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Genetic diversity in wild and laboratory populations of *Heterorhabditis bacteriophora* as determined by RAPD-PCR analysis

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Summary – Genetic variation in laboratory reared biocontrol agents may be reduced due to founder effect, inbreeding, and selection. We used random amplified polymorphic DNA (RAPD-PCR) to compare genetic variation in two strains of *Heterorhabditis bacteriophora*. One strain (IS5) was recently isolated from the field and the other strain (HP88) has been reared under laboratory conditions for over 10 years. For each strain, fifteen inbred lines were generated by eight cycles of selfing of a single hermaphrodite (reaching > 90 % homozygosity). Genomic DNA from each of the inbred lines was screened with fourteen decamer primers. Genetic variation was calculated based on average percentage similarity of DNA banding patterns and cluster analysis. The level of within population variation detected did not differ significantly between the two strains.

Résumé – ***Diversité génétique chez des populations sauvages et des populations élevées au laboratoire d'*Heterorhabditis bacteriophora *telle que révélée par l'analyse en RAPD-PCR*** – La variabilité génétique d'agents de contrôle biologique élevés au laboratoire peut avoir des causes limitées à l'effet d'établissement, les croisements internes et la sélection. Les auteurs ont utilisé l'analyse en RAPD-PCR pour comparer la variabilité génétique de deux souches d'*Heterorhabditis bacteriophora*, l'une (souche IS5) récemment isolée du champ, l'autre (HP 88) élevée au laboratoire depuis 10 ans. Pour chaque souche, quinze lignées consanguines ont été produites grâce à huit cycles d'autofécondation d'un seul hermaphrodite (atteignant ainsi plus de 90 % d'homozygotie). L'ADN génomique de chaque lignée consanguine a été testé à l'aide de quatorze amorces décamères. La variabilité génétique a été calculée en se fondant sur la moyenne des pourcentages de similarité des profils d'ADN et sur une analyse en grappes. Les niveaux de variabilité interne de chacune des populations ne diffèrent pas significativement entre eux.

Key-words : biological control, diversity, entomopathogenic nematodes, genetic variation, RAPD.

Organisms that reproduce under non-natural conditions may lose genetic variation (Hartl & Clark, 1989). Such a phenomenon may lead to a decline in fitness. For example, inbreeding has been reported to cause yield reductions in crop plants and losses in fecundity and survivability in rats (Hartl & Clark, 1989). Similarly, reduction in genetic variation during laboratory rearing of biological control agents can jeopardize the use of these organisms in pest control. For example, genetic changes in laboratory-reared parasitic Hymenoptera have been reported to cause deficiencies in fecundity and longevity (Geden *et al.*, 1992), and in host acceptance and suitability (Van Bergeijk *et al.*, 1989). Furthermore, conservation of genetic variation in biological control agents is required for the success of genetic improvement programs involving artificial selection (Gaugler, 1988).

Soil dwelling nematodes in the genus *Heterorhabditis* have many promising traits as biological control agents including wide host ranges, easy storage, non-toxicity to mammals, host seeking ability, and a symbiosis with a pathogenic bacterium (*Photorhabdus luminescens*) which enables the nematodes to kill their hosts rapidly (Georgis & Manweiler, 1994). The nematodes generally enter their host as infective juveniles and complete two to three life cycles before exiting (Poinar, 1990). The first generation adults of *H. bacteriophora* are exclusively hermaphroditic and subsequent generations are mixed automictic and hermaphroditic forms (Strauch *et al.*, 1994; Koltai *et al.*, 1995).

The genetic variation in *Heterorhabditis* spp. has been hypothesized to be low due to hermaphroditic reproduction (Downes & Griffin, 1996). The objec-

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tive of the present study was to evaluate genetic variation in two strains of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar: the recently isolated IS5 strain and the HP88 strain, which has been reared under laboratory conditions for over 10 years.

Materials and methods

NEMATODES

Genetic variation was characterized in two strains of *H. bacteriophora*. The HP88 strain of *H. bacteriophora* was originally isolated from a single scarab larva (*Phyllophaga* sp.) in Logan, Utah, USA in 1982 (Poinar & Georgis, 1990). Since that time the strain has been reared continuously *in vivo* or *in vitro* (Friedman, 1990). We obtained our population of this strain from Ecogen, Inc. (Langhorne, PA, USA).

The IS5 strain was isolated from the Negev desert region of Israel in 1994 (Glazer *et al.*, 1996). The nematodes were recovered from soil samples using larvae of the greater wax moth, *Galleria mellonella* (L.) in a live baiting technique (Fan & Hominick, 1991). After the recovery of the first sample of this isolate from that site, and the initial characterization of its heat tolerance ability (Glazer *et al.*, 1996), fifteen additional soil samples were taken from the same spot. The founding population of IS5 used for the present study was constructed by pooling the nematodes from these samples. The IS5 nematodes were then reared in *G. mellonella* larvae for six passages at 30 °C using standard laboratory protocols (Woodring & Kaya, 1988). Morphological examination and measurements of the adults and infective juveniles from IS5 did not distinguish this new isolate from other *H. bacteriophora* nematodes (Glazer *et al.*, 1996). Further evidence that IS5 is a strain of *H. bacteriophora* was recently obtained when we demonstrated, using genetic markers, that nematodes from IS5 and the HP88 strain of *H. bacteriophora* can cross breed to produce fertile progeny (Shapiro *et al.*, 1997).

CREATION OF INBRED LINES

The genetic variation of the nematodes was evaluated by creating fifteen inbred lines for each of the strains. Creation of inbred lines was based on the procedures described in Glazer *et al.* (1991). Briefly, infective juveniles were surface sterilized and inoculated onto Nematode Growth Medium (Brenner, 1974) in 50 cm Petri dishes. Prior to nematode inoculation, the medium had been seeded with symbiotic bacteria (*P. luminescens*). After approximately 72 h of incubation at 25 °C each of the resulting nematode progeny, which were exclusively hermaphrodites, was transferred to new medium (one nematode per Petri dish). This process was repeated seven times resulting in inbred lines that were highly (> 95 %) genetically homogeneous (Hartl & Clark, 1989). To obtain sufficient numbers of nematodes for DNA extraction, nematodes from the final round of selfing were twice passed through *G. mellonella* larvae.

DNA EXTRACTION AND PCR ANALYSIS

DNA extraction was based on protocols described by Hashmi *et al.* (1996). Approximately one million adult nematodes were crushed in liquid nitrogen and then digested in 10 ml extraction buffer for 1 hour at 50 °C (Hashmi *et al.*, 1996). Protein was removed by phenol extractions and DNA was precipitated in ethanol.

The DNA from each inbred line was analyzed using random amplified polymorphic DNA markers (RAPD) (Welsh & McClelland, 1990; Williams *et al.*, 1990). Total volume for each reaction was 20 µl. Each reaction mixture contained 2 µl of Promega (Madison, WI) 10X assay buffer (500 mM KCl, 100 mM Tris-HCl, and 1 % Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM of each of the four dNTPs, 1.5 µM decamer primer, 0.5 units Taq DNA polymerase (Promega), and 20 ng DNA. The following fourteen Operon (Alameda, CA, USA) primers were chosen because they were previously shown to be suitable for RAPD analysis of *Heterorhabditis* DNA (Hashmi *et al.*, 1996): A01, A02, A03, A13, A18, C06, C09, C11, C12, HO4, H15, H18, H19, and S16.

The mixtures were placed in a MJ Research Mini-Cycler programmed as follows: 94 °C for 1 min, 40 °C for 2 min, and 72 °C for 3 min, then 39 cycles of 94 °C for 1 min, 40 °C for 1.5 min, and 72 °C for 2 min. The PCR products were subjected to gel electrophoresis in 1.2 % agarose gels. The DNA banding patterns (stained with Ethidium Bromide) were photographed using a #88-5 Polaroid camera.

DATA ANALYSIS

Average percentage similarities in band sharing were used to compare the degree of genetic variation between strains (Shapiro *et al.*, 1991; Rus-Kortekaas *et al.*, 1994). A similarity matrix was generated for the analysis of each primer with each strain. These matrices were based on the percentage of shared bands in all pair-wise comparisons. The equation $2cxy/(Tx + Ty) \times 100$ (where Cxy is the number of common bands and Tx and Ty are the total number of bands in that particular pair-wise comparison) was used to generate the similarity matrices (Nei & Li, 1979). The average percent similarities from each matrix was subjected to a T test to determine if differences in genetic variation between the strains were statistically significant. The analysis was paired according to primers so that average variation between strains was compared

for each primer and variation among primers was not a factor.

Additionally, differences in genetic variation were illustrated with a cluster analysis using the average linkage method (Anon., 1985). The cluster analysis used genetic distance (1-similarity) which was calculated from similarity matrices. If the average distances form less clusters in one strain than in the other then the strain with less clusters contains less genetic variation.

Results

RAPD-PCR analysis resulted in banding patterns (Fig. 1) that were reproducible. The total number of bands produced from fourteen primers was 74 and 61 for the IS5 and HP88 strains, respectively. After screening with eleven of the fourteen primers, DNA from a few of the IS5 inbred lines was used up and the nematodes were no longer available. Therefore the last three primers (A01, A02, and A03) were screened with less than fifteen inbred lines for each strain (Table 1).

Occasionally DNA from a particular inbred line would not produce any PCR product. If this result was repeated then the inbred line was scored as having no bands in common with the other lines. The absence of PCR products occurred in less than 2 % of the reactions and never more than twice with any primer.

The average percent similarities calculated for each primer varied from 68 %-100 % and 40.9 %-100 % for HP88 and IS5, respectively (Table 1). Statistical analysis indicated that the overall average similarity was not significantly different among the inbred lines of HP88 and IS5 (T= 2.02, df=14, α = 0.05).

The degree of genetic variation is also depicted in the results of the cluster analysis (Table 2). The degree of genetic variation may be determined by the number of clusters at specific average genetic distances. The number of clusters differ between IS5 and HP88 in only three of the eleven distances illustrated (Table 2).

Discussion

We found the genetic heterogeneity of a recently isolated population of *H. bacteriophora* to be similar to a population that has been reared in the laboratory for a long period. Thus, in this case considerable genetic variation has been retained in the HP88 population even after an extended period of reproduction under laboratory conditions. This conclusion is supported

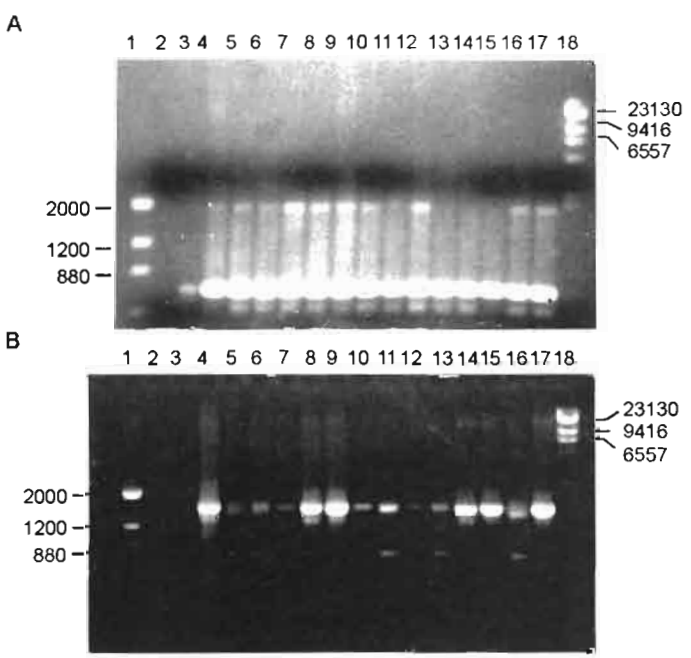

Fig. 1. *Examples of PCR products using Operon primer A13 and DNA from fifteen inbred lines of the HP88 (A) and IS5 (B) strains of* Heterorhabditis *sp. (Lane 1: Low weight DNA mass ladder (Giborcol, Inc.); Lane 2: A control containing the PCR mix minus DNA; Lanes 3-17: The fifteen inbred lines A-O; Lane 18: Lambda DNA cut with HindIII).*

Table 1. *Average percent similarity in band sharing.*

Operon primer	n	Nematode strain	
		HP88	IS5
C09	15	95.9	81.8
H18	15	81.4	81.1
A13	15	95.9	81.6
A18	15	77.6	100
C06	15	83.4	85.9
C11	15	86.1	86.7
C12	15	81.8	84.3
H04	15	93.0	58.0
H15	15	95.1	80.5
H19	15	68.0	68.7
S16	15	100	65.8
A01	12	69.9	65.1
A02	14	81.0	40.9
A03	14	87.6	84.9
Overall mean ± SE		85.5 ± 2.5	76.1 ± 3.84

DNA from n inbred lines of two strains of *Heterorhabditis* were subjected to RAPD-PCR analysis; subsequently, the average percent similarity was calculated from all pair-wise comparisons.
SE = Standard error of the mean.

Table 2. *Number of clusters at various average genetic distances.*

Distance	Nematode strain	
	HP88	IS5
1.0	1	1
0.9	2	2
0.8	2	2
0.7	2	2
0.6	2	2
0.5	3	2
0.4	3	3
0.3	3	8
0.2	7	14
0.1	14	15
0.08	15	15

DNA from n inbred lines of two strains of *Heterorhabditis* were subjected to RAPD-PCR analysis; subsequently, cluster analysis was made using the average linkage method.
Distance = 1 – similarity.

by a previous genetic study in which we have shown that an appreciable degree of genetic heterogeneity exists in the HP88 strain with respect to various traits such as desiccation tolerance, heat tolerance, host finding, and UV resistance (Glazer *et al.*, 1991). The genetic heterogeneity in HP88 can thus be used for selection studies (Glazer *et al.*, 1997). Better understanding of the source of genetic heterogeneity in a population requires information on the history of its development. The background information about the isolation of HP88 and about IS5 is rather limited, but the former apparently has passed through in a considerably narrower "bottle-neck" then the latter (see materials and methods).

Downes and Griffin (1996) hypothesized that genetic variation within populations of *Heterorhabditis* would be extremely low due to hermaphroditism. Therefore they suggested that the nematodes may be considered clonal, or nearly clonal, organisms. Further, a relatively limited dispersal combined with the low genetic variability would result in populations that are highly adapted to local environmental conditions. A high degree of specialization is indeed evident in the heat tolerant strain IS5 (Glazer *et al.*, 1996) and in cold tolerant isolates from Ireland (Griffin & Downes, 1991).

Attempts to characterize within population variation of entomopathogenic nematodes have heretofore included studies that analyzed variation in several phenotypic traits only (Glazer *et al.*, 1991). This is the first study to use molecular markers to characterize overall within population variation for an entomopathogenic nematode. More studies will be needed to determine if genetic variation of biological control agents decreases when they are reared under laboratory conditions over a long period. Verification of a loss in diversity can best be obtained by monitoring the genetic variation in a single population from the time it is collected until sufficient time has elapsed to make a decrease in genetic variation plausible.

Hopper *et al.* (1993) assert that the risk of loss in genetic diversity due to drift may be negligible as long as the populations size is not too small; furthermore, they argue that problems due to inbreeding in amphimictically reproducing biocontrol agents tend to be minimal. However, this conclusion is based on studies, with beneficial insects used for biological control, in which a relatively few number of generations were observed and mating was exclusively amphimictic (Hopper *et al.*, 1993). Because of their short generation time (about 5 days) and hermaphroditic reproduction *Heterorhabditis* spp. are likely to be more susceptible to problems of reduction of genetic diversity than other biocontrol agents.

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