Fundam. appl. Nematol., 1997, **20** (2), 197-205

The isolation of mutants of *Heterorhabditis megidis* (Strain UK211) with increased desiccation tolerance

Sean A. O'Leary and Ann M. Burnell

Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland.

Accepted for publication 16 December 1996.

Summary – Desiccation tolerance of infective juveniles of *Heterorhabditis megidis* strain UK211 has been improved using EMS mutagenesis. Two mutant screens were designed; a rapid desiccation screen (57 % RH, 210 min) and a slow desiccation screen (95 % RH, 8 days). The isolation of three mutant lines from the rapid desiccation screen is described. These mutant lines were more tolerant to dehydration at low relative humidities but not at high relative humidities. Their host-finding ability and tolerance to temperature extremes were comparable to that of the parental strain. However, they were more susceptible than the parental strain to treatment with anionic detergents while being more tolerant than the parental strain to treatment with cationic detergents. The mutants also displayed a slower rate of water loss during dehydration at 0 % RH. These results indicate that a change has occured in either the cuticle or the sheath of the mutant lines.

Résumé – *Isolement de mutants d'*Heterorhabditis megidis *(Souche UK211) ayant une tolérance accrue à la dessiccation –* La tolérance à la dessiccation de juvéniles infestants d'*Heterorhabditis megidis* souche UK211 a été accrue par mutagenèse à l'EMS. Deux schémas de mutation ont été établis : dessiccation rapide (57 % RH, 210 min) et dessiccation lente (95 % RH, 8 jours). L'isolement de trois lignées de mutants provenant de dessiccation rapide est décrit. Ces lignées de mutants sont plus tolérantes à la déshydratation à des humidités relatives basses, mais non dans le cas d'humidités relatives élevées. Leur capacité à rechercher un hôte et leur tolérance aux températures extrêmes sont comparables à celles de la souche parentale. Cependant, ces lignées sont plus sensibles que la souche parentale aux traitements à l'aide de détergents cationiques mais plus tolérantes dans le cas de détergents anioniques. Ces mutants montrent également une plus faible perte d'eau pendant une déshydratation à 0 % RH. Ces résultats démontrent qu'une modification s'est produite affectant soit la cuticule soit l'enveloppe de ces lignées mutantes.

Key-words : cuticle, desiccation, *Heterorhabditis,* infective juvenile, mutagenesis, mutant.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are important biological control agents of soil-borne insect pests (Klein, 1990). The infective stage of *Heterorhabditis* spp. is a third stage infective juvenile (IJ) which occurs free in the soil and harbours in its intestine cells of the bacterial symbiont *Photorhabdus luminescens*. Once the IJ enters a new insect host it releases *P. luminescens* cells into the insect haemocoel and these cells multiply and kill the insect by septicaemia, thereby creating conditions suitable for nematode development. The IJ initiates feeding, develops through the J4 stage and into an hermaphroditic stage. Subsequent generations produce amphimictic males and females (Dix *et al.,* 1991) and hermaphrodites (Zioni *et al.,* 1992; Strauch *et al.,* 1994). Once nutrients are depleted in the insect cadaver IJs are produced which leave the cadaver and enter the soil. They are non-feeding and remain in the soil until they infect a new host. IJs are the only stage to occur freely in the soil and *Heterorhabditis* IJs retain the J2 cuticle which acts as a sheath and aids in environmental protection (Campbell & Gaugler, 1991). Heterorhabditid IJs can be commercially produced using either a solid phase system (Bedding, 1984) or liquid fermentation (Friedman, 1990) and targetted insect pests are controlled in the field by inundative application of IJs (Ehler, 1990). However, there are many environmental factors such as high and low temperatures, UV light and desiccation which can limit the usefulness of EPNs as biological control agents in field situations (Gaugler, 1988; Kaya, 1990).

Many organisms, including some nematodes, can withstand complete desiccation by entering a condition known as anhydrobiosis in which their metabolism is fully arrested (Crowe *et al.,* 1992). When water becomes available again such organisms rehydrate and resume active metabolism. Entomopathogenic nematodes are not full anhydrobiotes and can only survive moderate levels of desiccation by entering into a quiescent state in which their metabolism is partly suppressed (Womersley, 1990). In anhydrobiotic nematodes preconditioning at high relative humidities such as 97 % RH is necessary if they are to successfully survive complete desiccation (Crowe & Madin, 1975; Madin & Crowe, 1975). The biochemical changes which occur in anhydrobiotic nematodes during this preconditioning period typically involve an increase in trehalose and glycerol levels and a reduction in lipid and glycogen levels. The suggested role for trehalose in anhydrobiotes undergoing desiccation is to replace bulk and structural water and to inhibit oxidative damage (for review, see Womersley, 1990). Glycerol is a cryoprotectant (Mab-

bett & Wharton, 1986) and has been implicated in the replacement of structural water during desiccation in eggs of the brine shrimp *Artemia salina* (Clegg, 1974) and in the plant parasitic nematode *Aphelenchus avenae* (Crowe *et al.*, 1979), although evidence also suggests that this may not be the case (Higa & Womersley, 1993). Popiel and Vasquez (1991) have shown that IJs of the EPN *Steinernema carpocapsae* produce glycerol and trehalose in response to osmotic desiccation but no data was presented for IJs of *Heterorhabditis*. Despite the fact that steinernematids have been shown to produce glycerol and trehalose in response to osmotic desiccation, they and heterorhabditids are susceptible to inactivation by evaporative desiccation (Begley, 1990; Kaya, 1990). This affects their viability following spray application for the control of insect pests in the soil, but it especially limits the usefulness of EPNs for the control of insect pests in other niches such as foliar surfaces (Gaugler, 1981). Therefore evening application of EPNs and the incorporation of anti-desiccants into the EPN spray have been considered for the control of insect pests in the foliar environment. However, the incorporation of the anti-desiccant Folicote into the EPN spray was not effective in controlling foliar pests at relative humidities below 60 % (Glazer *et al.*, 1992). The inabilility of EPN IJs to withstand desiccation also poses problems for storage of these nematodes prior to application. Extensive research has concentrated on formulations which can immobilize or partially desiccate IJs on specific carriers such as alginate gels or clay, thus reducing their activity and metabolism leading to an extended shelf life (Georgis, 1990). The development of strains of IJs with increased desiccation tolerance would be desirable therefore not only for IJ formulation and storage but also for application at higher temperatures and on foliar surfaces.

Attempts to isolate desiccation resistant strains of *H. megidis* by chemical mutagenesis are reported here. Two mutant screens were designed. The first was based on the dose mortality curve for the desiccation of *H. megidis* strain UK211 at 95 % RH (slow desiccation). This screen required the isolation of IJs which were capable of surviving 8 days at 95 % RH, a treatment which was lethal for the non mutagenized parental strain. Because of the moderate levels of desiccation stress which these conditions impose it was expected that mutants isolated in such a screen might have alterations in their biochemistry such as an increased capacity to synthesise trehalose and glycerol. The second mutant screen was based on the dose mortality curve for the desiccation of IJs of *H. megidis* strain UK211 at 57 % RH (rapid desiccation). During rapid desiccation the cuticle/sheath is the most important barrier against water loss, thus it was expected that mutants isolated from this screen would have an increased ability to retard water loss. Despite repeated attempts no mutants were isolated from the slow desiccation mutant screen. However, mutants were successfully isolated from the rapid desiccation mutant screen. The isolation and partial characterization of these mutants are described here.

Materials and methods

Source and maintenance of *Heterorhabditis megidis*

Heterorhabditis megidis strain UK211 was isolated in England by Dr. W. Hominick and a stock is maintained in the authors' laboratory. These nematodes were cultured at 20 °C *in vivo* in *Tenebrio molitor* larvae (Dunphy & Webster, 1989). Ten infected *T. molitor* larvae were placed on each water-trap (White, 1927) and each trap was harvested daily until production of IJs ceased. Samples from the total number of IJs to emerge were used for experimental procedures to avoid batch variance (O'Leary *et al.*, 1997).

Isolation and maintenance of *Photorhabdus luminescens*

Photorhabdus luminescens was isolated from the intestine of surface sterilised *H. megidis* strain UK211 IJs as described by Akhurst (1980). Primary colonies of the bacteria were identified by culturing on NBTA plates (Akhurst, 1986). Cultures were maintained on LB plates (Miller, 1992) at 20 °C until required.

Surface sterilization of IJs

IJs were incubated in 0.4 % Hyamine 2389 (BDH Chemicals) for 15 min and then rinsed five times with sterile water (Woodring & Kaya, 1988).

Desiccation tolerance of IJs

IJs (1 ml samples containing *ca.* 200 IJs) were vacuum-filtered onto 2.5 cm Whatman No. 1 filter paper discs. The discs were transferred to 3 cm Petri dishes which were then placed without their lids in glass desiccators containing the appropriate concentration of sulphuric acid to yield the desired relative humidity (Solomon, 1951). After the desiccation period, the Petri dishes were removed and the IJs were rehydrated with tap water at 20 °C. Percentage survival was assessed after a recovery period of 24 h at 20 °C.

Mutagenesis protocol

Surface sterilized IJs were applied to lipid agar plates streaked with the primary form of the symbiont bacterium *Photorhabdus luminescens* (Dunphy & Webster, 1989) and incubated at 20 °C for 48 h, by which time the IJs had developed into first generation J4 juveniles and young hermaphrodites. This population was washed off the plates, rinsed with sterile M9 buffer (Brenner, 1974), resuspended in 3 ml of M9 buffer containing 0.05 M ethyl methane sulphonate (EMS) and incubated at room temperature for 2 h (Brenner, 1974). Mutagenized worms were washed three times with sterile M9 buffer. Fifty mutagenised nematodes were then

transferred to each lipid agar plate and allowed to further develop through to second generation amphimictic adults and to continue developing until IJs were again produced due to nutrient depletion (This required a minimum of two generations). Each plate of IJs was screened separately for desiccation tolerance and where desiccation resistant IJs were detected only a single mutant line derived from a single IJ was established from each plate. Two screening protocols were used for mutant isolation, a rapid desiccation test (57 % RH for 210 min) and a slow desiccation test (95 % RH for 8 days).

Temperature tolerance of IJs

The temperature tolerance of IJs of the parental strain *H. megidis* strain UK211 and mutant lines was tested at four different temperatures (– 15 °C for 40 min, 2 °C for 240 h, 30 °C for 168 h, and 37 °C for 135 min) as described by O'Leary *et al.* (1997).

IJ infectivity

The infectivity of IJs of the parental strain *H. megidis* strain UK211 and mutant lines was measured in a Petri dish test as described by Griffin and Downes (1994). Insect mortality was assessed 2 days and 5 days post-infection. The results are presented as the percentage of *T. molitor* larvae infected by each strain.

IJ host finding capability

A modification of the assay described by Gaugler *et al.* (1989) was used to measure the host finding capability of IJs of the parental *H. megidis* strain and the mutant lines. Test plates were prepared by pouring 50 ml of 2 % agar into 9 cm Petri dishes. The centre of the lid contained an access port which could be sealed. The lid also contained two small openings at opposite sides of and of equal distance (3.5 cm) from the access port. These two openings contained 1 ml disposable tips, one of which contained two *Galleria mellonella* larvae, *i.e.,* the host tip. An attractant gradient was allowed to form for 1 h at 20 °C. IJs were applied via the access port and allowed to migrate for 1 h at 20 °C. The response of the IJs to the test was scored by determining the percentage of IJs in the following two regions of nine replicate plates, *i.e.,* those that *i)* accumulated under the host tip in a 1 cm diameter area and *ii)* accumulated under the control tip in a 1 cm diameter area. The IJs were applied to the centre of the test dishes. After 1 h, the agar discs located under the control and host tips were removed using a scalpel and were rinsed separately into conical tubes to recover IJs. The remaining agar on the test plate was rinsed repeatedly into 50 ml conical tubes to recover the remainder of the IJs. The number of IJs located under the control and host tips and the total number of IJs on the test plates were scored.

Measurement of the rate of water loss from IJs

Comparison of the rate of water loss from the parental strain and mutant lines of *H. megidis* was achieved using a technique first described by Ellenby (1975). It is based on the measurement of the refractive index of liquid paraffin coated nematodes. The refractive index of a nematode depends on its water content. When the refractive index of a nematode is equal to the refractive index of the surrounding liquid paraffin the worm appears invisible. Nematodes with the same refractive index as liquid paraffin have an estimated water content of *ca.* 20 % (Ellenby, 1975). A 50 ml suspension containing *ca.* 40 IJs was pipetted onto a glass slide. Excess water was removed with filter paper, while ensuring that the worms were kept separate from each other. The glass slide was quickly placed into a 350 cm^3 Perspex food box containing 100 g silica gel (Womersley, 1978). After the incubation period, the slide was removed and 30 ml liquid paraffin was pipetted onto the IJs which were then covered with a cover-slip. The number of visible and invisible worms was determined. Worms with the fastest rate of water loss become invisible before worms with a slower rate of water loss. As different parts of the worm dry at different rates (Womersley, 1978), the centre of the worm was used as a reference for invisibility. The worm was judged to be invisible when the area of invisibility at the centre extended across the width of the worm (Fig. 1).

Tolerance of IJs to detergents

IJs (*ca.* 200) were immersed in 4 % solutions of the cationic detergent sodium dodecyl sulphate (SDS) for 67 h, the zwitterionic detergent CHAPS pH 9.0 for 120 h and CHAPS pH 5.0 for 120 h, the neutral detergent Tween 20 for 216 h and the anionic detergent hexadecyltri-methylammonium bromide (Cetrimide)

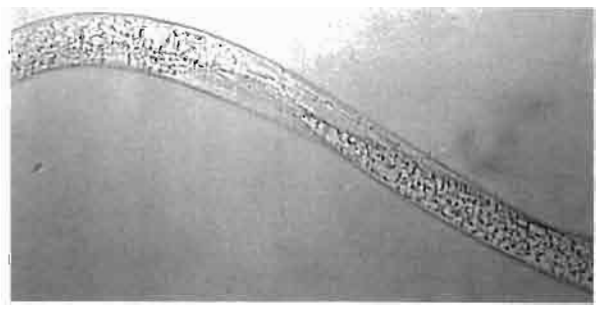

Fig. 1. *The centre of an IJ of* Heterorhabditis megidis *strain UK211 appears invisible when its refractive index is equal to that of the surrounding liquid paraffin. Since the refractive index of the IJ depends on its water content, this can be used as a convenient method for comparing the rate of water loss from different strains of EPN.*

for 48 h in 24-well micro-titre plates (n = 5). After the incubation period IJs were washed five times with distilled water and transferred to 3 cm Petri dishes. Percentage survival was assessed by microscopic observation of motility and response to probing after storage at 20 °C for 24 h. All detergents were obtained from Sigma, UK.

Statistical analysis

Data are presented as the mean + SE. ANOVA was performed on normalized data. The data was normalized using an arcsine transformation for proportions. The significance of difference was tested using Duncan's multiple range test at $P \leq 0.05$ significance level.

Results

Desiccation tolerance of IJs

The ability of IJs of *Heterorhabditis megidis* strain UK211 to survive exposure on filter paper to 95 % RH at 20 °C is presented in Fig. 2A. It was found that a period of 8 days was required under these conditions to kill all IJs. Thus desiccation at 95 % RH for 8 days was used as a lethal mutant screen. Pre-incubation at 95 % RH for 3 days increased the survival of IJs of *H. megidis* during desiccation at lower relative humidities (O'Leary & Burnell, unpubl.) indicating that the nematodes undergo physiological changes during slow desiccation which aid in protecting the nematode from the effects of water loss. However, these physiological changes are unable to protect the nematode fully from the effects of water loss and cannot even enable IJs to survive for longer than 8 days at 95 % RH. The second mutant screen was based on the dose mortality curve of *H. megidis* IJs in response to exposure on filter paper to 57 % RH at 20 °C (Fig. 2B). Desiccation at 57 % RH for 210 min was found to be lethal to IJs of *H. megidis*. Thus exposure to 57 % RH for 210 min was used as a rapid desiccation mutant screen.

Isolation of desiccation resistant mutant lines

Four IJs from the mutagenized population survived the slow desiccation mutant screen but none showed a heritable improvement in their desiccation tolerance at 95 % RH. Three of the five IJs that survived and reproduced following the rapid desiccation mutant screen (*i.e.*, lines 14, 15, and 16) displayed a heritable improvement in their response to rapid desiccation. However, when tested at higher relative humidities the mutant lines did not differ markedly from the parental strain (Fig. 3).

Partial physiological characterisation of desiccation resistant mutants

The rate of water loss from the mutant lines and parental strain *H. megidis* was compared. This was achieved by measuring the time at which maximum invisibility of the IJs immersed in liquid paraffin occurred.

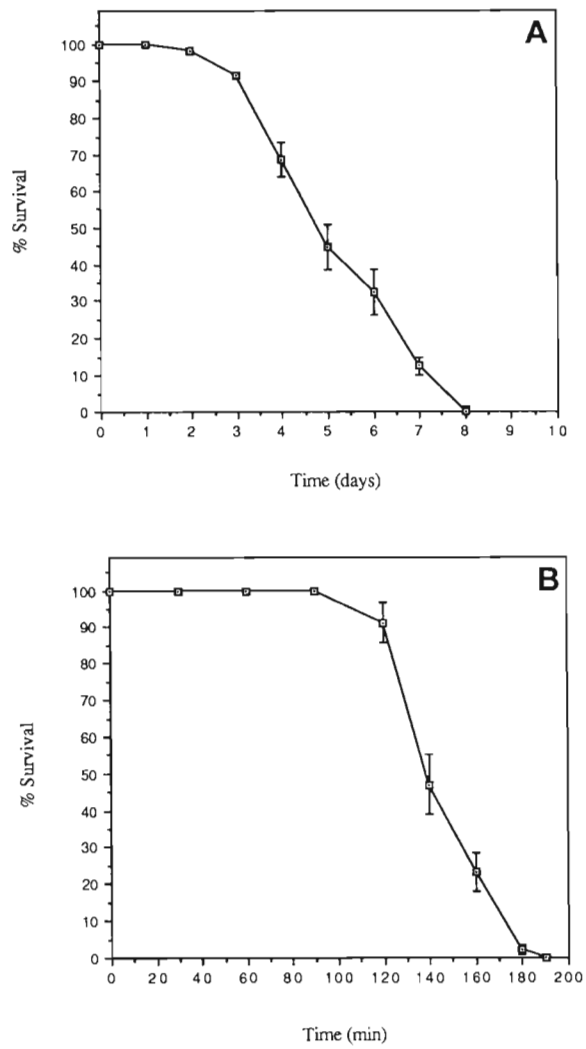

Fig. 2. *The dose response curve for desiccation tolerance of* Heterorhabditis megidis *IJs on filter paper exposed A : To 95 % RH at 20 °C and B : To 57 % RH at 20°C. (Each point represents the mean ± SE of five replicates.)*

Maximum invisibility for IJs of the mutant lines occurred later than for IJs of the parental strain and this indicates that IJs of the mutant lines have a lower rate of water loss than IJs of the parental strain (Fig. 4). This suggests that an alteration may have occurred in the cuticle of IJs of the mutant lines which retards the rate of water loss during desiccation. The surface properties of the sheath and cuticle of IJs of the mutant lines were therefore investigated using detergents.

Treatment of the parental strain and desiccation tolerant mutant lines with detergents (Fig. 5) demonstrated an altered tolerance of the mutants to detergent action. The response to detergents was found to be dependant on the charge of the detergent. IJs of the mutant lines 14,

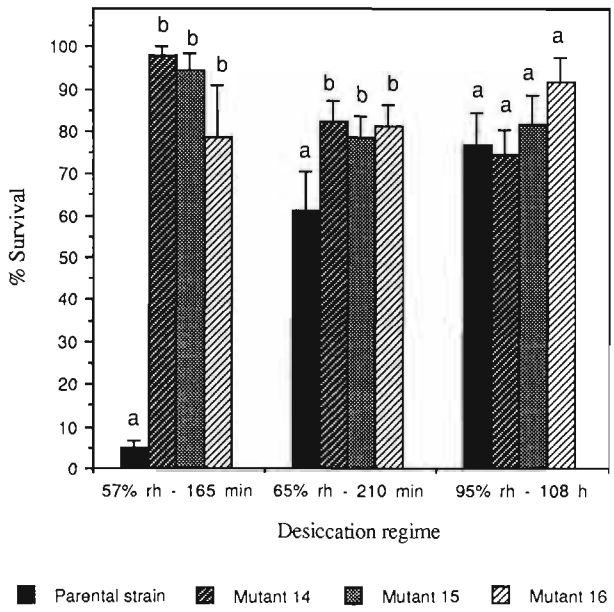

Fig. 3. *Percentage survival of IJs of the parental strain and the desiccation tolerant mutants of* Heterorhabditis megidis *desiccated at a range of relative humidities. (Each value represents the mean ± SE of five replicates; for each desiccation regime means followed by the same letter are not significantly different following Duncan's multiple range test;* $P \leq 0.05$*).*

15, and 16 showed increased susceptibility (relative to the parental strain) to the anionic detergents sodium dodecyl sulphate (SDS) and 3-([3-cholamidopropyl]-dimethylammonio)-1-propane-sulfonate (CHAPS, pH 9.0) but these IJs were more tolerant to the cationic detergents hexadecyltri-methylammonium bromide (Cetrimide) and CHAPS (pH 5.0) than were IJs of the unmutagenized parental strain. CHAPS is a zwitterionic detergent which acts as an anionic detergent at basic pH and acts as a cationic detergent at acidic pH. IJs of the mutant lines displayed the same tolerance as IJs of the parental strain to the neutral detergent Tween 20. The surface of nematodes is typically negatively charged (Maizels *et al.,* 1993) and so this increased susceptibility to anionic detergents may indicate that the surface of the mutant lines is less negatively charged than that of the parental strain. Such an alteration in charge on the surface of IJs of the mutant lines could cause them to be more susceptible to anionic detergents while rendering them more tolerant to cationic detergents.

IJs of the mutant lines 14, 15 and 16 did not display any alterations in their tolerance to a range of temperatures as compared to IJs of the parental strain (Fig. 6). In addition, the host-finding ability of the three mutant lines was comparable to that of the parental strain, *i.e.,* the percentage of IJs accumulating at the host tip in the agar plate bioassay was similar for all strains (Table 1).

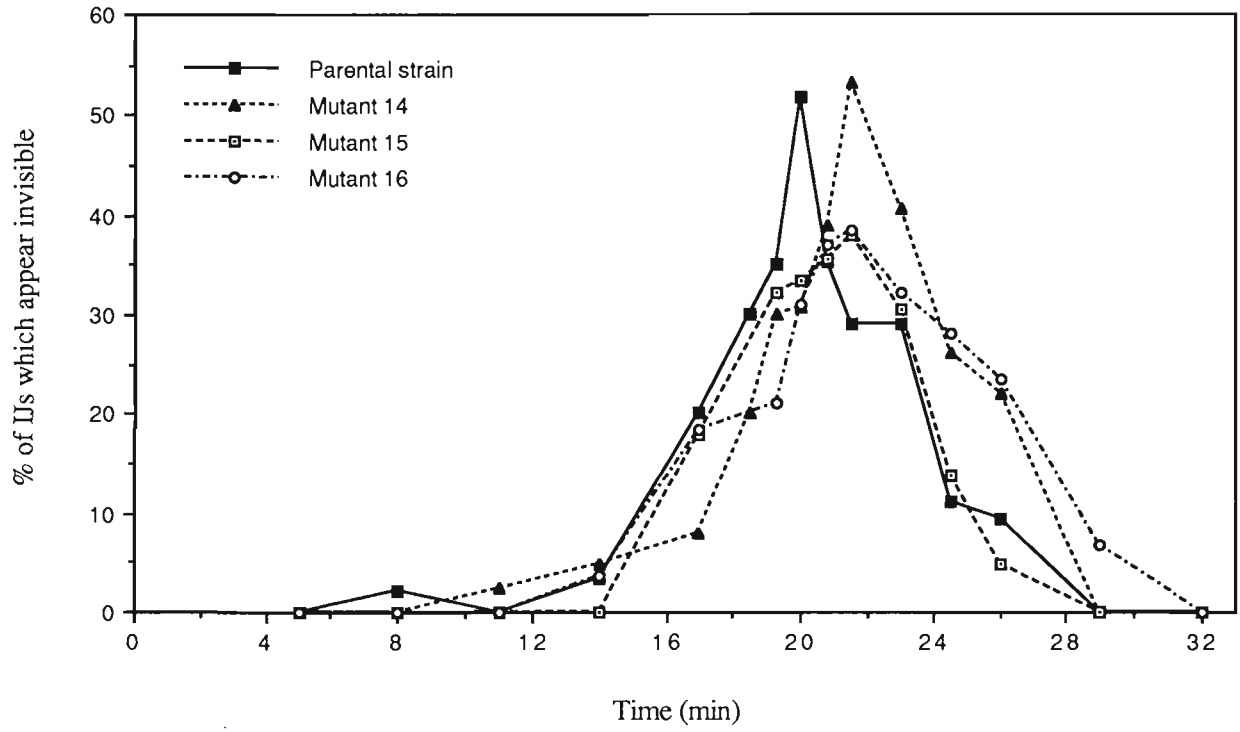

Fig. 4. *Comparison of the rate of water loss at 0 % RH from IJs of the parental strain and desiccation tolerant mutant lines of* Heterorhabditis megidis. *IJs with the fastest rate of water loss become invisible before IJs with a slower rate of water loss.*

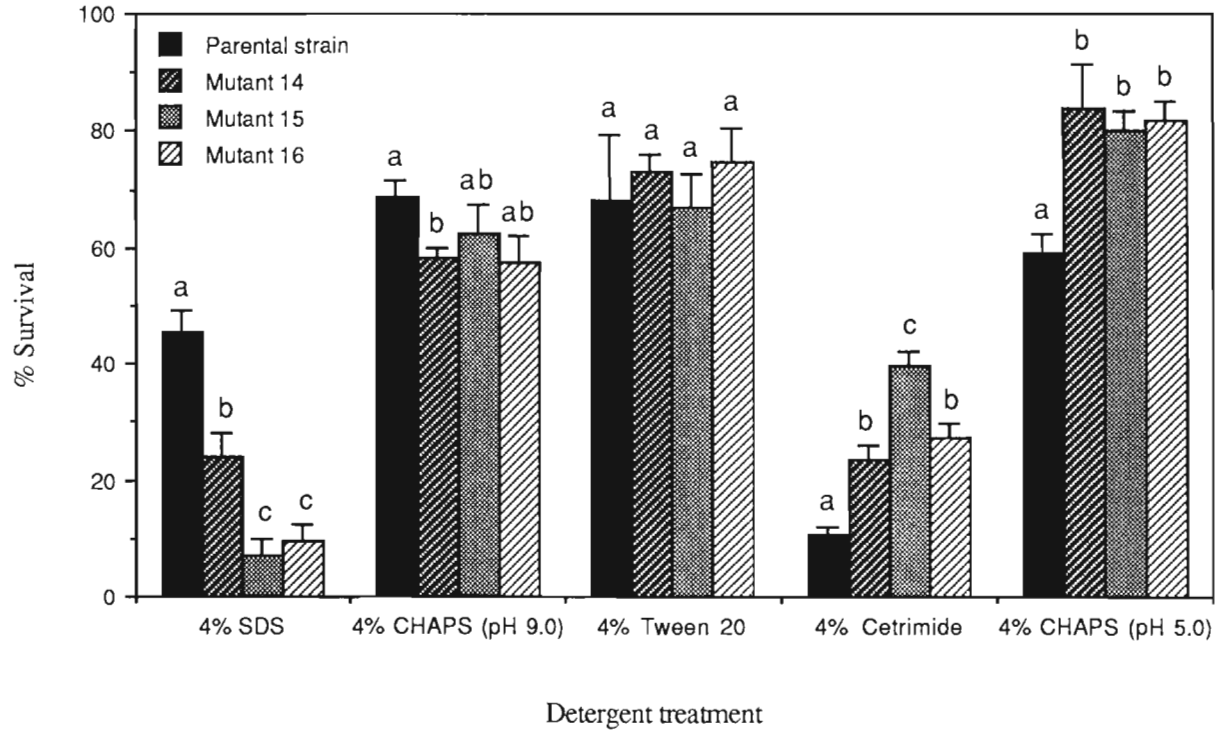

Fig. 5. *Mean percentage survival ± SE (n = 5) of IJs of the parental strain and desiccation tolerant mutant lines of* Heterorhabditis megidis *when incubated in the cationic detergent SDS, the zwitterionic detergent CHAPS pH 5.0 and CHAPS pH 9.0, the neutral detergent Tween 20 and the anionic detergent Cetrimide. (For each detergent tested means followed by the same letter are not significantly different following Duncan's multiple range test;* $P \leq 0.05$*).*

IJs of the mutant lines however appear to have increased infectivity, infecting more *T. molitor* larvae during a 24 h period at 20 °C than IJs of the parental strain (Table 2).

Discussion

It was not possible to isolate mutants from the 95 % RH slow desiccation screen. It was expected that mutants isolated in this screen might possess physiological alterations in their cellular biochemistry (*e.g.,* trehalose or glycerol biosynthesis) or possibly in the structure of their membrane phospholipids (Womersley, 1990). It is possible that mutants with a more favourable biochemistry for entry into anhydrobiosis could be isolated from lethal mutant screens involving higher humidities such as 98 % RH. However, pre-incubation at 95 % RH does increase the survival of IJs at lower relative humidities (O'Leary & Burnell, umpubl.) which suggests that biochemical adjustments can occur during this period of acclimation at 95 % RH. The failure to isolate mutants from the slow desiccation mutant screen might perhaps result from the possibility that the biochemical changes necessary for entry into anhydrobiosis are controlled by a number of genes (from one or more pathways) that are induced at different levels of dehydration. If this were the case then a mutation in a single gene in this pathway may be insufficient in itself to alter the desiccation phenotype. Higa and Womersley (1993) have shown that despite the induction of elevated trehalose levels in small aggregations of *Aphelenchus avenae* upon pre-incubation at 97 % RH for 72 h, survival at lower humidities was directly related to the rate of evaporative water loss rather than to trehalose content. They suggest that following trehalose synthesis, other adaptations need to be implemented before the nematode can successfully survive dehydration. Evidence suggests that these adaptations may involve alterations in membrane phospholipids during desiccation (Womersley, 1990) and that antioxidants are important during the induction of and recovery from anhydrobiosis (Gresham & Womersley, 1991).

The nematode cuticle not only acts as an exoskeleton but it also acts as a protective barrier to the external environment. Campbell and Gaugler (1991) have shown that the J2 sheath retained by the IJs plays a significant role in the ability of *H. bacteriophora* IJs to tolerate desiccation. Using mutagenesis we have improved the desiccation tolerance of *H. megidis* IJs to 57 % RH and the mutant lines which we isolated were

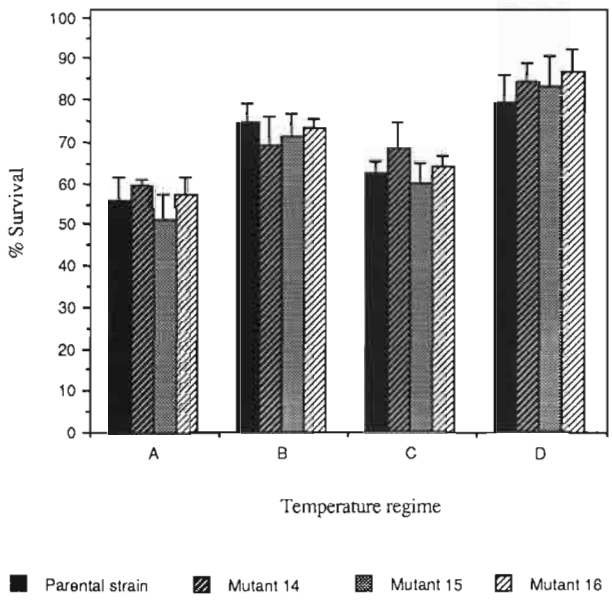

Fig. 6. *Mean percentage survival ± SE (n = 5) of IJs of the parental strain and desiccation tolerant mutant lines of* Heterorhabditis megidis *when exposed to four temperature regimes. A : – 15 °C for 40 min; B : 2 °C for 240 h; C : 30 °C for 168 h; D : 37 °C for 135 min. (No significant differences were found between the desiccation tolerant mutant lines and parental strain for each test temperature).*

Table 1. *The host-finding ability of IJs of the desiccation tolerant mutant lines and parental strain of* Heterorhabditis megidis *(Strain UK211). The mean percentage ± SE (n = 9) of IJs from each strain which accumulated at either the host tip or control tip is presented. Means followed by the same letter are not significantly different (Duncan's multiple range test,* $P \leq 0.05$*).*

	% IJs at host tip	% IJs at control tip
Parental strain	9.2 ± 2.3 *a*	0.9 ± 0.1 *b*
Mutant 14	7.9 ± 2.8 *a*	0.8 ± 0.1 *b*
Mutant 15	8.4 ± 3.1 *a*	0.9 ± 0.1 *b*
Mutant 16	8.7 ± 2.6 *a*	1.1 ± 0.2 *b*

found to have a slower rate of water loss than the parental strain at 0 % RH. However, desiccation of these mutant lines at higher humidities results in no improvement in the survival of the mutant lines over the parental strain. At low humidities, the rate of water loss from the IJs is rapid so the decreased rate of water loss in the mutant lines confers a survival advantage to them over the parental strain. At high relative humidities, the rate of water loss from the IJs is slow and under these conditions a decreased rate of water loss from the mutant strains appears not to offer an advantage over the parental strain. Work performed on the plant parasitic nematodes *Anguina agrostis* (Preston & Bird, 1987) and *Ditylenchus dipsaci* (Wharton *et al.*, 1988) suggests that the permeability barrier of the cuticle lies in the epicuticle. Because of the altered response of the mutant lines to detergents, it appears likely that the change responsible for the slower rate of water loss occurs in the external layer of the sheath (possibly the epicuticle) and may be related to surface charge.

Table 2. *Comparison of the mean infectivity ± SE (n = 10) of the parental strain and desiccation tolerant mutant strains of* Heterorhabditis megidis *at 20 °C. Means followed by the same letter are not significantly different (Duncan's multiple range test,* $P \leq 0.05$*).*

	Mortality of *Tenebrio molitor* larvae (%)	
	2 days post-infection	5 days post-infection
Parental strain	53.4 ± 4.1 *a*	59.8 ± 2.8 *a*
Mutant 14	72.3 ± 5.8 *b*	76.3 ± 3.2 *b*
Mutant 15	74.6 ± 4.1 *b*	79.5 ± 2.1 *b*
Mutant 16	72.4 ± 6.6 *b*	74.7 ± 5.6 *b*

Although the mutant lines do not display an increased host-finding ability they clearly cause greater insect mortality than the parental strain. Due to the nature of the assay it is possible that the IJs do not infect purely on the basis of their host-finding ability. Rather their ability to move across the damp filter paper or gain entry into the host might be important factors in determining their infectivity. It may be that the mutant strains caused greater insect mortality because of an increased ability to migrate or enter the host due to a change in the surface of their outer cuticle. This would not be unfeasible as the surface of some nematodes (*e.g.*, the infective stage of *Toxocara canis*) contains structures similar to mucins which suggests that the surface coat may form a lubricating layer which could facilitate greater ease of movement (Khoo *et al.*, 1991).

Phenotypes of importance in the infection process such as the temperature tolerance and host-finding ability of the IJs have not been impaired in the mutant lines which we have isolated. This is critical if mutagenesis is to be used as a tool for the genetic improvement of EPNs. The results presented here clearly demonstrate that the tolerance of EPN IJs to environmental extremes such as desiccation can be improved using EMS mutagenesis. The authors are at present investigating the outer cuticle of the mutant lines using fluorescent lipophilic probes in an attempt to elucidate the nature of the changes responsible for the mutant phenotype.

References

AKHURST, R. J. (1980). Morphological and functional dimorphism in *Xenorhabdus spp.*, bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. gen. Microb.*, 21 : 303-309.

AKHURST, R. J. (1986). *Xenorhabdus nematophilus* subsp. *poinarii* : Its interaction with insect parasitic nematodes. *Syst. appl. Microb.*, 8 : 142-147.

BEDDING, R. A. (1984). Large scale production, storage and transport of the insect parasitic nematodes, *Neoaplectana* spp. and *Heterorhabditis* spp. *Ann. appl. Biol.*, 104 : 17-29.

BEGLEY, J. W. (1990). Efficacy against insects in habitats other than soil. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL., USA, CRC Press : 318-336.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*, 77 : 71-94.

CAMPBELL, L. R. & GAUGLER, R. (1991). Role of the sheath in desiccation tolerance of two entomopathogenic nematodes. *Nematologica*, 37 : 324-332.

CLEGG, J. S. (1974). Biochemical adaptations associated with the embryonic dormancy of *Artemia salina*. *Trans. am. Microb. Soc.*, 93 : 481-490.

CROWE, J. H., HOEKSTRA, F. A. & CROWE, L. M. (1992). Anhydrobiosis. *A. Rev. Physiol.*, 54 : 579-599.

CROWE, J. H., & MADIN, K. A. C. (1975). Anhydrobiosis in nematodes : evaporative water loss and survival, *J. exp. Zool.*, 193 : 323-331.

CROWE, J. H., O'DELL, S. J. & ARMSTRONG, D. A. (1979). Anhydrobiosis in nematodes : Permeability during rehydration. *J. exp. Zool.*, 207 : 431-438.

DIX, I., BURNELL, A. M., GRIFFIN, C. T., JOYCE, S. A., NUGENT, M. J. & DOWNES, M. J. (1992). The identification of biological species in the genus *Heterorhabditis* (Nematoda : Heterorhabditidae) by cross-breeding second generation amphimictic adults. *Parasitology*, 104 : 509-518.

DUNPHY, G. B. & WEBSTER, J. M. (1989). The monoxenic culture of *Neoaplectana carpocapsae* DD136 and *Heterorhabditis heliothidis*. *Revue Nématol.*, 12 : 113-123.

EHLER, L. E. (1990). Some contemporary issues in biological control of insects and their relevance to the use of entomopathogenic nematodes. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL., USA, CRC Press : 1-19.

ELLENBY, C. (1975). A simple method of comparing the rate of drying of nematode worms. *Nematologica*, 21 : 115-116.

FRIEDMAN, M. J. (1990). Commercial production and development. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 153-170.

GAUGLER, R. (1981). Biological control potential of neoaplectanid nematodes. *J. Nematol.*, 13 : 241-249.

GAUGLER, R. (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agric., Ecosyst., Envir.*, 24 : 351-360.

GAUGLER, R., CAMPBELL, J. F. & MCGUIRE, T. R. (1989). Selection for host-finding in *Steinernema feltiae*. *J. Invert. Pathol.*, 54 : 363-372.

GEORGIS, R. (1990). Formulation and application technology. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 173-187.

GLAZER, I., KLEIN, M., NAVON, A. & NAKACHE, Y. (1992). Biological control of three cotton pests by canopy sprays of entomopathogenic nematodes. *J. econ. Ent.*, 85 : 73-82.

GRESHAM, A. T. & WOMERSLEY, C. Z. (1991). Modulation of catalase activity during the enforced induction and revival from anhydrobiosis in nematodes. *FASEB*, 5 : 682.

GRIFFIN, C. T. & DOWNES, M. J. (1994). Recognition of low temperature active isolates of the entomopathogenic nematode *Heterorhabditis*. *Nematologica*, 40 : 106-115.

HIGA, L. M. & WOMERSLEY, C. Z. (1993). New insights into the anhydrobiotic phenonemon : The effects of trehalose content and differential rates of evaporative water loss on the survival of *Aphelenchus avenae*. *J. exp. Zool.*, 267 : 120-129.

KAYA, H. K. (1990). Soil ecology. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 93-115.

KHOO, K. H., MAIZELS, R. M., PAGE, A. P., TAYLOR, G. W., RENDELL, N. L. B. & DELL, A. (1991). Characterization of nematode glycoproteins : the major *O*-glycans of *Toxocara* excretory-secretory antigens are *O*-methylated trisaccharides. *Glycobiology*, 1 : 163-171.

KLEIN, M. G. (1990). Efficacy against soil-inhabiting insect pests. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 195-210.

MABBETT, K., & WHARTON, D. A. (1986). Cold tolerance and acclimation in the free-living nematode, *Panagrellus redivivus*. *Revue Nématol.*, 9 : 167-170.

MADIN, K. A. C. & CROWE, J. H. (1975). Anhyrobiosis in nematodes : carbohydrate and lipid metabolism during rehydration, *J. exp. Zool.*, 193 : 335-346.

MAIZELS, R. M., BLAXTER, M. L. & SELKIRK, M. E. (1993). Forms and functions of nematode surfaces. *Expl Parasit.*, 77 : 380-384.

MILLER, J. H. (1992). *A short course in bacterial genetics : a laboratory manual and handbook for* Escherichia coli *and related bacteria.* New York, USA, Cold Spring Harbour Laboratory Press, 466 p.

O'LEARY, S. A. CHUBB, M. & BURNELL, A. M. (1997). The effect of day of emergence from the insect cadaver on the behaviour and environmental tolerances of IJs of the entomopathogenic nematode *Heterorhabditis megidis* (Strain UK211). *Parasitology* (in press).

POPIEL, I. & VASQUEZ, E. M. (1991). Cryopreservation of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. *J. Nematol.*, 23 : 432-437.

PRESTON, C. M. & BIRD, A. F. (1987). Physiological and morphological changes associated with recovery from ana-

biosis in the dauer larva of the nematode *Anguina agrostis*. *Parasitology,* 95 : 125-137.

SOLOMON, M. E. (1951). Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. *Bull. ent. Res.,* 42 : 543-554.

STRAUCH, O., STOESSEL, S. & EHLERS, R.-U. (1994). Culture conditions define automictic or amphimictic reproduction in entomopathogenic rhabditid nematodes of the genus *Heterorhabditis*. *Fundam. appl. Nematol.,* 17 : 575-582.

WHARTON, D. A., PRESTON, C. M., BARRETT, J., & PERRY, R. N. (1988). Changes in cuticular permeability associated with recovery from anhydrobiosis in the plant parasitic nematode, *Ditylenchus dipsaci*. *Parasitology,* 97 : 317-326.

WHITE, G. F. (1927). A method for obtaining infective nematode larvae from cultures. *Science,* 66 : 302-303.

WOMERSLEY, C. Z. (1978). A comparison of the rate of drying of four nematode species using a liquid paraffin technique. *Ann. appl. Biol.,* 90 : 401-405.

WOMERSLEY, C. Z. (1990). Dehydration survival and anhydrobiotic potential. *In :* Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 117-137.

WOODRING, J. L. & KAYA, H. K. (1988). *Steinernematid and heterorhabditid nematodes : a handbook of biology and techniques.* Southern Cooperative Series Bulletin No. 331, Arkansas Agric. Exp. Statn, Fayetteville, AK, USA, 25 p.

ZIONI (COHEN-NISSAN), S., GLAZER, I. & SEGAL, D. (1992). Life cycle and reproductive potential of the nematode *Heterorhabditis bacteriophora* strain HP88. *J. Nematol.,* 24 : 352-358.