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Inhibition of egg hatch of the potato cyst nematode *Globodera rostochiensis* by chitinase-producing bacteria

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Abstract

Plant-parasitic nematodes are major agronomic pests. Purified commercial chitinase inhibited egg hatch of the potato cyst nematode, *Globodera rostochiensis* (Ro1) *in vitro* by up to 70% when compared with an untreated control. A screening strategy was devised to isolate chitinase-producing bacteria from a soil with no documented history of damage due to potato cyst nematodes in the last 30 years and that was cropped with potato cv. 'Kerr's Pink'. Only 137 of 3,200 bacterial isolates tested for chitinase production on chitin agar plates were chitinase-positive (i.e. about 4%). All the chitinase-producing bacteria tested *in vitro* could reduce the hatch of *G. rostochiensis* eggs, some by up to 90% compared with the controls. One of these strains, M1-12, was identified as *Stenotrophomonas maltophilia* and a second strain UP1 was classified as a *Chromobacterium* sp. based on morphological and biochemical tests. The inoculum level and the incubation time influenced the degree of inhibition of egg hatch of *G. rostochiensis* by M1-12 and UP1 *in vitro*. An initial cell density of 10^6 CFU ml^{-1} or greater and an incubation time of two weeks was needed to inhibit egg hatch. The longer UP1 was allowed to act on the eggs of *G. rostochiensis* the greater the level of inhibition. Strains M1-12 and UP1 also reduced the ability of *G. rostochiensis* to hatch in soil microcosms planted with potato seed tubers cv. 'Désirée'. The inhibition of egg hatch of *G. rostochiensis* by chitinase-producing bacteria is suggested as a biocontrol strategy for the defence of potato crops from potato cyst nematodes.

Introduction

Nematodes are major agricultural pests of potatoes, and include the potato cyst nematodes *Globodera rostochiensis* (Ro1–Ro5) and *Globodera pallida* (Pa1–Pa3). Cyst nematodes are classified individually using a nomenclature system developed by Kort et al. (1977). The designations Ro1-Ron and Pa1-Pan are used for *G. rostochiensis* and *G. pallida*, respectively, depending upon their ability to multiply on particular potato hosts (Kort et al., 1977; Zaheer et al., 1993). Yield losses vary according to soil type and nematode densities. Economic crop failure can happen when the nematode population in soil is high enough. Large nematode populations cause stunting, early senescence and proliferation of lateral roots. As most of the damage to potato plants occurs below ground level it is difficult to diagnose a potato cyst nematode infection in the field. *G. rostochiensis* juveniles are stimulated to hatch by potato root exudates (Clarke and Perry, 1977). They then enter the host plant roots, feed, and develop through a series of three moults. Female nematodes increase in size, rupture the root cortex and are exposed in the rhizosphere. The cyst is formed from the body wall of the dead female, following fertilisation by the male and contains the eggs. The ability of the cyst of *G. rostochiensis* to survive on farm tools, machinery, clothing, infected dried plant material and in soil in the absence of the host crop explains why it is difficult to fully eradicate *G. rostochiensis*.

Historically, potato nematode control has involved crop rotation. However, this strategy has become less attractive where economics demand specialisation and intensification. Chemical nematicides are effective but are very toxic to humans and are environmentally harmful. For instance, 1,2-dibromo-3-chloropropane

has been banned in the United States. This situation has intensified the search for natural soil enemies of potato-parasitic nematodes. The selection of soil fungi as biocontrol agents of nematodes gave interesting results (Pramer, 1964; Sayre, 1986) but their efficacy is often limited by their poor ability to compete with the resident microbiota (Kerry, 1990). Furthermore, a number of fungi antagonistic to parasitic nematodes are potentially pathogenic to animals.

Certain parasitic bacteria can reduce nematode mobility (Stirling, 1984), while other bacteria are antagonistic and can synthesise compounds lethal to plant-parasitic nematodes (Oostendorp and Sikora, 1990; Sikora, 1991; Spiegel et al., 1991). One of the main constituents of the eggshell of *G. rostochiensis* is chitin (Clarke and Hennessy, 1976). Chitin has been added to nematode-infested soils in an attempt to increase the natural chitinolytic bacterial population. However, it was determined that while the expected increase did occur, the observed nematicidal effect may have been due, at least in part, to the release of ammonia as a breakdown product of the added chitin (Spiegel et al., 1987). Interestingly, although root-knot nematodes may not utilise chitinase in the hatching process, chitinase has been shown to interfere with the hatch of *Meloidogyne hapla* (Mercer et al., 1992) resulting in early emergence of juveniles that may be less able to survive in soil. The effect of chitinase on *G. rostochiensis* is unknown.

In this study, chitinase-producing bacteria were isolated from soil and their ability to interfere with the hatching process of *G. rostochiensis* was investigated. No soil clearly documented as being suppressive was available for study. Therefore, a field site in which cyst nematode damage to potato crops had not been detected during the previous 30 years, cropped with potato, was used to isolate the bacteria.

Materials and methods

Effect of chitinase on egg hatch of G. rostochiensis in vitro

Purified chitinase [EC 1.4.3.5] from Sigma was dissolved in 20 ml sterile 50 mM sodium phosphate buffer ($Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot 2H_2O$), pH 6.6 (McKenzie and Dawson, 1969) to reach a concentration of 0.42 units of chitinase per ml. A one-ml volume of chitinase solution was added to 20 ml of 50 mM sodium phosphate buffer, pH 6.4 in a 100 ml Erlenmeyer flask. Cysts of *G. rostochiensis* were obtained from a single generation of the potato cyst nematode using potato cv. 'Kerr's Pink'. In order to ensure that diapause was complete cysts were stored at 25 °C for 4–5 months and were subsequently placed on moist filter paper for 7 days at 18 °C. Twenty-five cysts were introduced into the flask. The flask was incubated at 12 °C for 8 weeks. At fortnightly intervals six cysts were removed aseptically using a sterile Pasteur pipette and placed in 200 μl of 0.3 mM picrolonic acid, an artificial hatching factor, for 28 days (with gentle shaking on alternate days). Picrolonic acid simulates the effect of potato root exudates on nematode eggs, exposure to which is a prerequisite for hatch (Clarke and Shepherd, 1966). At weekly intervals throughout the four-week period counts of hatched eggs per cyst were made by enumeration of hatched juveniles and fresh picrolonic acid was added. The cysts were also crushed in 100 μl of quarter-strength Ringer's solution (Oxoid) and the ratio of empty eggs (hatched) to intact eggs (unhatched) was determined microscopically, using three 20-μl volumes of crushed cyst suspension. At the end of the four-week period the total, cumulative number of observed eggs hatched was calculated. Buffer without chitinase was used as a control. The total number of eggs capable of hatching at the start of the experiment was 246 (+/– 25) eggs per cyst. The experiment was carried out in triplicate with two groups of three cysts assayed per replicate.

Isolation of chitinase-producing bacteria

Bacteria were isolated from the surface horizon of a brown podzolic soil located near Cobh (Co. Cork, Ireland). The field has been cropped with potato every three years for the last 30 years, in rotation with essentially barley and sugarbeet, and was sown with potatoes cv. 'Kerr's Pink' at the time of sampling. Crop damage due to cyst nematode activity was not observed throughout the 30-year period. Furthermore, while it is recognised that a level of approximately 10-20 nematode eggs per g of soil is required for disease pressure only 2–3 eggs per g were detected in this soil.

The soil was extracted and serially diluted in quarter-strength Ringer's solution and dilutions were plated onto quarter-strength Tryptic Soy Agar (Sigma) supplemented with the antifungal compound cycloheximide (100 μg ml^{-1}). The plates were incubated at 12 °C for 72 h. 3,200 bacterial colonies were chosen at random and purified by subculturing (12 °C) on solid glucose Minimal Medium (glucose MM) consisting of

5 g glucose, 4 g casamino acids (Difco), 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.2 g $mgSO_4 \cdot 7H_2O$, 10 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeCl_3 \cdot 6H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$ per litre. Plates were incubated at 12 °C and colonies were checked for chitinase production by replica-plating onto solid chitin Minimal Medium (chitin MM). Chitin MM was prepared similarly to glucose MM, except that glucose was replaced with 4 g chitin (Sigma) and 0.2 g N-acetyl-D-glucosamine per litre. The latter compound is the monomeric unit of chitin and its presence at a low concentration induces chitinase production in fungi (St Leger et al., 1986). Preliminary results in this study showed that N-acetyl-D-glucosamine induces chitinase production in bacteria. The plates were incubated at 12 °C for 14 days.

In vitro *selection of chitinase-producing bacteria antagonistic to potato cyst nematodes*

Chitinase-producing bacteria that resulted in a zone of clearing greater than 1 cm in diameter on the plates were tested for their effect on egg hatch of *G. rostochiensis in vitro*, as follows. Bacteria were grown overnight at 28 °C with shaking in Tryptic Soy Broth (Sigma). The cells were washed twice in quarter-strength Ringer's solution and a 1%-inoculum was transferred into liquid glucose MM. The cultures were incubated at 28 °C with shaking until turbid (OD of 1.5 at 600 nm). The cells were washed twice in quarter-strength Ringer's solution. The cell suspensions were diluted 10 fold in the same solution and one-ml volumes (i.e. 10^6 to 10^7 CFU) were added into 100 ml Erlenmeyer flasks containing 20 ml of 25 mM sodium phosphate buffer, pH 6.6 and 15 cysts of *G. rostochiensis*. The flasks were incubated at 12 °C for 21 days and were shaken by hand on alternate days to ensure aeration. The cysts were removed and incubated in the presence of picrolonic acid, and hatched eggs were counted, as described earlier. Cysts in sterile buffer were used as a control. The egg hatch bioassay was carried out in duplicate and two groups of three cysts were assayed per replicate.

Characterisation of chitinase-producing bacterial strains M1-12 and UP1

Two chitinase-producing bacterial isolates that influenced egg hatch of *G. rostochiensis in vitro* were selected for further study and were characterised using biochemical tests. Tests performed included Gram staining, oxidase production, catalase production and proteinase production on skim milk plates. Skim milk powder (20 g l^{-1}) was autoclaved at 10 PSI for 10 min and added post-autoclave to Luria Bertani agar (Maniatis et al., 1982) (3 g l^{-1}) containing yeast extract (2 g l^{-1}). Pigment production was investigated on Luria Bertani agar and on Chrome Azurol S indicator agar (Schwyn and Neilands, 1987). Bacteria were compared microscopically on the basis of cell motility in wet mounts. Strain M1-12 was also studied using the API-20-NE identification kit (API System, BioMérieux, La Balme les Grottes, France).

Effect of strains M1-12 and UP1 on egg hatch in vitro

Strains M1-12 and UP1 were used to investigate the effect of bacterial inoculum level and incubation time on egg hatch. The effect of inoculum level was studied with the egg hatch bioassay described above except that eight cysts were added per flask and 50 mM sodium phosphate buffer was used. The bacteria were added to the sodium phosphate buffer at the inoculum level of 10^4, 10^6 or 10^8 CFU per ml buffer.

The effect of incubation time on the ability of these strains to inhibit egg hatch was studied at weekly intervals over a four-week period. Strain M1-12 was added at 10^7 CFU per ml buffer whereas strain UP1 was used at 10^6 CFU per ml buffer. A total of 25 cysts instead of 15 cysts were used for each of the two Erlenmeyer flasks used.

In both experiments, controls included uninoculated buffer containing cysts. Each experiment was carried out in duplicate and two groups of three cysts were assayed per replicate for egg hatch.

Effect of strains M1-12 and UP1 on egg hatch in soil microcosm experiments

The soil used in this experiment was sampled from the surface horizon of a brown podzolic soil located at Fota (Co. Cork, Ireland), at about 10 km from the Cobh site where soil used to isolate chitinase-producing bacteria was collected. Both soils belong to the same pedon and display essentially similar physico-chemical characteristics (data not shown), but the Fota soil has not been cropped with potatoes for at least 10 years. The soil was passed through a 5-mm mesh screen and was mixed with a fine sand. The sand was previously washed eight times in tap water and dried at 37 °C. The sand was autoclaved for 60 min on two consecutive days before being mixed with the soil in a 1:4 ratio [dry w/dry w]. Cysts of *G. rostochiensis* were added to

Table 1. Effect of purified chitinase on egg hatch of *G. rostochiensis in vitro*

Conditions of incubation	Egg hatch (hatched eggs per cyst)	
	At two weeks	At four weeks
Control[1]	183 (4)[2] ***a***[3]	147 (23) ***a***
Presence of chitinase	70 (21) ***b***	57 (17) ***b***

[1] The total number of eggs capable of hatching prior to the start of the experiment was 246 (+/– 25) per cyst.
[2] SD.
[3] Bold letters in italics are used to indicate statistical relationship between data.

the soil-sand mixture (hereafter referred to as soil) at a concentration of 30 cysts per 100 g soil. Bacterial cell suspensions of strains M1-12 and UP1 were added singly to soil at the inoculum level of 10^7 CFU per g soil and the soil was mixed thoroughly. About 300 g of soil were used per pot and the water content of the soil was adjusted to 22% w/w water content using distilled water. The pots were put in a growth chamber (12 °C; 16-h photoperiod) and were watered by spraying with distilled water every three days. After 6 or 16 weeks, seed tubers of potato cv. 'Désirée' were planted (one tuber seed per pot). After a further 9 weeks (i.e. on weeks 15 or 25), which is considered insufficient for new cyst generation (Foot, 1977), the cysts were extracted from soil as described by Winfield et al. (1987). In each replicate, five samples of cysts with five cysts per sample were assayed for hatch of the nematode, as described above, and the % of hatched eggs was determined. In addition to strains UP1 and M1-12, the experiment was carried out with M56-13, a bacterial isolate from Cobh soil obtained on quarter-strength Tryptic Soy Agar. Strain M56-13 does not produce chitinase on chitin MM plates and does not affect hatch of *G. rostochiensis in vitro*. The fourth treatment consisted of an uninoculated control in which soil was amended with cysts but was not inoculated with bacteria. The experiment was carried out in triplicate.

Statistical aspects

Data were analysed by analysis of variance, using procedures of the Statistical Analysis System (SAS Institute, Cary, NC). When appropriate, treatments were compared using Fisher's least significant difference test. All analyses were conducted at $P = 0.05$.

Results

Effect of chitinase on egg hatch

Incubation of cysts of *G. rostochiensis* in the presence of purified chitinase enzyme for two weeks significantly inhibited egg hatch *in vitro* compared with the untreated control (Table 1). A similar extent of egg hatch was found with the untreated control after two and four weeks of incubation. Extending the incubation time to six or eight weeks did not influence the extent of chitinase-mediated inhibition of egg hatch (data not shown).

Isolation of chitinase-producing bacteria

A total of 3,200 bacterial soil isolates were screened for their ability to produce chitinase on solid chitin MM and 137 bacteria (i.e. about 4%) produced chitinase. Among the latter, 39 bacteria (i.e. about 30% of them) displayed a zone of clearing of one cm or more in diameter on chitin MM, and they were selected for further analysis.

Effect of chitinase-producing bacteria on egg hatch in vitro

Thirty-nine chitinase-producing bacteria were tested for inhibition of egg hatch of *G. rostochiensis in vitro.* All 39 chitinase-producing bacteria significantly inhibited egg hatch, although to different extents (Figure 1). Chitinase-negative bacterial strain M56-13 had no effect on the ability of eggs to hatch *in vitro* compared with the uninoculated control. Inhibition of egg hatch *in vitro* by the chitinase-producing bacteria was not correlated with their ability to produce chitinase on solid chitin MM *in vitro* (Figure 1). A total of 26 of the 39 bacteria were characterised on the basis of Gram reaction and their production of oxidase and catalase to investigate whether the ability to inhibit nematode egg hatch *in vitro* was a biocontrol trait found within a particular genotypic background only. Results indicated that a wide range of bacteria from the Cobh soil inhibited egg hatch *in vitro* (Table 2).

Two of the most effective isolates in this assay (i.e. UP1 and M1-12) inhibited egg hatch almost completely (Figure 1). Strain M1-12 was identified as *Stenotrophomonas* sp. on the basis of the following characteristics: small Gram-negative motile aerobic rods, catalase positive, oxidase positive, yellowish colony appearance, inability to use asparagine as the

Table 2. Effect of 26 chitinase-producing bacteria on egg hatch of *G. rostochiensis in vitro*

Bacterial characteristics			Number bacteria studied	Egg hatch *in vitro* (hatched eggs per cyst)[1]			
Gram reaction	Production catalase	Production oxidase		Min.	Max.	Mean	CV (%)
+	+	–	3	2	34	20.7	81
+	–	+	1			43	
+	–	–	1			51	
–	+	+	10	3	76	31.5	74
–	+	–	9	18	66	43.4	37
–	–	+	2	8	26	17	75
		Total[2]	26	2	76	34.5	58

[1] 112 and 90 eggs hatched per cyst in the absence of inoculum and when incoculated with chitinase-minus strain M56-13, respectively.
[2] Results for 26 chitinase-producing bacteria randomly chosen from the 39 chitinase-producing bacteria that resulted in a zone of clearing greater than 1 cm in diameter on chitin MM.

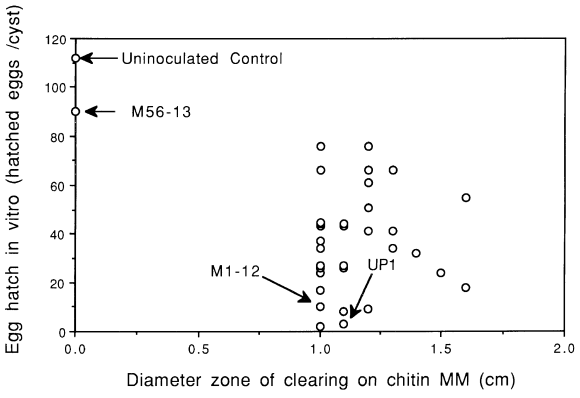

Figure 1. Relationship between chitinase production on chitin MM agar and inhibition of egg hatch of *G. rostochiensis in vitro* for 39 chitinase-producing bacteria.

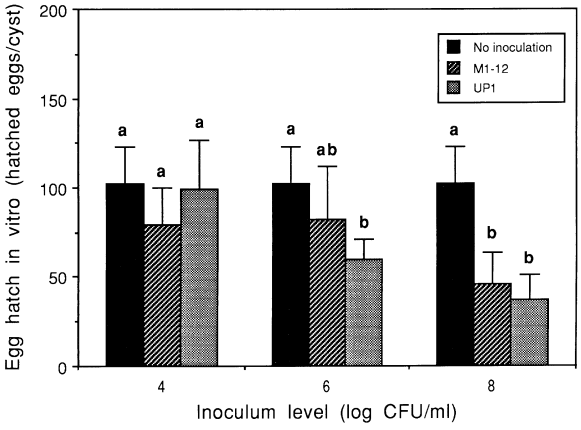

Figure 2. Effect of inoculum density of M1-12 and UP1 on egg hatch of *G. rostochiensis in vitro*. The duration of incubation was three weeks. Vertical bars represent the standard deviation of data. Treatments resulting in differences that were statistically significant at $P = 0.05$ are indicated with different letters at each inoculum level.

sole nitrogen source and ability to hydrolyse gelatine and Tween 80. M1-12 was further classified as a strain of *S. maltophilia* by the API-20-NE bacterial identification kit (98% accuracy).

Strain UP1 was characterised as *Chromobacterium* sp. on the basis of the following characteristics: Gram-negative motile aerobic rods, oxidase and catalase positive, production of a purple pigment and tough membranous colony morphology with gelatinous texture on solid Luria Bertani medium.

Effect of Stenotrophomonas *M1-12 and* Chromobacterium *UP1 on egg hatch* in vitro

Strains M1-12 and UP1 were used to investigate further the effect of chitinase-producing bacteria on the egg hatch ability of *G. rostochiensis in vitro*. The relationship between the initial inoculum level of each strain and the inhibition of egg hatch after three weeks was statistically significant (Figure 2). M1-12 inhibited egg hatch compared with the uninoculated control when the strain was introduced at 10^8 CFU ml^{-1} but not at 10^6 CFU ml^{-1}. Strain UP1 at 10^6 CFU ml^{-1} reduced egg hatch but had no effect when inoculated at 10^4 CFU ml^{-1}.

The duration of incubation of cysts in the presence of the bacteria influenced the extent of egg hatch (Figure 3). *Chromobacterium* UP1 and *Stenotrophomonas* M1-12 (introduced at respectively 10^6 and 10^7 CFU ml^{-1}) did not reduce egg hatch at one week. Inhibition

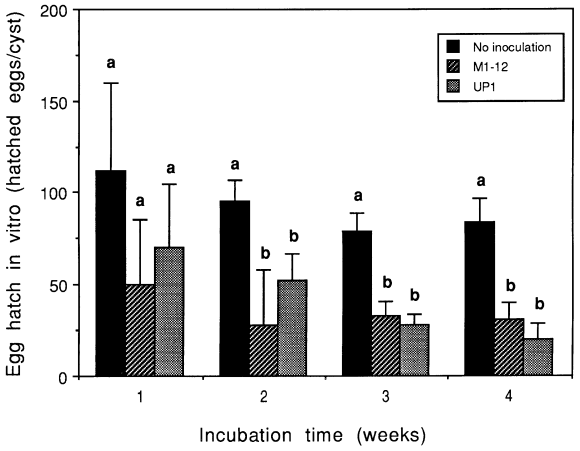

Figure 3. Effect of incubation time on the ability of M1-12 (10^7 CFU ml^{-1}) and UP1 (10^6 CFU ml^{-1}) to affect egg hatch of *G. rostochiensis in vitro*. Vertical bars represent the standard deviation of data. Treatments resulting in differences that were statistically significant at $P = 0.05$ are indicated with different letters at each incubation time.

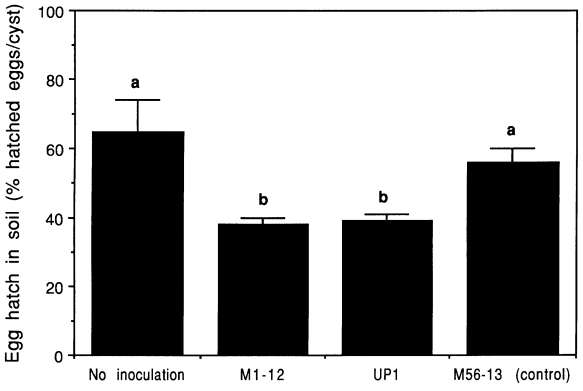

Figure 4. Influence of strains M1-12 and UP1 on the egg hatch of *G. rostochiensis* in soil at 25 weeks. Vertical bars represent the standard deviation of data. Treatments indicated with the same letter were not significantly different ($P = 0.05$).

of egg hatch by UP1 increased from 54% to 78% from week 2 to week 4, but M1-12 inhibited egg hatch to the same extent from week 2 to week 4.

Effect of Stenotrophomonas *M1-12 and* Chromobacterium *UP1 on egg hatch ability of* G. rostochiensis *in soil*

M1-12 and UP1 significantly reduced the ability of eggs of *G. rostochiensis* to hatch at 25 weeks after inoculation of soil planted with potatoes when compared with the uninoculated control (Figure 4). The chitinase-negative strain M56-13 had no influence on egg hatch. Incubation for 15 weeks resulted in essentially similar effects of treatments on egg hatch (data not shown).

Discussion

Recently, antagonistic bacteria have received increased attention as potential biocontrol agents against nematode pests (Sikora and Hoffmann-Hergarten, 1993; D. Cronin, unpubl.). The egg shell of the nematode *G. rostochiensis* contains chitin and in this work purified commercial chitinase proved successful in reducing the extent of egg hatch of *G. rostochiensis in vitro* (Table 1). Therefore, chitinase-producing bacteria were isolated from a soil in which cyst nematode damage was absent. While it was not determined whether this absence of crop damage was due to nematode suppression by a biotic component or was linked to physical/chemical soil properties, an attempt was made to identify chitinase-producing bacteria capable of interfering with the hatching process of the potato cyst nematode *G. rostochiensis.*

A total of 3,200 bacteria were isolated from the Cobh soil and studied for chitinase production. Simple characterisation of 26 of the best 39 chitinase-producers, on the basis of Gram reaction and production of oxidase and catalase, showed that chitinase-production was a biocontrol trait found in very diverse genetic backgrounds in bacteria isolated from the Cobh soil (Table 2). The 39 chitinase-producers were tested for their ability to inhibit egg hatch of *G. rostochiensis in vitro*. The only carbon and nitrogen sources present in the test system were constituents of the cysts/eggs and/or compounds diffusing from them. All 39 chitinase-producing bacteria tested significantly inhibited egg hatch of *G. rostochiensis* when compared with the uninoculated control, but a chitinase-negative bacterium in this work (i.e. strain M56-13) did not. This suggests that chitinase-producing bacteria could have a role to play in the biocontrol of *G. rostochiensis*. Mercer et al. (1992) demonstrated that chitinase treatment of root-knot nematode eggs resulted in an increase of egg hatch, and also in the development of aberrant eggs which were altered in egg shape. However, the chitinase-producing bacteria tested in this study differed in their ability to affect egg hatch of *G. rostochiensis in vitro*. This could result from differences in the amount or the type of chitinase produced

(Mercer et al., 1992), and/or from other, unrelated factors (Westcott and Kluepfel, 1993). Indeed, there was no correlation between the level of egg hatch inhibition *in vitro* and the quantity of chitinase produced on chitin MM agar (Figure 1), indicating that chitinase may not be the only factor to contribute to the ability of these bacteria to inhibit egg hatch of *G. rostochiensis*. The ability to inhibit egg hatch efficiently *in vitro* was found among different types of chitinase-producing bacteria isolated from the Cobh soil, suggesting that the overall level of chitinase activity in soil is a biocontrol property resulting from the contribution of a diverse microbial community.

The ability of M1-12 and UP1 to inhibit egg hatch of *G. rostochiensis in vitro* was further investigated. The ability of the strains to inhibit egg hatch depended on the inoculum level and higher inoculum levels increased the level of inhibition (Figure 2). The duration of the incubation also influenced the rate at which each strain significantly inhibited the egg hatch of *G. rostochiensis* (Figure 3). The incubation time each strain needs to affect the egg hatch of *G. rostochiensis* is a very important factor as it determines how long prior to sowing the bacteria should be incorporated into the soil.

In order to evaluate whether resident bacteria present in/on the cyst were involved in the effect of M1-12 and UP1 on egg hatch, an effort was made to surface-sterilise the cysts. The cysts were incubated in a 2% sodium hypochlorite solution for five min and rinsed three times in sterile deionised distilled water. This process reduced the number of bacteria present in/on cysts from 10^6 to 10^2 CFU cyst^{-1} without affecting nematode viability (data not shown). Both M1-12 and UP1 (incubated for 21 days at 10^7 and 10^6 CFU ml^{-1} buffer, respectively) inhibited egg hatch of partially surface-sterilised cysts of *G. rostochiensis* compared with the uninoculated control (data not shown), suggesting that the resident bacteria were not required for successful inhibition of egg hatch.

The capacity of strains UP1 and M1-12 to inhibit the ability of eggs of *G. rostochiensis* to hatch was evaluated in soil. Both inhibited egg hatch ability of *G. rostochiensis* compared with an uninoculated control (Figure 4) although to a lesser extent than *in vitro*. A strain that did not inhibit egg hatch *in vitro*, M56-13, did not inhibit egg hatch ability in soil. UP1 and M1-12 inhibited the hatching ability of *G. rostochiensis* similarly at 15 and 25 weeks in soil infested with the cyst nematode, indicating that the inhibition of egg hatch ability in soil is independent of time between 15 and 25 weeks. The observed reduction of nematode egg hatch, while significant, is probably insufficient to greatly affect nematode population dynamics in the soil environment, but it may be enough to reduce plant damage and so improve crop yields.

Previous work on chitinase-producing bacteria has focused on their ability to control phytopathogenic fungi (Ordentlich et al., 1988; Kobayashi et al., 1995). The results of the current investigation indicate that chitinase-producing bacteria isolated from soil can inhibit egg hatch of *G. rostochiensis* and represent a promising alternative for the control of the potato cyst nematode. A biocontrol strategy is proposed, based on the use of chitinase-producing bacteria as soil inoculants, with the objective of inhibiting egg hatch of the nematode. Future work will evaluate the biocontrol ability of chitinase-producers M1-12 and UP1 in fields infested by the potato cyst nematode.

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