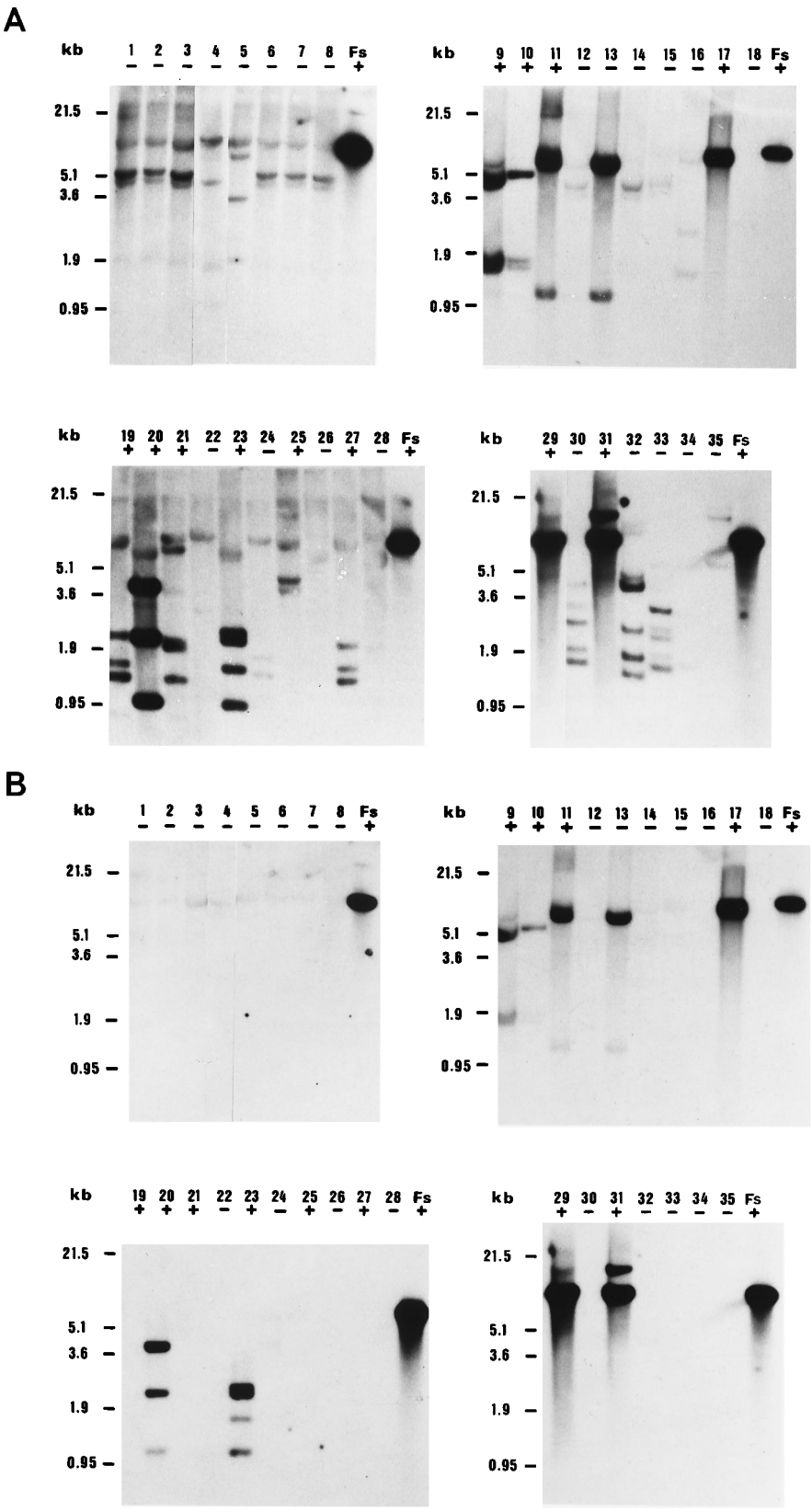
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FIG. 3. Southern blot analysis of *Sal*I-restricted genomic DNA showing sequences homologous to *esyn1* of *F. scirpi*. (A) Blots were washed at moderate stringency (2× SSPE plus 0.1% SDS at 50°C). Lanes: Fs, *F. scirpi* ETH 1536 J5; 1, *F. sambucinum* R-2633; 2, *F. sambucinum* R-5390; 3, *F. sambucinum* R-5455; 4, *F. sambucinum* R-6354; 5, *F. sambucinum* R-7570; 6, *F. sambucinum* R-7843; 7, *F. sambucinum* NRRL 13500; 8, *F. sambucinum* NRRL 13503; 9, *F. lateritium* BBA 65090; 10, *F. merismoides* BBA 64329; 11, *F. sambucinum* BBA 63933; 12, *F. sambucinum* R-583; 13, *F. sambucinum* R-5690; 14, *F. sambucinum* R-6112; 15, *F. sambucinum* R-6380; 16, *F. proliferatum* BBA 63625; 17, *F. avenaceum* BBA 64338; 18, *F. equiseti* BBA 64814; 19, *F. oxysporum* f. sp. *pisi* BBA 62057; 20, *F. acuminatum* BBA 62148; 21, *F. oxysporum* f. sp. *lupini* BBA 62334; 22, *F. solani* f. sp. *pisi* BBA 62420; 23, *F. tricinctum* BBA 62446; 24, *F. udum* BBA 62451; 25, *F. sambucinum* BBA 62397; 26, *F. ensiforme* BBA 64683 Schneider; 27, *F. oxysporum* f. sp. *batatas* BBA 64952; 28, *F. solani* BBA 64953; 29, *F. arthrosporioides* BBA 64134; 30, *F. oxysporum* f. sp. *lycopersici* BBA 62060; 31, *F. avenaceum* BBA 62168; 32, *F. oxysporum* var. *redolens* BBA 62390; 33, *F. inflexum* BBA 63203 Schneider; 34, *F. crookwellense* BBA 64297; 35, *F. compactum* BBA 65671. The sizes of marker DNA fragments (*Hin*dIII-*Eco*RI-digested λ DNA) are indicated on the left. (B) Blots were washed at high stringency (0.1× SSPE plus 0.1% SDS at 65°C). Lanes are identical to those in panel A.

[Fig. 3B]), but hybridization was detected with most of the enniatin producers and the nonproducer *F. sambucinum* R-5690. No hybridization was detected under these conditions with enniatin producers *F. sambucinum* BBA 62397 (lane 25) and the formae specialis of *F. oxysporum*, f. sp. *pisi* BBA 62057 (lane 19), f. sp. *lupini* BBA 62334 (lane 21), and f. sp. *batatas* BBA 64952 (lane 27).

Virulence of *Fusarium* species on potato tuber tissue. Since enniatins cause necrotic lesions of potato tuber tissue, all 36 strains were tested for virulence on slices of potato tubers. All of the more virulent (20 to 250% relative virulence) strains produced a muddy brown rot, and the less virulent (10 to 20%) strains produced a dry brown rot of the superficial tissue layers. Of the 36 strains examined, 23, including all strains from potato and 2 from sweet potato (*Ipomoea batatas*), had a relative virulence of at least 10% of that of the positive control strain *F. sambucinum* BBA 62397 (Table 1). Of the strains tested, *F. ensiforme* BBA 64683, which is a known potato pathogen (20a), was the only one whose virulence exceeded that of the *F. sambucinum* control. Of the 13 enniatin-producing strains, only 4 (*F. sambucinum* BBA 62397, *F. scirpi* ETH 1536, *F. avenaceum* BBA 64338, and *F. arthrosporioides* BBA 64134) affected potato tubers strongly (38 to 100% virulence); none of these 4 strains synthesized more than 180 μg of enniatin per ml. Three enniatin producers, *F. lateritium* BBA 65090, *F. merismoides* BBA 64329, and *F. avenaceum* BBA 62168, were less virulent (12 to 15%). Two *F. sambucinum* strains, NRRL 13500 and BBA 62397, differ in enniatin production but have similar virulence profiles on potato tuber tissue.

DISCUSSION

This is the first survey about enniatin production, the presence of enniatin synthetase gene homologs, and the virulence of a large number of *Fusarium* strains. Of the 36 *Fusarium* strains isolated from a wide variety of plants, 13 were able to synthesize enniatins in culture. Enniatins could be recovered from potato tuber tissue infected by six of these strains. Interestingly, all the strains synthesizing enniatins at levels of more than 100 μg/ml were isolated from cereal plants. Bishop and Ilsley (3) suggested that the production of enniatin is a useful criterion for confirming the identity of *Fusarium* strains. In our study, many different fusaria synthesized enniatins, indicating that enniatin production is widespread in this genus.

In general, the DNA of enniatin-producing strains showed strong hybridization to an enniatin synthetase (*esyn1*) gene fragment of *F. scirpi*, with the exception of *F. sambucinum* BBA 62397 and the formae specialis of *F. oxysporum*, which produced small amounts of enniatins (<100 μg/ml) in culture. Interestingly, *F. sambucinum* R-5690 did not produce enniatin but exhibited strong DNA homology to the *esyn1* gene. The lack of enniatin production in some *Fusarium* species exhibiting DNA homologies to the *esyn1* gene is not necessarily due to a general inability to synthesize enniatin. Enniatin production might be very low and not detectable by the assay used or might be influenced by differential responses to environmental conditions and differences in regulatory circuits. Furthermore, the existence of a structural gene family cannot be excluded.

A 1,476-bp DNA fragment (accession number Z48743 of the EMBL nucleotide sequence database) from *F. sambucinum* BBA 63933 producing enniatins A and A_1 (22) was isolated by PCR. Nucleotide sequence determination revealed an identity of 88.5% to the corresponding part (nucleotides 5890 to 7366) of the *esyn1* gene of *F. scirpi*. The deduced amino acid sequence showed 91% identity (6). Additionally, a 1,004-bp DNA fragment of the *esyn1* gene of the enniatin B producer *F. avenaceum* BBA 64338 was amplified by PCR. Sequencing of the PCR product showed the nucleotide sequences of *esyn1* fragments of *F. avenaceum* and *F. scirpi* (nucleotides 4882 to 5886 of *F. scirpi esyn1*) to be 99.9% identical (16), indicating that the structural genes of enniatin synthetases in these strains exhibit a high degree of similarity.

In contrast to *Fusarium* species, in which most of the enniatin-nonproducing strains exhibited at least low DNA homologies to the *esyn1* gene fragment of *F. scirpi*, the HC-toxin synthetase gene in *Cochliobolus carbonum* was detected only in isolates of the fungus that produce the toxin (21, 26). The cyclic tetrapeptide HC-toxin is, like enniatins, synthesized by a large, multifunctional enzyme. Unlike enniatins, HC-toxin is host selective and enables only toxin-producing isolates to infect particular genotypes of maize.

From the results of our virulence assays, it is clear that enniatin is not essential for the successful infection of potato tuber tissue by *Fusarium* strains. Pathogenicity is probably a complex process, depending on the expression of many genes, and plant resistance mechanisms may involve several distinct defense responses. Desjardins et al. (8, 9) proposed that the detoxification of the potato phytoalexins lubimin and rishitin by *Fusarium* isolates is insufficient to ensure virulence on potato tubers since some strains metabolizing rishitin and lubimin in vitro are not highly virulent. Mutants of *F. sambucinum* R-6380 that did not produce the host-nonspecific phytotoxic trichothecenes were obtained by disruption of the gene encoding trichodiene synthase. The virulence of the non-trichothecene-producing strains was significantly reduced on parsnip root slices but not on potato tubers (9).

Enniatin production, although not required for the infection of potato tuber tissue by *Fusarium* species, could still play a role in the pathogenicity process of the strains that do produce it. Enniatin-nonproducing cultures of *F. scirpi* ETH 1536 were less virulent on potato tubers than was the enniatin producer (16). Together with the cloned enniatin synthetase gene (*esyn1*), the characterization of a wide variety of *Fusarium* strains with respect to enniatin production, *esyn1* homologs, and virulence should enable a critical genetic analysis of whether enniatins are in fact involved in the pathogenicity process.

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