In vitro mass production of *Pasteuria penetrans* (Mankau) Sayre and Starr for the management of *Meloidogyne incognita* (Kofoid and White) Chitwood

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ABSTRACT

Plant parasitic nematodes are parasitized and preyed by a variety of soil organisms which includes predatory nematodes, fungi, bacteria, viruses, protozoans, turbellarians, tardigrades, mites and other microarthropods. One such spore forming and an obligate parasite is *Pasteuria penetrans*. The major limitation in the use of *Pasteuria* spp. is their inability to grow in the absence of host nematodes. *In vivo* systems although proven are unlikely to be adopted for large scale application in most farming systems. Attempts were made to culture the organism in *in vitro*. Among the symbiotic bacteria used for mass production co-culturing with *Enterobacter* proved to be successful in culturing this obligate organism in a specially developed medium for mass multiplication.

Key words: *Pasteuria penetrans, Meloidogyne incognita, in vitro,* mass production.

INTORDUCTION

Pasteuria (Metchinkoff, 1888) is a Gram positive endospore forming bacterium. It is one of the most promising biocontrol agents for many nematodes that cause extensive damage to field crops, vegetables, turf grass and ornamentals. The bacterium propagates within the pseudocoelom of the infected nematode host and thus resulting in loss of fecundity (Bird, 1986). The nematodes serve as an amplification medium for the infective spores. Each infected root knot nematode female contains an average of 2-2.5 million spores which are eventually released into the soil. The ability to form spores is a significant advantage in formulating this organism.

MATERIALS AND METHODS

Isolation of *Enterobacter cloacae*

One hundred spores encumbered J_2 were inoculated in tomato plants grown for a week in tumbler pots. The inoculated plants were maintained for 15 days in the glass house. The plants were uprooted and root system was harvested and incubated in cytolase solution (25 %) for 48 h (Chen *et al*., 1996). The roots were placed in 100 mesh and jet of water was directed towards the root to dislodge the milky white females. The females were carefully collected and rinsed in double distilled water for 10 times. The infected females were squeezed between the cover slips and observed under the microscope to confirm that the bacterium was in vegetative stage. The body content was streaked on nutrient agar plates. Single colonies which appeared after 24 h were separately streaked on slants. Primary identification was made by Gram staining, which stained negative. The strains were confirmed as *Enterobacter* through enterotube test (Martin *et al*., 1971). The bacterium was sub cultured on NA slants. Symbiotic bacteria from *Steinernema glaseri* and *Heterorhabditis indica* were isolated by the procedure given by Heungens *et al*. (2002).

Preparation of co culture Medium

A special co culture liquid medium was prepared with simple modifications of medium reported by White *et al*. (2006). The composition of co culture medium is Glucose (50 g), Egg yolk (10 ml), Yeast extract (1g), $CuSo_4.5H_2O$ (1g), $MnSO_4.4H_2O$ (1g), $ZnSo_4$ (2 g), H_3Bo_3 (0.25g), $FeSo_4$ (0.5g), KNo_3 (1g), Micronutrient solution (10ml), Multivitamin tablet (500 mg) and Double distilled water (1000 ml) with pH 5.5.

The isolate of *Enterobacter cloacae* (M1) symbiotic bacteria of entomopathogenic nematodes *viz*., *Xenorhabdus poinarii*. (*S. glaseri*) (M2) and *Photorhabdus luminescens*. (*H. indica*) (M3) were inoculated in the co culture medium. After 48 h, the medium was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and filtered through bacterial filter and the medium is made free of any bacterial cells. To the culture filtrates the vegetative stages of *P. penetrans* collected from infected females were added @ 5 crushed females per flask. Observations were made at 25, 35, and 45 DAI by drawing 5ml of aliquot. The experiment was replicated five times.

RESULTS AND DISCUSSION

Inoculation of vegetative stages of *P. penetrans* in the culture flask turned the medium turbid. *P. penetrans* in vegetative stage began to multiply in all the media. Mycelia like structures were found during the early stages. Vegetative stages were observed 25 DAI in all the media. After 35 DAI, vegetative stages were present in M1 and M2 and no vegetative structures were present in M3. In M2 medium, some replications did not show any vegetative stage. Spores were present in M1 after 45 DAI. Some replications showed both the presence of spores and vegetative stages. Spores settled at the bottom of the flask like a precipitate. The spore count of M1 medium was 1×10^4 spores per flask (Table 1). The culture filtrates

Table 1. Multiplication of *P. penetrans* in different media

Media	DAI	Vegetative stages	Spores	Spore load per 50 ml medium
	25	+	-	-
	35	+	-	-
	45	-,+	+	1×10^4
	25	+	-	-
	35	-,+	-	-
	45	-	+	1000
	25	+	-	-
	35	-	-	-
	45	-	+	100

+ present,- absent

M1 - Culture filtrate of *Enterobacter,cloacae*

M2 - Culture filtrate of *X. poinarii*

M3 - Culture filtrate of *P. luminescens*

of the *E. cloacae* supported a moderate growth of *P. penetrans*. In M2, lysis of vegetative structures was noticed and only 1000 spores were produced per flask. Similar reactions were recorded in M3 medium also. The M2 and M3 media supported poor multiplication of *P. penetrans*. Various media had been tested for their ability to support the growth of isolates of *P. penetrans* but they can maintain only the vegetative cells and no spore production was observed (Reise *et al*., 1988). Bishop and Ellar (1991) screened several microbial media but resulted in failure. After a long time multiplication of this organism is now partially successful. *In vitro* mass production of *P. penetrans* is simple when compared to other methods. The culture filtrates of the *M. incognita* associated bacterium supports the growth of the *P. penetrans*. The biochemical composition of culture filtrate is yet to be defined. The isolate of *E. cloacae* which was isolated in the present study supported a moderate growth of *P. penetrans*. Still efficient isolate is to be isolated. The symbiotic bacterium obtained from entamopathogenic nematodes *viz., S. glaseri* and *H. indica* supported moderate and poor growth of *P. penetrans* respectively. The antibiotics produced by these bacteria should be the limiting factor for multiplication of *P. penetrans*. *P. luminescens* culture filtrate digested the vegetative stages of *P. penetrans*. The late vegetative stages resistant to digestion formed spores. The culture filtrates of *E. cloacae* contains a "helper factor" which helps to maintain and produce spores of *P. penetrans* as reported in WO01/11017A2 (Gerber and White, 2001).

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