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Research Note

Influence of laboratory culturing of *Paecilomyces lilacinus* (Thomson) Sams. and *Pochonia chlamydosporia* Zare *et al.* on spore viability and infectivity against *Meloidogyne incognita* Chitwood eggs

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ABSTRACT: **Two native isolates each of *Paecilomyces lilacinus* (Thomson) Sams. (PDBC PL55 and PDBC PL58) and *Pochonia chlamydosporia* Zare *et al.* (PDBC VC56 and PDBC VC57) were subjected to repeated sub-culturing for ten generations at 28 ± 1°C with intervening resting periods in refrigerated conditions to evaluate the effect on their spore viability and infectivity. Significant decrease in spore viability was noticed from generation IX in case of *P. lilacinus* isolates (PDBC PL55 and 58), while, *P. chlamydosporia* (PDBC VC56 and 57) isolates recorded significant reduction in spore viability from generation VII. The infectivity of the *P. lilacinus* and *P. chlamydosporia* isolates under study were on par with each other in all the generations, which recorded significant reduction in tenth generation. Between the spore viability and infectivity of the isolates under report, spore viability was more influenced by repeated sub-culturing than infectivity.**

KEY WORDS: Infectivity, *Meloidogyne incognita*, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, spore viability, sub-culturing

The common antagonistic fungi such as *Paecilomyces lilacinus* and *Pochonia chlamydosporia*, originally isolated from soil in natural forms undergo several cycles of sub-culturing in the laboratory or production unit before they reach the final commercial formulation. Although several methods of culture preservation are available, the working cultures and cultures for short-term storage are maintained on standard mycological agar based media at 10 °C for a few weeks to a few months, depending on isolate or species, which in due course lose spore viability. Many fungi and bacteria are known to attenuate during successive sub-culturing resulting in the loss of virulence and also variation in conidiation characteristics (Matsuyama and Yamaguchi, 1979; Ezuka, 1980; Samsinakova and Kalalova, 1983; Jenkins *et al.*, 1998). The common method to overcome this problem is to pass the pathogen through the host at intervals (Hall, 1980), which is

cumbersome and complicated in soil-inhabiting microorganisms like nematodes and their propagules. The knowledge of the behaviour of isolates regarding their virulence and spore viability during sub-culturing will help in optimizing this process. Among various antagonistic fungi reported so far, the egg/ egg mass/ cyst parasitic saprophytic fungi, *viz. Paecilomyces lilacinus* and *Pochonia chlamydosporia* were successful in reaching commercial formulation stage for the control of plant parasitic nematodes (Swarup and Gokte, 1986; De Leij and Kerry, 1991; Stirling, 1991; Nagesh and Reddy, 2003). However, studies are lacking on the effect of repeated laboratory culturing of the nematode antagonistic fungi on their spore viability and infectivity. The objective of this study was to evaluate whether repeated culturing with intervening resting period in refrigerated conditions influenced the spore viability and infectivity of two native isolates each of *Paecilomyces lilacinus* and *Pochonia chlamydosporia* from the collections of Project Directorate of Biological Control, Bangalore.

Organisms, media and culture conditions

Two native isolates each of *Paecilomyces lilacinus* (PDBC PL55 and 58) and *Pochonia chlamydosporia* (PDBC VC56 and PDBC VC57) grown and maintained on potato dextrose broth plus glycerol at –20° C were used in the experiment. The stock starter cultures of *P. lilacinus* isolates and their subsequent generations were cultured on PDA (2%) at 30° C for production of conidia, while *Po. chlamydosporia* was cultured on corn meal agar (CMA) at 28 ± 1°C for 15-18 days for complete spore production.

Collection of eggs from egg masses of *M. incognita*

Meloidogyne incognita (Kofoid and White, 1909) Chitwood 1949 used in this study was obtained from single egg mass of tomato (*Lycopersicon esculentum* Mill. cv. Pusa Ruby) galled roots. Nematode inoculum was prepared by extracting *M. incognita* eggs from galled tomato roots using sodium hypochlorite technique (Hussey and Baker, 1973).

Methodology for obtaining generations

The fungal isolates from storage (PD broth-glycerol media stored at –20°C) were inoculated in to sterile Petri-plates containing PDA and CMA, for *P. lilacinus* and *Po. chlamydosporia* isolates, respectively, and incubated at 28 ± 1°C for 3 weeks and designated as generation 1. The Petri-plates with fully sporulated fungi were given an intervening storage (resting) period of 7 days at 8°C (in a refrigerator). The fungal spores of *P. lilacinus* and *Po. chlamydosporia* isolates after resting period were inoculated on to fresh PDA or CMA, respectively, and incubated at 28 ± 1° C for 3 weeks and designated as generation 2. This was followed by intervening resting period of 7 days at 8° C.

These steps were repeated till 10 generations were obtained. Four replications were maintained for each isolate and observations taken on each fungal culture from each generation, separately.

Nine-mm discs were cut from each Petri-plate containing the isolates of the fungi (21 days old) using a cork borer. These discs were placed in a test tube containing 10 ml of sterile distilled water and a drop of Tween 80 (80%) and vortexed vigorously in a laminar flow. Depending on the spore concentrations, the stock suspensions were serially diluted and the spores were counted under a microscope (40X) using a haemocytometer. Further the spore concentration per ml or per 9mm disc were rechecked by measuring the optical density of the spore suspension at 650 nm (2.5×10^6 spores ml^{-1} corresponds to A650 of 0.5) using a UV-Vis scanning spectrophotometer (Biomate 5, Thermospectronic) (Kirk *et al.*, 1978). The dilutions were uniformly fixed for all the isolates of *P. lilacinus* or *Po. chlamydosporia*, respectively. One hundred µl of fixed dilution of the spore suspension pipetted in to sterile Petri-plates containing solidified PDA with Rose Bengal and streptomycin sulfate and incubated at 28 ± 1 °C for the colonies to grow. The spore viability of the fungal isolates of each generation was compared with the discs obtained from respective starter cultures on PDA from which generation 1 was obtained.

Infectivity of the fungal isolates was observed on a thin layer (≈1mm) of water agar medium (0.8%) in Petri-plates (diam 5cm) to each of which a plug/ disc of inoculum (diam 9mm) taken from the edge of developing fungal cultures on PDA and CMA (*P. lilacinus* and *Po. chlamydosporia*, respectively). On water agar the mycelial growth of each strain was similar and gradual. 0.5 ml of a suspension containing ≈100 eggs was spread over the medium and the plates were incubated at 12° C. The eggs were observed for infection at 40 X magnifications at 24 hours interval and the number of infected eggs was recorded. All the treatments were replicated five times.

Treatment effects (repeated culturing) were subjected to analysis of variance (ANOVA) and the treatment means were separated using least significant difference (LSD).

Spore viability and infectivity of the fungi against the eggs of root-knot nematodes

Spore viability of all the four isolates, *viz.* PDBC PL55, PDBC PL58, PDBC VC56 and PDBC VC57 was cent per cent in the starter cultures (Table 1). Spore viability showed a gradual decline from generation III in all isolates except *Po. chlamydosporia* isolate, PDBC VC57 (100 % in generation III), which recorded decline from Generation IV. Significant decrease in spore viability was noticed only from generation IX in case of *P. lilacinus* isolates (PDBC PL55 and 58), while; *Po. chlamydosporia* (PDBC VC56 and 57) isolates

Table 1. Effect of repeated lab culturing of *P. lilacinus* and *Po. chlamydosporia* isolates on their spore viability and infectivity

Generation	Spore viability (%)				Infectivity of eggs (%)			
	P. lilacinus		*Po. chlamydosporia*		*P. lilacinus*		*Po. chlamydosporia*	
	PDBC PL55	PDBC PL58	PDBC VC57	PDBC VC58	PDBC PL55	PDBC PL58	PDBC VC57	PDBC VC58
Starter	100.0^{b}	100.0^{b,c}	100.0^{c,d}	100.0^{c}	98.5^{b}	96.5^{a,b}	99.0^{a,b}	94.0^{a,b}
I	99.0^{b}	99.0^{b,c}	100.0^{c,d}	100.0^{c}	98.0^{a}	96.5^{a,b}	98.5^{a}	96.0^{a,b}
II	100.0^{b}	100.0^{b,c}	100.0^{c,d}	100.0^{c}	98.0^{a}	97.0^{a,b}	98.5^{a}	95.0^{a,b}
III	98.5^{b}	98.0^{b,c}	98.5^{c,d}	100.0^{c}	98.0^{a}	96.5^{a,b}	98.0^{a}	94.0^{a,b}
IV	95.0^{b}	96.5^{b}	98.0^{c,d}	98.0^{c}	98.0^{a}	97.0^{a,b}	99.0^{a,b}	96.0^{a,b}
V	94.5^{a,b}	95.5^{b}	98.0^{c,d}	97.0^{c}	97.5^{a}	97.0^{a,b}	98.5^{a}	95.0^{a,b}
VI	95.0^{b}	95.0^{b}	98.0^{c,d}	96.0^{c}	94.0^{a}	92.5^{a}	96.0^{a}	94.5^{a,b}
VII	94.0^{a,b}	93.0^{b}	96.0^{c}	95.0^{b,c}	93.5^{a}	92.0^{a}	91.5^{a}	92.0^{a}
VIII	94.0^{a,b}	89.5^{a,b}	90.0^{c}	87.5^{b}	93.0^{a}	91.0^{a}	90.5^{a}	90.5^{a}
IX	90.0^{a}	82.5^{a}	80.5^{b}	78.5^{a}	89.5^{a}	88.0^{a}	85.0^{a}	84.5^{a}
X	84.5^{a}	79.0^{a}	72.5^{a}	71.0^{a}	86.0^{a}	86.0^{a}	84.0^{a}	81.5^{a}
SEM±	2.22	1.98	1.44	1.75	2.88	2.66	3.42	2.92
CD (P=0.05)	9.0	7.7	6.1	7.5	12.1	10.1	14.7	12.2

*Significant at P = 0.05

NS – Non-significant

Means followed by the same letter within a column are not significantly different.

recorded significant reduction in spore viability from generation VII. Spore viability was significantly low in all the four isolates in the generation X, indicating that the repeated sub-culturing influenced spore viability. Between the isolates of the two fungi, PDBC PL55 and PDBC VC56 recorded higher or equal spore viability at all generations over their respective counterparts.

The isolates of *P. lilacinus* (PDBC PL55 and 58) and *Po. chlamydosporia* (PDBC VC56 and 57) recorded an infectivity of 98.5, 96.0, 99.0 and 94.0 per cent, respectively (Table 1), which showed decrease with the number of generations cultured. However, significant reduction in infectivity of these fungi was observed in generation X. The infectivity of the *P. lilacinus* and *Po. chlamydosporia* isolates under study were almost on par with each other, respectively, in all the generations. The results presented demonstrate that there is a decline in spore viability and infectivity of the isolates after 8-10 repeated sub-culturing.

Information on the effect of repeated sub-culturing and laboratory storage on spore viability and infectivity of antagonistic fungi are essential cardinals for their use in biological control programmes. This helps to identify the generation from which there is a significant reduction in spore viability and infectivity so that it can be recycled through the host to restore these desirable parameters, and a suitable preservation technique for specific period of storage can be worked out.

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