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A microscopic analysis of role of conidia and chlamydospore during germination process in *Fusarium udum*

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Abstract

This study is the report of *Fusarium udum conidia*, which are produced behind the active hyphal growth zone during filamentous growth. Monokaryons of *F. udum* have for a long time been known to constitutively produce abundant uninucleate asexual spores (conidia). These spores are sickle shaped, and because of this shape, they are called conidia. Chlamydospores are large, thick-walled spores of variable forms and with condensed cytoplasm. Filaments of *F. udum* produce both intercalary and terminal chlamydospores. During germination of the chlamydospore, a single germ tube displaces the spore pore cap. Similarly, a single germ tube is formed by the conidium. Nuclei within the germ tubes divide, and septa may be formed between the daughter nuclei; alternatively, the germ tubes may stay aseptate through several cycles of nuclear division. Multinucleate side branches emerge, while septum formation occurs in the older portions of the germ tubes to yield cell segments with one or only a few nuclei. This side branches contained new conidia.

Keywords: *Fusarium udum*, Conidia, Chlamydospore, Germination, Wilt

Introduction

Fusarium udum causes wilting in *Cajanus cajan*. It can persist in soil for several years in the form of resisting body known as chlamydospores in absence of the host. Biological functions ascribed to these chlamydospores differ between species. For example, desiccation-resistant chlamydospores of *P. cinnamomi* are produced within plant roots during drought and are transported in root fragments or soil, germinating to cause infections when warm, moist conditions are encountered. When chlamydospores of the nematode-trapping fungus *D. flagrans* are fed to domesticated animals, they can survive passage through the alimentary tract and reduce the number of parasitic nematode larvae that develop from eggs in the feces, thus preventing clinical disease (Ballario *et. al.*, 1998). In addition, the chlamydospore developmental phase of *Aspergillus parasiticus* has been associated with increased aflatoxin production, while chlamydospores of *Fusarium* species are the principal means of long-term survival during unfavorable periods in the soil and play an important role as the primary inocula infecting plants (Ahmed and Miles, 1970; Asgeirsdottir, *et. al.*, 1995). Chlamydospores have also been observed in human fungal pathogens such as *Candida albicans*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis* (Ballario and Macino, 1997)

The environmental indications for chlamydospore formation for various fungi are usually species specific and include nutrients (Anderson, 1971), osmolarity, light (Bieszke, *et.al.*, 1999), pH, temperature, air (Anderson, 1971), drug treatment, and plant stimulants. Thus, although chlamydospores are produced by a broad assortment of fungi, their mechanisms of formation, biological functions, and molecular regulation remain enigmatic.

Here we describe the identification of chlamydospores in the *F. udum*. It can be readily isolated from the soil or infected plant root of *Cajanus cajan*. While many soilborne fungi produce chlamydospores as long-lived survival structures under hostile environmental conditions. Here we describe the formation of a morphological structure associated with *F. udum* that is providing the documentation of such structures in this organism.

Materials and methods

Isolation of fungal pathogen

The cropping areas of Betul district in Madhya Pradesh, India were surveyed and wilt prone area were marked. Samples of diseased root parts and seeds were randomly collected and fungal pathogen *Fusarium udum was* isolated from infected roots of pigeon pea (*Cajanus cajan*) following the water agar technique. This technique was used for the isolation of external and internal mycoflora of the diseased seeds and infected roots showing reddish brown lesions on the hypocotyls. The external surface mycoflora was isolated by directly placing the seeds and infected plant material on the water agar medium containing 2% agar. For the isolation of internal mycoflora, seeds and diseased roots were surface sterilized by soaking them in 0.5% sodium hypochlorite solution for 3-5 min. Samples were then rinsed in several changes of sterile

water to remove the traces of sodium hypochlorite, and placed on the surface of pre-incubated water agar plates containing Penta-chloro-dinitrobenzene (PCNB). After incubation at 28°C for one week, plates were observed for the appearance of various fungi.

Identification of Fungal Pathogens

Different fungal isolates from infected seeds and plant materials were grown on the potato dextrose agar (PDA) medium. The fungal cultures were maintained on potato dextrose agar (PDA) medium and characterized following illustration and description of standard mycological literature and comparing with standard cultures obtained from the division of Plant Pathology, Indian Institute of Pulses Research (IIPR), Kanpur, India.

Pathogencity test

After identification *F. udum,* culture was tested for the establishment of pathogeneeity on *C. cajan*. Healthy seeds of pigeon pea were surface sterilized by 0.5% sodium hypochlorite solution and wrapped in seed germination paper and incubated for one week at 28°C. After seedling emergence, these were artificially inoculated by spraying aqueous suspension of Chalmydospore and conidia of *F. udum*. Thereafter, seedlings were transferred in to the earthen pots containing 5 kg steam sterilized soil. The pots were covered with polythene bags to avoid the contamination. Control plants were treated with sterilized distilled water. Symptoms of fusarium wilt on the root parts of the plants were observed. Infected plant parts were again used for the re-isolation of *F. udum* and the culture was later compared with that of parental culture of *F. udum*. Isolated fungal cultures of *F. udum* was maintained on PDA medium at 4 °C.

Microscopy

Cells were grown on PDA medium on the top of slides and kept for incubation at 28 °C. These slides were observed after every 6 h of incubation till complete mat of fungus was not grown.

Results and discussion

A clearer understanding of the reproductive modes and survival structures of fungal pathogens in the environment is of great importance with regard to their ecology and epidemiology. Budding, conidiation, sporulation, fragmentation of hyphae, and conversion of hyphal elements into chlamydospores are common modes of reproduction. In some soilborne fungi, chlamydospores have been documented to have a role as survival structures. This study is the report of *F. udum* chlamydospores, which are produced behind the active hyphal growth zone during filamentous growth. Monokaryons of *F. udum* have for a long time been known to constitutively produce abundant uninucleate asexual spores (conidia). These spores are

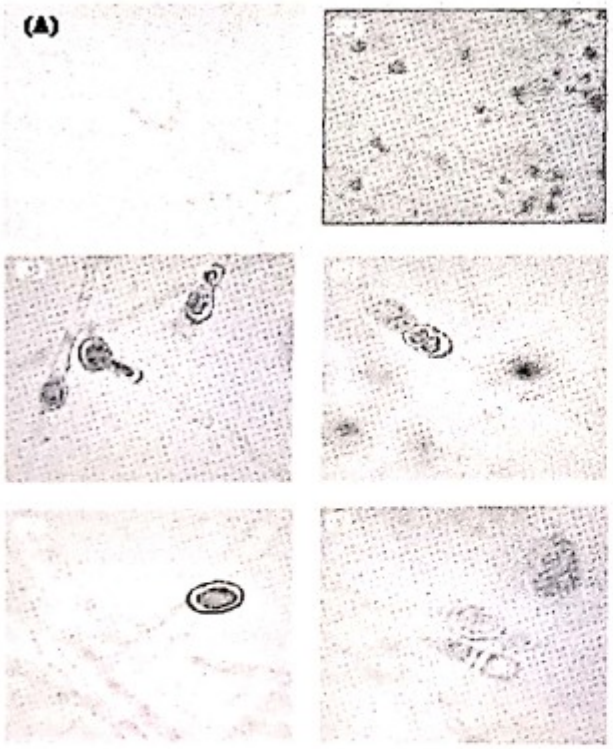

Fig 1: Macroconidia and microconidia of Fusarium udum (A), Different types of chlamydospores produced in Fusarium udum (B to F)

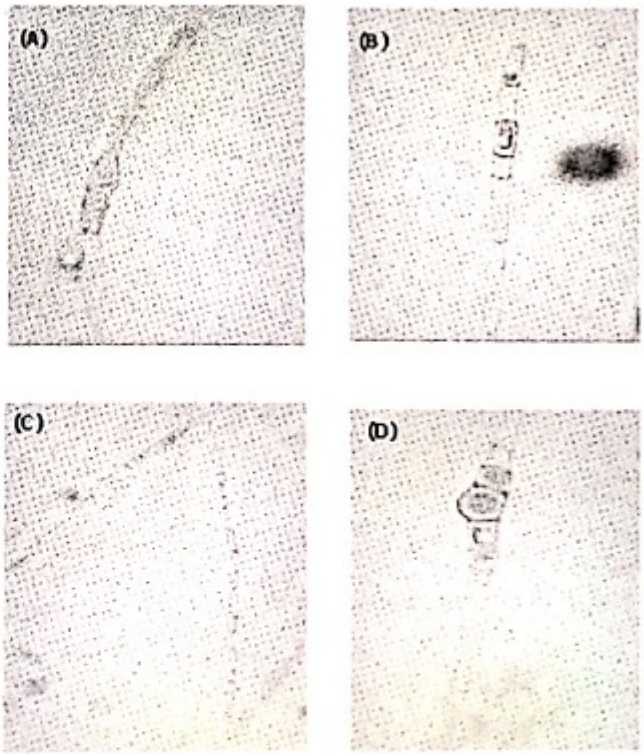

Fig 2: Different stages of chlamydospore formation by Fusarium udum from multinucleate hyphae (A to D). Young chlamydospores in the submerged mycelium of monokaryon

sickle shaped, and because of this shape, they are called conidia (Fig 1). Conidia are formed predominantly in the aerial mycelium and can regularly be detected in the submerged mycelium of agar culture (Fig 1a).

As we have found in our study, chlamydospores are large, thick-walled spores of variable forms and with condensed cytoplasm (Fig 1b). The generation mode of this type of spore in *F. udum* is not well documented. In general, chlamydospores may arise endogenously in cells of the vegetative hyphae following compression of the cytoplasm (chlamydospores in the strictest sense) or by transfer of the compressed cytoplasm from a hyphal cell into a bud (Connerton and Knes, 1994). Filaments of *F. udum* produce both intercalary and terminal chlamydospores (fig 2), which are potentially fully functional (independent from the mycelium) and physiologically active, as they are capable of generating new branches. Whether the energy stored in the chlamydospores is for their own survival and reproduction or to support proficient conidia production and/or maturation is an important unanswered question. Chlamydospore are proposed to be the propagules for *F. udum* dispersal and infection, and it is likely that chlamydospore are also long-term survival structures in nature.

Development starts with the protrusion of a young condia. Conidia germinate and give rise a complete met of fungus (Fig. 3a). Despite their poor cytological description, chlamydospores have been used to collect component monokaryons from dikaryotic mycelia. Chlamydospores from dikaryons generally contain one nucleus of each parental type. Dikaryotic chlamydospores germinate with either one germ tube or two germ tubes, one at each end. When two germ tubes are present, each will receive one nuclear type. Monokaryons arise from germination of the haploid binucleate chlamudospore or, alternatively, from germination of the uninucleate haploid conidia. During germination of the chlamydospore, a single germ tube displaces the spore pore cap (Fig. 3b). Similarly, a single germ tube is formed by the conidium (fig. 3c). Nuclei within the germ tubes divide, and septa may be formed between the daughter nuclei; alternatively, the germ tubes may stay aseptate through several cycles of nuclear division. Multinucleate side branches emerge (Fig 3d), while septum formation occurs in the older portions of the germ tubes to yield cell segments with one or only a few nuclei. This side branches contained new conidia (Fig. 3e). However, the description of this fungus is still not complete. There is need to carry out research work on developmental processes by physiological and genetic studies and to analyze the influences of the environment on development of this fungus.

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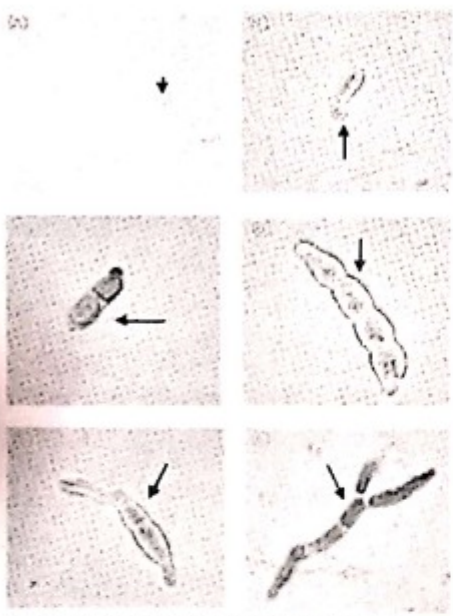
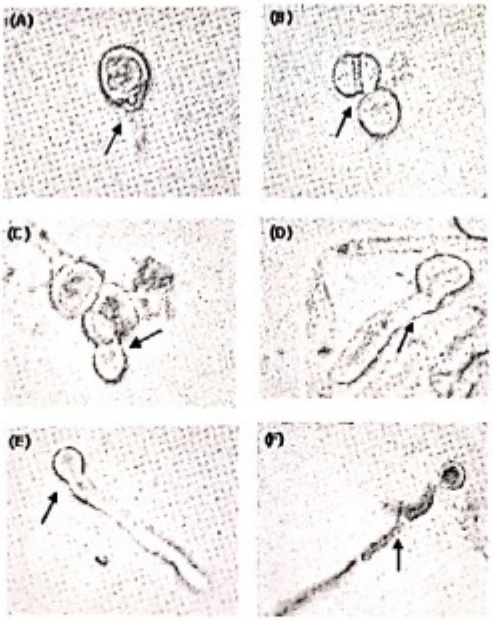


Fig 3I: Different strage of conidia



3 II: Chlamydospore germination of Fusarium udum during favorable

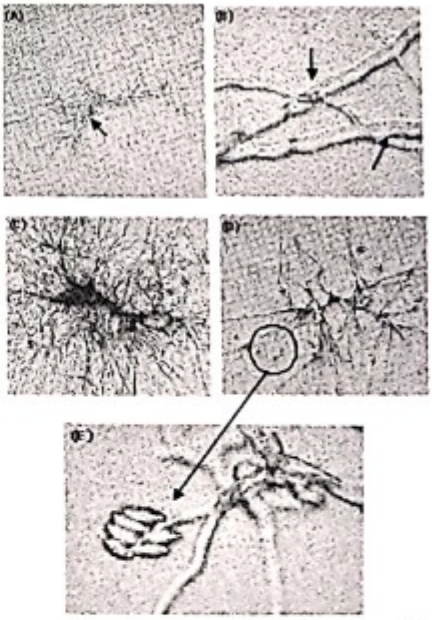


Fig 4: Formation fo mycelia after germination of conidia and chalmydospores of *Fusarium udum* (A), fusion of hyphae (B) resulting in formation of complete mat (C) and conidia produced at hyphal tips (D and E) of *Fusarium udum*.

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