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Genetic Nature, Stability, and Improved Virulence of Hybrids from Protoplast Fusion in *Beauveria*

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Abstract. Genetic improvement of two different strains of the entomopathogenic fungus *Beauveria bassiana* for more effective control of *Ostrinia nubilalis* and *Leptinotarsa decemlineata* was obtained by crosses with the insecticidal toxin-producing strain *Beauveria sulfurescens*. Protoplast fusion between diauxotrophic mutants resulted in the recovery of some stable prototrophic fusion products. The low levels of virulence of the wild type strain *B. bassiana* 28 isolated originally from *L. decemlineata* were enhanced both on *L. decemlineata* and *O. nubilalis* for one of the hybrids obtained (FP 8) from the cross *B. bassiana* 28 × *B. sulfurescens* 2. Fusion product 25 obtained from the cross between *B. sulfurescens* and the highly pathogenic strain *B. bassiana* 147 showed a three-day reduction in the LT_{50} towards *O. nubilalis*. Southern blot hybridization with nine probe–enzyme combinations were conducted on genomic DNAs from the original wild strains, parental mutant strains, and fusion products. Additive banding patterns or unique banding pattern of either parental strain was observed in five hybrids, indicating their status as recombinant and/or partially diploïd. Combination of RFLP markers indicative of both parental genomes was never observed with fusion product FP 25. The stability of the virulence following passage through insect-host and stability of molecular structure for the fusion products FP 8 and FP 25 suggest that asexual genetic recombination by protoplast fusion may provide an attractive method for the genetic improvement of biocontrol efficiency in entomopathogenic fungi.

Introduction

Parasexual recombination not only facilitates genetic analysis in asexually reproducing fungi, but also provides an important tool in strain improvement of bioprotectant fungi. Because of the great potential of *Beauveria* strains in the biological control of pests, the attempts at controlled protoplast fusion to initiate the parasexual cycle are increasing in importance [4]. Paris [12] first reported the production of heterokaryons by protoplast fusion between auxotrophic mutants of *Beauveria tenella*. Since then, both intraspecific and interspecific fusions and parasexual

recombination have been reported in other members of the genus *Beauveria* [4, 6, 10, 11, 17]. However, reports on intra- and interspecific protoplast fusion between mutants of various entomogenous fungi have not discussed genetic improvement in the virulence of these fungi; generally, fusion products showed intermediate or reduced pathogenicity, or at best were similar to the parent wild-type isolates [16]. The main aim of our study was to increase the virulence of two different strains of *B. bassiana* towards *Ostrinia nubilalis* and *Leptinotarsa decemlineata* by hybridization with a *Beauveria sulfurescens* strain known for its specific production of a high entomopathogenic toxin [8]. The molecular analysis of fusion products by Southern hybridization with various DNA probes, and the analysis on their stability through successive pathogenic cycles are presented.

Materials and Methods

Strains and Culture Conditions

The following *B. bassiana* strains from the INRA culture Collection, La Minière, France, were used: *B. bassiana* 28 selected on the basis of its virulence against the Colorado potato beetle *L. decemlineata* [2]; *B. bassiana* 147 selected on the basis of its virulence against the European corn borer *O. nubilalis* [14]. The strain *B. sulfurescens* 2 was a gift of M.J. le Petit, Marseille University, France, 1981 (may be same strain as *Sporotrichum sulfurescens* CBS 209.27 and *Beauveria bassiana* ATCC 7159). Virulence of strains *B. bassiana* 28 and *B. bassiana* 147 towards *O. nubilalis* are low and very high, respectively, whereas the toxicogenic strain *B. sulfurescens* 2 is nonpathogenic toward *O. nubilalis* and *L. decemlineata.* Culture media were complete agar medium (CM) and minimal medium (MM) previously described by Riba and Ravelojoana [15]. Incubation was at 25°C throughout. Double auxotrophic mutants, *B. bassiana* 147-20-1 requiring methionin and adenine, *B. bassiana* 28-14-554 requiring adenine and panthotenic acid, and *B. sulfurescens* 12-431 requiring lysine and leucine, were selected after treatment of wild strains with sequential mutagenesis: 3% ethylmethane sulfonate (EMS), 2 h incubation, and 10% N-methyl-N′-nitro-N nitrosoguanidine, 2 h incubation, as described by Riba and Ravelojoana [15]. Mutants were cultured and maintained on minimal medium amended with necessary vitamins (5 $\mu g\ ml^{-1}$), amino acids (200 $\mu g\ ml^{-1}$), or nucleotides (50 $\mu g\ ml^{-1}$).

Protoplast Isolation

Mycelium cultivated for 24 h on complete medium with a cellophane disk was harvested, washed and resuspended (40 mg ml^{-1}) in isotopic buffer (0.6 M KCl, 0.1 M sodium phosphate, pH 5.8), before treatment with 0.75 mg g^{-1} sterile β-glucanase (Glucanex, Novoferment; Rueil-Malmaison, France) for 4 h with gentle shaking. The protoplasts were separated from mycelial debris by filtration through a sterile 30-μm nylon muslin and finally collected by centrifugation at 5000 *g* for 15 min and washed twice in 2 ml 0.6 M KCl.

Fusion Experiments

About 10^7 protoplasts of each *Beauveria* strain were mixed by centrifugation (5000 *g* for 15 min) and the pellet was incubated in 1 ml 20% polyethylene glycol (PEG 6000) containing 0.01 M $CaCl_2$ and 0.05 M glycine (pH 7.5) for 30 min at 25°C. Protoplasts were washed twice with 0.6 M potassium phosphate buffer, and 0.1-ml aliquots of the protoplast suspension were plated on minimal medium agar supplemented with 20% sucrose (MMS). Parental protoplasts were subjected separately to the same PEG treatment and regeneration process.

Analysis of Progeny Genotypes

A part of the cross progeny was screened by plating on minimal medium to select prototrophic progeny. In one experiment the regenerated mycelium did not sporulate; so, it was scraped from plates into water, homogenized in a vortex mixer, and plated onto MM. To test mitotic stability of progeny genotypes, each putative prototrophic colony obtained on MM was purified from single conidia and transferred onto CM. This procedure was repeated for at least four serial single colony passages onto CM. Several colonies produced large parental sectors during conidiogenesis cycles while others conserved the same phenotype. These putative stable prototrophic fusion products were submitted to the haploidizing agent benomyl at the concentration of 0.75 ppm [3]; thereafter, 100 individual conidia of each of these fusion products were assayed for phenotypic stability on MM.

Pathogenicity of Prototrophic Fusion Products

Pathogenicity towards the European corn borer, *O. nubilalis,* by the prototrophic fusion products was compared to that of the original wild type strains *B. bassiana* 28 and *B. bassiana* 147 and diauxotrophic parental stains. Newly emerged fifth instar larvae were dipped in conidial suspensions and insect mortality was assessed daily [14]. Pathogenicity towards *L. decemlineata* of fusion products from the cross *B. bassiana* 28 × *B. sulfurescens* 2 was compared to that of the parental strains *B. bassiana* 28 and *B. bassiana* 28.14-554 by spraying conidial suspensions on third instar larvae of *L. decemlineata* [2].

Concentrations of conidial suspensions were 1.10^8 and 4.10^6 conidia ml^{-1}, respectively, in the crosses *B. bassiana* 28 × *B. sulfurescens* 2 and *B. bassiana* 147 × *B. sulfurescens* 2, and each treatment consisted of 4 replicates of 20 larvae. To assess virulence of the spore suspensions, probit analysis was used to estimate the time taken to kill 50% of the insect population (LT_{50}).

DNA Isolation and Molecular Analysis

Genomic DNA prepared from parental strains and from stable fusion products grown in liquid medium were subjected to Southern hybridization analysis. DNA from each isolate was digested separately with the restriction enzymes *Pst* I, *Hind* III, and *Eco*R I. The restriction enzymes were chosen in order to obtain different restriction profiles that allowed a molecular characterization of the parental strains. Digestions were conducted according to the manufacturer's instructions. DNA isolation, DNA electrophoresis, Southern blotting, prehybridization, and hybridization conditions were as described by Daboussi et al. [1].

Probes were obtained from partial libraries made in pUC 19 out of DNA fragments from *B. bassiana* 147 strain, digested with *Sau* 3A or *Eco*R I. Probe 24-5 (7.5 kb) was hybridized to DNAs with *Pst* I and *Hind* III, probes T1-37 (2.5 kb), T2-22 (1.5 kb), T7-12 (0.9 kb), and 600-3 (600 bp) were hybridized to DNAs digested with *Pst* I, probe T7-40 (1 kb) to *Hind* III digested DNAs, and probes 600-6 (600 bp) and 600-7 (600 bp) to *Eco* RI digested DNAs. Molecular analyses were performed in duplicate.

Stability after Parasitic Growth on Insects

Three prototrophic fusion products and their corresponding parental wild type strains were passed through four disease cycles on successive generations of the host insect *O. nubilalis.* After each disease cycle, single spores recovered from the cadavers of insects were cultured on CM before inoculation of a new insect generation. After 4 generations, 100 individual conidia were plated on minimal medium to determine the percentage of prototrophic conidia. Virulence of each prototrophic fusion product after four disease cycles on *O. nubilalis* was compared to that of the original isolate. To examine genotypic stability, DNA was isolated from single conidial cultures and analyzed by the Southern blot procedure.

Entomotoxic Activity

Fusion product 25 and the parental strains *B. bassiana* 147 and *B. sulfurescens* 2 were grown as a submerged culture in MY 20 medium (maltose, 4%; pancreatic peptone, 1%; yeast extract, 20%). Cultures were incubated (100 rpm) for 4 days at 25°C and filtered through Whatman paper (Prolabo, France). Filtrate was then concentrated tenfold by precipitation of proteins in ammonium sulfate (600 g l^{-1}) and centrifugation (9000 tr min^{-1}, 30 min) and fungal biomass evaluated. After 24-h dialysis against water at 4°C and sterilization by filtration (0.22 μm), 20 fifth instar larvae were injected with 10 μl filtrate. Dead larvae were checked daily for 3 days.

Results

Fusion of protoplasts from diauxotrophic mutant strains of *B. bassiana* and *B. sulfurescens* resulted in the recovery of prototrophic fusion products at a frequency ranging from 1×10^{-6} to 1×10^{-7}; no colonies appeared on MM from protoplasts of only one of the fusion partners, suggesting that at the concentrations used no back mutations occurred. One to four weeks were usually required before progeny were sufficiently large to be visible. Among the 13 putative fusion products obtained from the two cross experiments of *B. bassiana* 147 × *B. sulfurescens* 2, only three of these fusion products continued to grow rapidly when subcultured on CM; they were indistinguishable in appearance from the prototrophic strains they were derived from and did not produce sectors upon subculturing. Progeny from the cross *B. bassiana* 28 × *B. sulfurescens* 2 were recovered from a small, wrinkled colony that developed very slowly with very distorted mycelium and sparse sporulation. By vortexing this fusion product and subculturing on MM, ten spontaneously prototrophic colonies were selected. After four transfers on nonselective medium, the morphology of these colonies remained stable. In the same way, fusion products obtained from the different crosses were morphologically stable and showed no sign of sectoring when benomyl was added to CM.

Analysis of mitotic stability on MM, pathogenic activity and molecular analysis were conducted on a random choice of three stable fusion products of the cross *B. bassiana* 28 × *B. sulfurescens* 2 and the three selected from the cross *B. bassiana* 147 × *B. sulfurescens* 2. All one hundred single conidial isolates from each fusion product exhibited prototrophic phenotype by subculturing on minimal medium after four serial single colony passages onto CM.

Pathogenic activity of parental strains and stable fusion products was assessed by bioassays under standardized conditions. The different LT_{50} values are shown in Table 1. Whatever the cross, no insects were infected when spore suspensions of the wild strain *B. sulfurescens* 2 or parental mutant strain *B. sulfurescens* 12-431 were applied to larvae of *O. nubilalis* or *L. decemlineata.* Reduction in pathogenicity was associated with the introduction of auxotrophic markers in wild strains; diauxotrophic mutants of the parental strains *B. bassiana* 28 and *B. bassiana* 147 appeared to be weakly pathogenic strains. The pathogenicity of the different fusion products varied. Assessment of the pathogenicity on *O. nubilalis* of fusion products from the cross *B. bassiana* 28 × *B. sulfurescens* 2 showed that the estimated LT_{50} values were high, i.e., greater than 20 days for FP 3 and FP 5, whereas the third fusion product FP 8 showed a lower LT_{50} than the parental wild strain *B. bassiana* 28. In the same way, the lowest LT_{50} value on *L. decemlineata* was

Table 1. Virulence of parental strains, mutant strains, and fusion products of *B. bassiana* and *B. sulfurescens* against *Ostrinia nubilalis* and *Leptinotarsa decemlineata*. Eighty insects were infected per treatment by immersion in a conidial suspension (1.10^8 conidia ml^{-1} in the cross *B. b.* 28 × *B. s.* 2 and 4.10^6 conidia ml^{-1} in the cross *B. b.* 147 × *B. s.* 2)

Strain	Time taken to kill 50% of the insects (LT_{50})		
Cross B. b. 28 × *B. s* 2	*Ostrinia nubilalis*	*Ostrinia nubilalis* (four disease cycles)	*Leptinotarsa decemlineata*
B. b. 28	16.8 ± 3.0	11.9 ± 0.8	14.2 ± 1.0
B. s. 2	>25	ND[a]	>25
B. b. 28-14-554	>25	ND	>25
B. s. 2-12-431	>25	ND	>25
FP 3	>25	ND	12.1 ± 1.1
FP 5	20.6 ± 3.6	13.9 ± 1	13.2 ± 1.1
FP 8	6.6 ± 0.7	5.4 ± 0.8	10.4 ± 0.9
Cross *B. b.* 147 × *B. s.* 2			
B. b. 147	8.2 ± 0.3	6.4 ± 0.7	
B. s. 2	>25	ND	
B. b. 147.20.1	>25	ND	
B. s. 2 12.431	>25	ND	
FP 19	>25	ND	
FP 20	>25	ND	
FP 25	4.7 ± 0.3	6.1 ± 0.7	

[a] ND, not determined

observed with this fusion product, whereas the LT_{50} values for FP 3 and FP 5 were of the same magnitude as that of the wild strain *B. bassiana* 28.

Fusion products FP 19 and FP 20 from the cross *B. bassiana* 147 × *B. sulfurescens* 2 were weak pathogens compared to the wild strain *B. bassiana* 147; conversely, FP 25 exhibited a higher virulence than the wild strain, which was selected because it causes high levels of corn borer mortality in a short period of time.

Genomic DNAs prepared from original wild strains, parental mutant strains, and different fusion products were subjected to Southern blot hybridization. Polymorphisms existed within the parental strains for each of the nine probe–enzyme combinations tested. Examples of the different RFLP patterns are shown in Fig 1. DNA from *B. bassiana* 28, *B. bassiana* 28 14-554, *B. bassiana* 147, *B. bassiana* 147-20-1, the three fusion products FP 19, FP 20, and FP 25 was digested with *Pst*I and produced two bands at 3.5 kb and 5.5 kb that hybridized to the probe 24-5, while isolates of *B. sulfurescens* 2 and *B. sulfurescens* 2 12-431 displayed a different pattern with bands at 3.5 kb and 7 kb. Patterns of the three fusion products from the cross *B. bassiana* 28 × *B. sulfurescens* 2 (FP 3, FP 5, FP 8) shared these two fragment lengths, but also yielded a third band (5.5 kb) that was present in original wild and parental mutant strains of *B. bassiana*. These RFLP patterns showed that hybridization had taken place with bands characteristic of both parents readily distinguishable in the fusion products. Interpreation of biparental banding patterns was supported by results from the probe–enzyme combinations pT2-22 *Pst*I, p600-3 *Pst*I, p24-5 *Pst*I, and p600-7 *Eco*R I (Table 2). Nevertheless, four other probe–enzyme combinations revealed a unique banding pattern characteristic of the parental strain *B. bassiana*, and the combination p600-6 *Eco*R I revealed the same banding pattern as that of the parental strain *B. sulfurescens*;

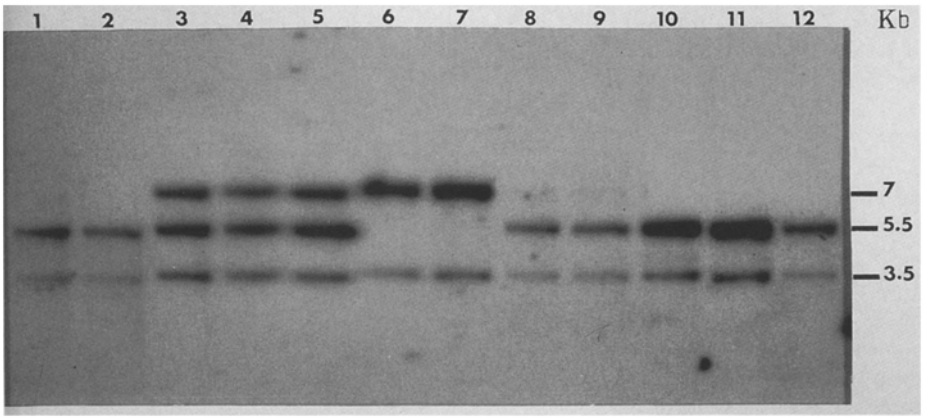

Fig. 1. Restriction fragment length polymorphism patterns of wild strains, parental mutant strains, and fusion products. *Pst* I digested genomic DNA fragments were hybridized with the probe 24-5 (7.5 kb). Wild strains: lane 1 (*B. b.* 28), 6 (*B. s.*), 11 (*B. b.* 147); parental mutant strains: lane 2 (*B. b.* 28-14-554), 7 (*B. s.* 12-431), 12 (*B. b.* 147-20-1); fusion products from cross *B. b.* 28 × *B. s.* 2: lane 3 (FP 3), 4 (FP 5), 5 (FP 8); fusion products from cross *B. b.* 147 × *B. s.* 2: lane 8 (FP 19), 9 (FP 20), 10 (FP 25).

these RFLP patterns are indicative of the partially diploid structure of the hybrids from the cross *B. bassiana* 28 × *B. sulfurescens* 2. Profiles of the fusion products from the cross *B. bassiana* 147 × *B. sulfurescens* 2 revealed only parental banding patterns; RFLP banding patterns for FP 19 and FP 20 showed bands characteristic of the parental strain *B. bassiana* with five probe–enzyme combinations, whereas the banding patterns obtained by Southern hybridization with the five other probe–enzyme combinations appeared identical to that of the parental strain *B. sulfurescens*. In every case, the banding pattern of FP 25 appeared identical to that of the parental strain *B. bassiana*; so the different RFLP markers used have not established the hybrid status of FP 25.

As the fusion product FP 25 showed a reduction in the LT_{50} compared to that of the highly virulent parental strain *B. bassiana* 147, a comparison of the entomotoxic activity of parental wild strain, parental mutant strain, and FP 25 was carried out. Toxicity was evaluated by the percentage of larval mortality determined three days after injection of a dialyzed culture of each isolate. Levels of *O. nubilalis* mortality were, respectively, 100% and 55% for the toxinogenic parental strains *B. sulfurescens* 2 and *B. sulfurescens* 2 12-431, and 10 and 20% for the parental strains *B. bassiana* 147 and *B. bassiana* 147-20-1, whereas 70% of larvae were killed in the same time by the entomotoxic activity of FP 25. Compared to the low level obtained for the *B. bassiana* strains, this high level of mortality suggested that FP 25 would have acquired the whole or part of the entomotoxic activity from the parental toxinogenic strain *B. sulfurescens*.

The pathogenic stability of the fusion products FP 5, FP 8, and FP 25 was evaluated through infestation of the insect host *O. nubilalis* followed by reisolation of single conidial isolates from mycosed cadavers; four successive inoculation/reisolation experiments were performed. Single conidial isolates from mycosed cadavers revealed the mitotic stability of the studied fusion products.

Table 2. Comparison of banding patterns obtained by Southern blot analysis using various probe–enzyme combinations: b: specific pattern of parental strain *B. bassiana* s: specific pattern of parental strain *B. sulfurescens* bs: bi-parental banding pattern (*B. bassiana* and *B. sulfurescens*)

Probe–enzyme combination	Wild and parental strains						Fusion products					
	B.b. 28	B.b. 28 14-554	B.b. 147	B.b. 147 20-1	B.s. 2	B.s. 2 12-431	FP3	FP5	FP8	FP19	FP20	FP25
p 24-5 *Pst*I	b1	b1	b1	b1	s1	s1	b1s1	b1s1	b1s1	b1	b1	b1
p T1-37 *Pst*I	b2	b2	b2	b2	s2	s2	b2	b2	b2	s2	s2	b2
p T2-22 *Pst*I	b3	b3	b3	b3	s3	s3	b3s3	b3s3	b3s3	s3	s3	b3
p T7-12 *Pst*I	b4	b4	b5	b5	s4	s4	b4	b4	b4	s4	s4	b5
p 600-3 *Pst*I	b6	b6	b7	b7	s5	s5	b6s5	b6s5	b6s5	b7	b7	b7
p 24-5 *Hind*III	b8	b8	b8	b8	s6	s6	b8s6	b8s6	b8s6	b8	b8	b8
p T7-40 *Hind*III	b9	b9	b9	b9	s7	s7	b9	b9	b9	b9	b9	b9
p 600-6 *Eco*R I	b10	b10	b10	b10	s8	s8	s8	s8	s8	s8	s8	b10
p 600-7 *Eco*R I	b11	b11	b11	b11	s9	s9	b11s9	b11s9	b11s9	s9	s9	b11

Virulence of FP 5 and FP 8 after four disease cycles on insect hosts were lower or corresponded closely to the values obtained in the corresponding previous experiments, whereas virulence of FP 25 was similar to that of the wild strain *B. bassiana* 147.

DNAs from these three fusion products and original parental strains were digested with *Eco*R I and *Pst* I, subjected to Southern blotting, and probed with p 600-7, p T1-37, and p 24-5. The original configuration of the banding patterns was retained in all cases, indicating that no significant rearrangement had occurred through mitosis and passage through the host insect.

Discussion

This is the first report of fusion between strains of *B. sulfurescens* and *B. bassiana*. The cell fusion protocol was successful as prototrophs were recovered by protoplast fusion from paired complementary diauxotrophic mutants of each strain but not from the parental mutant itself. Fusion frequency was low compared to that recorded previously in *B. bassiana* fusion experiments [6], but similar to that obtained in other studies involving interspecific crosses in the genus *Beauveria* [17]. This low yield of fusion products could be explained by both vegetative incompatibility observed between *B. bassiana* and *B. sulfurescens* strains used in this study and genetic distance relating their genomes (unpublished results). Segregation of fusion products appeared to take place rapidly, since the first plating from fusion products onto nonselective complete medium generated apparently stable segregants. Three representative fusion products from the different interstrain crosses were analyzed for mitotic stability by subculturing onto nonselective medium for four generations then assayed for prototrophy on MM. Prototrophy and colony morphology were similar in the original progeny and all single-conidial isolates prepared from it. Similar stability was observed when pathogenic fusion products were propagated for four disease cycles on the host insect *O. nubilalis,* and no additional segregation was seen following exposure to the haploidizing agent benomyl. This stable prototrophic growth could suggest the hybrid status of fusion products selected from the crosses *B. bassiana* 28 × *B. sulfurescens* 2 and *B. bassiana* 147 × *B. sulfurescens* 2; Gadau and Lingg [4] pointed out that both diploids and aneuploids typically grow faster than heterokaryons on minimal medium, and on complete medium diploids are typically more stable than heterokaryons. Analysis of RFLP patterns confirmed that hybridization had taken place with bands characteristic of both parents being readily distinguishable in the fusion products FP 3, FP 5, and FP 8 from the cross *B. bassiana* 28 × *B. sulfurescens* 2, and FP 19 and FP 20 from the cross *B. bassiana* 147 × *B. sulfurescens* 2; conversely, recombinant RFLP markers were never observed with FP 25 from the cross *B. bassiana* 147 × *B. sulfurescens* 2. Single locus and multilocus RFLP data indicated that at least part of the genome of both parents were present in five hybrids. Recombinant status of FP 19 and FP 20 is demonstrated by the unique banding pattern of either parental strain that was observed whatever the probe–enzyme combination used. Moreover, the additive banding pattern observed, especially for FP 3, FP 5, and FP 8 with four probe–enzyme combinations, suggests the partially diploid structure of these hybrids. These different data indicate that whole genomes from both parents had not been

successfully integrated into the progeny but gave some evidence for genetic recombination. As recombination of genetic characteristics from both parents is indicative of parasexuality, we can argue that a heterokaryon was formed, and subsequently, a diploid due to nuclear fusion occurred following protoplast fusion. The diploid state of the parasexual cycle would be almost transient; parameiosis [18] would occur spontaneously resulting in either recombinants or heterozygous diploid intermediates. Appearance of novel isozyme banding patterns in interspecific progeny following protoplast fusion has also been seen in the genera *Aspergillus* [7] and *Pseudocercosporella* [5].

RFLP patterns indicative of a heterozygous diploid or recombinant were not detected for FP 25. It is possible that small portions of DNA from the parental strain *B. sulfurescens* are present but not detected with the nine probe–enzyme combinations used. The high level of entomotoxic activity of FP 25 is consistent with this hypothesis. However, as the toxin of the parental strain *B. sulfurescens* has not been purified, we cannot definitely rule out the possibility that genetic recombination in the parental strain *B. bassiana* may be bringing together genes in appropriate combination to lead to higher virulence. A similar explanation proposes that the weak virulence toward *O. nubilalis* of hybrids FP 3, FP 5, FP 19, and FP 20 could be due to the loss of a gene or genes. Conversely, the hypervirulence of FP 8 toward *L. decemlineata* and *O. nubilalis* could be explained by RFLP patterns that are indicative of expression of both parental genomes in the hybrid. Fast kill of insects combined with stability of the virulence following passage through the insect host, and stability of molecular structure observed for FP 8 and FP 25 are important advantages for biocontrol strains. Additional studies on the genetic nature of these fusion products are currently underway; karyotype comparison of intact chromosomes between parentals and fusants and use of probes for genomic mapping will allow us to analyze in depth the genetic basis of improved biocontrol ability. Taking into account the great genetic variability of the species *Beauveria* [9, 13], some strains having a wide spectrum of activity whereas other strains are able to control only specific insects, it can be assumed that mitotic recombination via protoplast fusion provides an attractive method for the genetic improvement of biocontrol efficiency in these entomopathogenic fungi. Moreover, combined with molecular analysis of genetic structure of the fusion products, this methodology should provide tools for exploration of genes involved in enhancement of virulence.

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