J. Mycopathol. Res. **44**(2) : 219-223 (2006)

Some abiotic and biotic factors influencing formation and germination of chlamydospores of *Fusarium udum*

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Formation and germination of chlamydospores are two very important phases in the life cycle of pathogenic soil borne *Fusarium* spp.

Chlamydospores of *Fusarium udum*, the causal organism of wilt of pigeonpea, were easily produced under stress conditions in the laboratory. Semisynthetic media favoured better formation of chlamydospores than synthetic media. Chlamydospores were produced in larger numbers when smaller amount of nutrient was supplied. Addition of the soil bacterium, *Bacillus subtilis* enhanced chlamydospore formation. Water extract from soil also induced chlamydospore formation, the formation being influenced by soils of different locations.

For germination of chlamydospores some energy source is required. Dextrose and DL-Aspartic acid had a profound stimulating effect on the germination of chlamydospores of *F. udum*, germination increased with an increase in the concentration of these chemicals. There was differential supporting effect of root exudates of different host plants on germination of chlamydospore. Germination percentage was very high in the root exudate of a susceptible cv of pigeonpea. Germination percentage was significantly lower in the root exudates of a resistant cv, as well as some non-host plants. Higher humidity supported better chlamydospore germination.

Key words : *Fusarium udum*, chlamydospore formation, germination, factors affecting

INTRODUCTION

Formation and germination of chlamydospores are two very important phases in the life cycle of pathogenic soil borne *Fusarium* spp. In absence of the host, mycelial cells and conidia rapidly undergo lysis and are destroyed in soil by the antifungal activities of the vast number of soil microorganisms which include fungi, actinomycetes, bacteria etc. Under such unfavourable conditions for the growth of the pathogenic Fusaria, the vegetative hyphae and conidia of these fungi are transformed into thick walled resing structures, chlamydospores, in which state these fungi are known to survive for a considerable period of time in soil.Several workers have demnonstrated the existence of commonly pathogenic Fusaria in soil as chlamydospores (Trujillo and Snyder, 1963; French and Nielson, 1966). Pal and Sen Gupta (1996) have reported chlamydospores production in soil by *Fusarium udum*, the causal organism of pigeonpea wilt.

On return of favourable conditions with the sowing of congenial host plants, the chlamydospores surviving in soil, germinate by production of germ tubes and active growth stage of the fungus is revived. The hyphe next infects the nearby host roots and the pathogenic state is reestablished.

Some abiotic and biotic factors influencing the formation and germination of the chlamydospores of *Fusarium udum*, the causal organism of pigeonpea wilt, under laboratory conditions is dealt in this paper.

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MATERIALS AND METHODS

Chlamydospore formation

An isolate of *Fusarium udum* Bult., isolated from a wilt infected pigeonpea plant was used for the present study. Single spore isolate of the fungus was maintained in potato dextrose agar slants at 5°C and subculturing was made at monthly intervals.

Media (liquid) used for the experiment on the effect of medium on chlamydospore formation were : Czapek Dox (modified) $NaNO_3$–2.0 g ; K_2HPO_4–1.0 g ; $MgSO_4$, $7H_2O$–0.5 g ; KCl–0.5 g ; $FeSO_4$, $7H_2O$-0.01 g; Sucrose-30.0 g; dist. water-1000.0 ml), Sucrose casamino (Sucrose-15.0 g; K_2HPO_4-10.0 g, MgSO4 $7H_2O$-0.5 g, hydrolysed casein 4.5 g; dist. water-1000.0 ml), Potato dextrose broth (potato decoction-200.0 ml; dextrose-20.0 g; dist water – 800.0 ml); and Oat meal broth (Oat-30.0 g, dist water to make the volume 1000.0 ml).

For all the other experiments 0.1% dextrose solution was used as the basal medium and except otherwise mentioned 20.0 ml of the sterilized medium were taken in 100 ml Erlenmeyer flasks.

For studying the effect of soil bacteria on chlamydospore formation, an isolate of *Bacillus subtilis*, isolated from soil and supplied by Prof. B. K. Dey, Department of Soil Microbiology, was used, A suspension of the 7 day old bacterial culture was made in 5 ml sterile distilled water and aseptically added to 7 day old *F. udum* culture in the basal medium.

Soil samples used in this experiment were collected from different agroclimatic zones of West Bengal : Gangetic alluvial (Kalyani, Dist. Nadia), red lateritic (Bolpur, Dist. Birbhum) and Tarai (Pundibari, Dist. Coochbehar). The soil up to plough depth (15 cm) was collected, air dried, grounded, sieved through a 2 mm sieve to remove all course particles. Twenty five g of each of the soil samples were added separately in 100 ml of sterile distilled water in Erlenmeyer flasks and kept overnight. The soil suspensions were then thoroughly shaken in an electric shaker for half an hour and filtered through cheese cloths. The soil extracts thus obtained were centrifuged at 1500 rpm for 10 minutes and supernatants were collected. Ten ml of the soil extract thus obtained was added to 10 ml of 2 % sterilized dextrose solution in 100 ml Erlenmeyer flasks.

Mycelial discs (6 mm diameter) of a 7 day old *F. udum* culture, grown on PDA at 28°C, were cut by means of a sterilized cork borer and inoculated into 100 ml Erlenmeyer flasks containing different test media and incubated at 28°C. After 15 days growth the mycelial mats were collected by filtering through folded bandage cloth and washed two times in sterile distilled water. The mycelial mats were blended separately in a Waring blender for 1 min for separation of the chlamydospores. The chlamydospore suspensions were centrifuged at 100 rpm 2 min. The supernatants were discarded and to the chlamydospores settled at the bottom 25 ml of distilled water was added to make chlamydospore suspensions. The number of chlamydospores produced was determined by a haemacytometer. Five replications were taken for each treatment.

Chlamydospore germination

Root exudates were collected following the method described by Dasgupta and Sen Gupta (1988).

Chlamydospore germination was studied by placing a drop of the test fluid in the cavity of a double grooved glass slide by means of a sterilized micropipette. The test fluid drop was allowed to air dry and a drop of chlamydospore suspension was placed in the cavity containing the dried test fluid.

The grooved slides were then rested on two glass rods in moist chambers prepared by lining Petridishes with moistened filter papers and incubated for 24 h at 28°C. The slides were then examined under a microscope for observing chlamydospore germination. A drop of lactophenol cotton bule was added to the groove to facilitate clarity of observation.

Humidity in the moist chambers was adjusted by adding different concentrations of glycerol (in water) instead of water only (Scharpf, 1964).

RESULTS AND DISCUSSION

Chlamydospore formation

The composition of the medium in which the fungus was grown may have some influence on chlamydospore formation. In the present study significantly larger numbr of chlamydospores of *F. udum* were produced in semisynthetic media, namely potato dextrose broth and oatmeal broth than the synthetic media (Table 1). Synthetic media, being more balanced and easily assimiliable, probably supported better hyphal growth and conidia formation than chlamydospores which are mainly produced under stress conditions. Influence of growth media on chlamydospore formation by *Fusarium* spp. has also been demonstrated by Huang *et al.* (1983).

Table 1 : Effect of different media on formation of chlamydospores

Media	Chlamydospores produced (x 10^5/ml.)
Sucrose casamino	2.95
Potato dextrose broth	4.25
Oatmeal broth	5.30
Cazapek-Dox	2.65
C.D. (P=0.05)	0.81

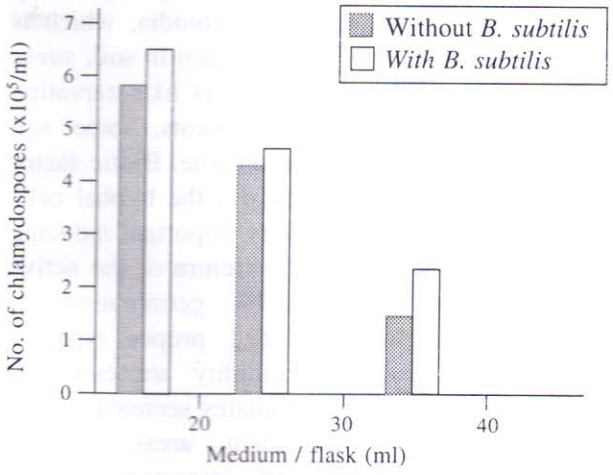

Fig. 1 : Effect of amount of nutrient and ammendment of *B. subtilis* on chlamydospore formation

Significantly increased number of chlamydospores were produced when lesser amount of energy, in the form of dextrose, was provided (Fig. 1). Mayers and Cook (1972) induced chlamydospore production in *F. solani* by abrupt removal of organic carbon substrate from the medium.

Chlamydospore production was further increased by addition of the soil bacterium. *Bacillus subtilis* in the medium for growth. Singh and Singh (1983) also reported increased production of chlamydospores by *F. udum* in presence of soil bacteria.

Addition of soil extracts in basal medium very much enhanced chlamydospore formation (Table 2). However, soils from different locations had varying effect on inducing chlamydospore formation. The effect was particularly pronounced when soil extract of Pundibari and Kalyani were used. Chlamydospore formation by *Fusarium* spp. had been considered as a form of fungistasis by Ford *et al.* (1970) and such fungistasis may be influenced by the nature of microorganisms present in a particular soil. Some abiotic factors like nutritional status of the soil, soil pH etc. might also influenced chlamydospore formation. It might be noted in this context that the extract of soils having pH towards neutral side and lower nutritional status favoured chlamydospore formation (Table 2).

Table 2 : Effect of soil extracts on chlamydospore formation

Location	Soil type	pH	Organic C (%)	Total N (%)	No. of chlamydospores (x10^5/ml.)
Kalyani	Silty loam	7.2	0.51	0.05	2.28
Bolpur	Sandy loam	6.3	0.26	0.02	2.25
Pundibari	Loam	5.5	1.60	0.15	1.60
Control					1.15
C.D. (P=0.05)					0.36

Chlamydospore germination

Chlamydospores of *Fusarium* spp. require some energy source for germination. In distilled water germination of the chlamydospores of *F. udum* was nil and in tap water it was negligible. An energy source in the form of dextrose (carbon) or DL-aspartic acid (amino acid) had a profound stimulating effect on germination of chlamydospores and with an increase in the dose of these chemicals germination also increased (Table 3). With 1-2 % dextrose germination was 100 per cent, while with 2 % aspartic acid it was more than 95 %. Earlier Toussoun and Snyder (1961) observed that chlamydospores of *F. solani* formed in unsterilized soil did not germinate in presence of water alone.

Table 3 : Effect of dextrose and DL-Asparitic acid on the germination of chlamydospores of *F. udum*

Treatment	Dose (%)	Chlamydospores germination (%) ±SE
Dextrose	0.1	22.55 (28.25)[1] ± 2.63
	0.5	93.75 (82.50) ± 6.25
	1.0	100.00 (90.00) ± 0.00
DL-Aspartic acid	0.1	92.67 (78.82) ± 4.46
	0.2	95.82 (83.41) ± 5.00
Tap water		1.38 (3.43) ± 0.48
Distilled water		0.92 (2.77) ± 0.03
C.D (P=0.05)		9.13

[1]Figures in parenthesis are transformed angular values.

Table 4 : Effect of root exudates on the germination of chlamydospores of *F. udum*

Crop		Germination of chlamydospores (%) ±SE
Pigeonpea	(cv ICP 11286)	83.64 (66.31)[1] ± 2.37
	(cv ICP 8863)	26.56 (31.01) ± 1.19
Rice	(cv IR – 36)	18.56 (24.36) ± 2.38
Jute		92.67 (78.82) ± 4.46
Tap water		95.82 (83.41) ± 5.00
Tap water		1.38 (3.43) ± 0.48
C.D (P=0.05)		19.96

[1]Figures in parenthesis are transformed angular values.

Nutrient for germination of chlamydospores of *Fusarium* spp. in soil is provided by root exudates of plants growing in the neighbouring zone and this had been demonstrated in case of *F. oxysporum* f. sp. *pisi* (Whalley and Taylor, 1976). In *in vivo* studies Chakraborty *et al.* (1992) demonstrated about 5 times higher germination of the chlamydospores of *F. udum* in soil in the vicinity of the roots of a susceptible pigeonpea cultivar as compared to that of a resistant cultivar. Root exudates contained a diverse number of chemicals including amino acids, organic acids and vitamins. They, however, varied qualitatively and quantitatively from host to host and some may even contain some chemicals toxic to some micro-organism (Rangawami and Balasubramanian, 1963). The differential nature of the root exudates of different host plants thus exerted some influence on the germination of chlamydospores and selective host infection. The root exudates of several crops were found to enhance the germination of Chlamydospores of *F. udum* when compared with that of tap water. However, the enhancement was different in root exudates of different crops. Chlamydospore germination in the root exudates of a susceptible cv of pigeonpea was remarkably higher, about 3 times higher than that of a resistant cv. Germination percentage in the root exudates of the resistant cv was a little higher than in the root exudates of some other crops (rice & jute) studied, but the differences were not statistically significant.

Table 5 : Effect of humidity on germination of chlamydospores of *F. udum*

Relative humidity (%)	Germination of chlamydospores (%) ±SE
25.00	7.31 (13.44) ± 2.51
45.00	12.78 (20.78) ± 1.89
65.00	13.55 (18.46) ± 5.61
80.00	17.83 (24.25) ± 2.60
92.00	19.65 (25.02) ± 2.41
100.00	22.55 (28.95) ± 2.63
C.D (P=0.05)	5.10

[1]Figures in parenthesis are transformed angular values.

High humidity favoured good germination of Chlamydospores (Table 5). However, between 45 and 65 % RH differences in germination was not significant. At 25 % RH germination was very low.

From the foregoing observations it is evident that unfavourable conditions are required for formation and germination of the chlamydospores of *Fusarium* spp. For formation of chlamydospores by conversion of hyphal cells and conidia, which is essential for survival of the pathogen in soil, stress conditions caused by abiotic factors like starvation and insufficient supply of nutrients, some soil factors etc. play very important role. Biotic factor like soil bacteria, which destroys the hyphal cells and conidia in soil, is also very important inducing chlamydospore formation. For return of the active phase of the pathogen by germination of chlamydospores sufficient and proper type of nutrient, and also high humidity are essential. Among the biotic factors exudates secreted by the host roots in the adjacent areas of the chlamydospores play a very important role in influencing the germination of the chlamydospores in the rhizosphere.

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(Accepted for publication March 27 2006)