
Factors affecting chlamydospore formation by *Fusarium udum*, the causal organism of wilt of pigeonpea

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Effect of some factors on the production of chlamydospores by *Fusarium udum*, the causal organism of wilt of pigeonpea, was studied in culture medium. Of the 5 culture media tested, maximum chlamydospore production was recorded in Czapek Dox medium, followed by oat meal broth and potato dextrose broth. Chlamydospores were produced in larger numbers when smaller amount of nutrient medium was supplied. The addition of the soil bacterium, *Bacillus subtilis* resulted in an increase in chlamydospore production. Water extracts from soil induced chlamydospore production, the effect being most pronounced in extracts of soil from Dhupguri and Kalyani.

Key words : *F. udum*, stimulatory factors, chlamydospore production

INTRODUCTION

Wilt of pigeonpea [*Cajanus cajan* (L.) Millsp.], caused by *Fusarium udum* Butl. is the most serious and destructive disease of the crop. In absence of the host fusarial pathogens survive in soil as dormant, thick walled resting chlamydospores for a considerable period of time, while the thin walled hyphae and conidia are lysed and rapidly disappear from soil (Lockwood, 1968). Several workers have demonstrated the existence of commonly pathogenic *Fusarium* spp. in soil as chlamydospores (Nash *et al.*, 1961; French and Nielson, 1966; Cook, 1968). Chlamydospore formation by *Fusarium* spp. in soil has been considered a form of fungistasis (Ford *et al.*, 1970).

In the present paper, results on the effect of certain factors that influence chlamydospore formation of *F. udum* have been presented.

MATERIALS AND METHODS

A pure culture of *F. udum*, isolated from the stem of a wilt infected pigeonpea plant was used throughout the studies. The fungal culture was multiplied in PDA medium and stored at 5°C for future use.

For studying the effect of different media on chlamydospore formation, the test fungus was grown for 7 days at 28°C on PDA in sterilized petridishes. Six mm diameter mycelial discs were cut from the petridish by means of a sterilized cork borer and one disc was

inoculated into a 150 ml Erlenmeyer flask containing 30 ml of sterilized liquid test medium and incubated at 28°C. The media used were sucrose casamino (sucrose - 15 g, KH₂PO₄ - 1 g, MgSO₄ - 0.5 g, hydrolysed casein - 4.5 g, dist. water - 1 L), host extract + potato broth (peeled potato - 200 g (decoction), host decoction dextrose (host stem - 20 g, dextrose - 20 g, dist. water - to make 1 L), potato dextrose broth (peeled potato - 200 g (decoction), dextrose - 20 g, dist. water - to make 1 L.), oat meal broth (oat - 30 g, dist. water - 1 L), Czapek Dox (NaNO₃ - 2 g, K₂HPO₄ - 1 g, MgSO₄ - 0.5 g, KCl - 0.5 g, FeSO₄ - 0.01 g, sucrose - 30 g, dist. water - 1 L). Three flasks were taken for each culture medium. After 15 days the growth in the flask was taken out by filtering through folded bandage cloth and washed twice in sterile distilled water. The fungal growth was then blended in a waring blender with 50 ml sterile distilled water for 1 min for separation of the chlamydospores produced. The chlamydospore suspension thus produced was then centrifuged at 100 r.p.m. for 2 min. The supernatant was discarded, the chlamydospores settled at the bottom of the centrifuge tubes were collected and a suspension in 50 ml sterile distilled water was prepared. The number of chlamydospores produced in each culture medium was determined by haemocytometer counts.

For convenience and easy preparation, other experiments were conducted in potato-dextrose broth.

For studying the effect of soil bacteria on chlamydospore formation by *F. udum*, a suspension of *Bacillus subtilis* was prepared in 5 ml sterile distilled water and was added to a 7 day old culture of *F. udum*, grown in potato dextrose broth (35 ml) in 150 ml Erlenmeyer flasks at 28°C. After 15 days chlamydospores were collected and counted.

For collection of soil extract 25 g of the soil sample (air dried and powdered) was added to 100 ml of distilled water in 250 ml Erlenmeyer flasks and kept for 12 h. The flasks were continuously shaken in a electric shaker. The extracts were collected by filtering through filter papers. Ten ml of extract of a soil sample was added to 30 ml of potato dextrose broth in a 150 ml Erlenmeyer flasks and inoculated with *F. udum* in the usual way and incubated at 28°C. After 15 days chlamydospores were collected and counted as in the other experiments.

RESULTS AND DISCUSSION

Several workers have postulated various factors as influencing chlamydospore formation by *Fusarium* spp., such as unfavourable conditions (Park, 1954; Cochran and Cochran, 1971), starvation (Meyers and Cook, 1972) or composition of culture medium including C:N ratio (Hsu and Lockwood, 1973 ; Hilderbrand and McCain, 1978; Huang, *et al.*, 1983).

In the present study also composition of the medium was found to have considerable effect on chlamydospore formation by *F. udum*. Maximum chlamydospore production was observed in Czapek Dox medium, followed by oat meal broth and potato dextrose broth (Table 1).

Meyers and Cook (1972) observed in their studies the induction of chlamydospore formation in *F. solani* f.sp. *phaseoli* and *F. solani* f.sp. *pisi* by abrupt removal of the organic carbon substrate. In the present study it was observed that chlamydospores of *F. udum* were produced in larger numbers when smaller amount of nutrient medium was

supplied (Table 2), indicating probably that the fungus is capable of active growth as long as adequate nutrient is available and with the exhaustion of the nutrient in the medium the hyphal cells are readily converted into chlamydo spores, the dormant resting structures.

Table 1. Effect of different liquid media on production of chlamydo spores by *F. udum*

Medium	No. of chlamydo spores ($\times 10^5$ /ml)
Sucrose casamino	2.95
Host extract + potato broth	2.65
Potato dextrose broth	4.82
Oatmeal broth	5.30
Czapek-Dox	5.92

Table 2. Effect of amount of growth medium (potato dextrose broth) on chlamydo spore formation by *F. udum*

Medium/ flask (ml)	No. of chlamydo spore ($\times 10^5$ /ml)	Increasing (%) in chlamydo spore formation (compared to growth in 40 ml medium)
20.0	5.60	348
30.0	4.25	240
40.0	1.25	

A few workers reported increased chlamydo spore production by *Fusarium* spp. in presence of soil bacteria (Ford and Trujillo, 1967 ; Singh and Singh, 1983). It was observed in the present study also that the addition of the soil bacterium, *Bacillus subtilis*, resulted in an increase in chlamydo spore production by *F. udum*. The effect was more pronounced with increased amount of culture medium which probably favoured more rapid multiplication of the bacterium.

Table 3. Effect of the soil bacterium, *Bacillus subtilis*, on the production of chlamydo spores by *F. udum*

Medium/ flask (ml)	No. of chlamydo spores ($\times 10^5$ /ml)		Increase (%) in chlamy- do spore production
	without <i>B. subtilis</i>	with <i>B. subtilis</i>	
20.0	5.60	6.15	8.03
30.0	4.25	4.62	8.70
40.3	1.25	2.03	62.48

Ford *et al.* (1970), on the other hand, suggested that soils contain some chlamydo spore inducing factor(s) irrespective of the nutrient status and such chlamydo spore inducing factor(s) might be the metabolites of soil microorganisms. Results of the present studies indicated (Table 4) that soil extracts of various locations had differential stimulating effect

on chlamydospore production by *F. udum*. Thus, while the chlamydospore inducing effect was very pronounced in the extracts of soils from Dhupguri and Kalyani, it was not so marked in the extract of soils from Bolpur. Soils from different locations naturally vary in various biotic and abiotic factors. Opgenorth and Endo (1985) emphasised the importance of abiotic factors of soil in influencing chlamydospore production by *F. oxysporum* f. sp. *apii*. It thus appears that both biotic, as well as abiotic factors of soil, extracts of which were used, influenced the production of chlamydospore formation by *F. udum*.

Table 4. Effect of soil extracts on production of chlamydospores

Soil sample (location)	Soil type	Soil pH	No. of chlamydospores ($\times 10^5$ /ml)	Increase (%) in chlamydospore over control
Dhupguri (Dt. Jalpaiguri)	Loam	5.5	2.28	+ 82.4
Kalyani (Dt. Nadia)	Silty loam	5.9	2.25	+ 80.2
Bolpur (Dt. Birbhum)	Sandy loam	5.2	1.40	+ 12.0
Control (PDB- 40 ml)			1.25	

Stimulus for chlamydospore formation by *F. udum*, a very important means of survival of the fungus, thus, appears to be influenced not by a single factor, but several factors.

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