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Review Article

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Stress Induced Microcycle Conidiation in Fungi

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ABSTRACT
Microcycle conidiation (MC) is a kind of survival mechanism in fungi reproducing asexually to produce a lot of spores in stress to withstand themselves unfavorably. In MC, new conidia are often formed on conidiophores from the parent conidia with the arrest of a mycelial phase. The conidia thus produced are synchronous in nature. The present review is an attempt to discuss the same phenomenon in certain fungi with their biological significance, practical applications and future research trajectories in the light of recent researches in the field of applied mycology and medical microbiology.

Keywords: Fungi, Stress, Microcycle Conidiation, Survival Mechanism, Synchronized Spores, Physiology, Biochemistry, Biocontrol

Introduction

Microcycle conidiation in filamentous fungi has been defined as the recapitulation of conidiation following conidial germination without an intervening phase of mycelial growth. The advantage of this form of conidiation for studying the biochemical and physiological aspects of transition from vegetative to the conidiating state of fungi are now being fully developed with several microcycle systems [1-4].

As fungus spores are agents of dissemination and propagation of the species, the study of spore germination and its physiology and biochemistry are fundamental to both successful disease control as well as perpetuation of the species. The biochemical events during spore germination are the targets of chemical control of fungi and our knowledge of them needs to be extended for a more effective approach of the problem. Further, it must not be overlooked that most submerged mycelial cultures are heterogenous in morphological form and any subsequent analysis can only be a summation of various physiological states. In part, some of these problems can be overcome by imparting some degree of synchronous control of the developmental process [5].

The age of conidium may well be an important factor in determining synchronous development. Microcycle conidiation results in the production of a morphologically distinct unit comprising the parent conidium connected to the spore bearing apparatus which may also arise from an abbreviated germ tube and the second generation conidia. Conidia produced by the microcycle techniques are similar in size and can undergo repeated microcycle conidiation without any change in the ability to revert to a normal developmental pattern [2-3].

Further, MC is a kind of survival strategy to encounter the unfavorable conditions. In MC, the fungal spores are germinated with the use of minimal nutritional requirements and humidity over a broad spectrum of temperatures. Quite interestingly, MC provides an ability to reproduce the certain fungi efficiently even on some abiotic substances like bioconcretes. The spores thus produced are escaped quickly to promote the spore dispersal serving as a good source of inoculum. Further, as the mass production of synchronous conidia is a prerequisite for various physiological and biochemical studies, industrial fermentation or as biological control agent, MC represents a good source of inoculum for the same. However, there are other fungi in which MC is suppressed involving genetic manipulation of some specific genes for the process of microcycle conidiation [3,6-8].

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The medical applications of fungal microcycle conidiation mainly focused on the potential of entomopathogenic fungi for biocontrol of insects. The entomopathogenic fungi like *Metarhizium acridum* and *Beauveria bassiana* are being explored for the same purposes. A gene Mmc has been involved for the microcycle conidiation in *Metarhizium anisopliae*. This is an insect biocontrol agent to control a variety of insects in agriculture.The role of chitin deacetylase (CDA) in MC conidial mass production, their viability and virulence have been investigated in B.bassiana [9,10]. An endochitinase, MaCts1 has been involved in conidial germination, conidial yield, stress tolerances and microcycle conidiation in micafungin-induced cell wall. Further, damage stimulates morphological changes consistent with microcycle conidiation in *Aspergillus nidulans*. More studies are still required to elucidate the biological steps in microcycle conidiation in nature [11,12].

The present review discusses about the microcycle conidiation in fungi in the light of recent research done so far in the field of basic microbiology and medical mycology including discovery and occurrence, induction of MC, morphology and ultrastructure, physiology and biochemistry and the genes involved in the survival phenomenon of MC in fungi.

Discovery and Occurrence of MC in Fungi

Microcycle conidiation was first reported as early as 1980 but the specific term was finally given by Anderson and Smith, 1971 & 1972 [13,14]. The widespread occurrence of microcycle conidiation in fungi have documented the genetic potential in response to environmental challenges highlighting the phenomenon as an adaptive mechanism in different environmental conditions. This is a bypassing of conidial development with the lack of mycelial growth in fungi. The conidia are either produced directly by budding from germ tubes or conidial cells. These synchronized conidia are rather produced fastly in huge amounts. They are synchronized in shape, diameter and weight. The conidia thus produced are successfully used in any physiological and biochemical studies and are collected dry and pure, devoid of water or any other solvent with the help of an improved vacuum collector for fungal spores especially designed by the author previously for the same purposes in 1980 [15].

Further, after the discovery of MC in fungi, researchers have tried more than 100 fungal species for the study of MC including saprophytic, plant pathogenic and entomopathogenic insect fungi. Some of them are as *Aspergillus niger, Penicillium urticae, P. digitatum, P. italicum, Clostidium candidum, Geotrichum candidum, Helminthosporium sativum, H. spiciferum, Colletotrichum gloeosporioides, Blastocladiella emersonii, Neurospora crassa, Paecilomyces varioti, Beauveria bassiana and Trichoderma* [1,3,6,10,16-19].

Induction of MC in Fungi

Microcycle conidiation have been recorded and described in several fungi responding to different environmental conditions. This is employed in various filamentous fungi diverging from the traditional life cycle. The phenomenon circumvented the hyphal growth directly producing a simplified conidiophore. This abbreviated developmental cycle is often represented by a reduced or completely absent mycelial phase, documenting a highly potent tool reproducing asexually under stress like nutrient depletion, extreme temperatures, high density of inoculum potential and hydrogen ion concentration [14,16,18,20].

The elevated temperature stress has been one of the most frequently used inducers for the microcycle conidiation in fungi. Temperature has been shown to give the most dramatic control of the process [1,13,18]. During the process of microcycle conidiation the elevated temperatures are used to suppress the germ tube formation, so that direct giant cells are produced. After returning to the normal temperature conidiophores are directly produced from the giant cells in a synchronous manner [20].

Induction of microcycle conidiation by nutrient limitation has also been recorded in several fungal species. The initial studies demonstrating the microcycle conidiation concept in *Aspergillus niger* were obtained by using a combined temperature and nutrient manipulation. Following the *Aspergillus* studies several variations of the system have been developed to induce forms of microcycle micro and macroconidiation. The density of inoculum potential also serve as the inducer for MC in fungi. It has been reported that high inoculum density cause MC in *Colletotrichum gloeosporioides* on solid medium [1,14,16,18].

Further, as the phenomenon of microcycle conidiation is exploited to achieve the synchronization of spores in fungi, this is also triggered by pH of the medium in *Penicillium italicum* [21]. Inhibitory conditions created by spore crowding can also result into microcycle sporogenesis [3,22]. High salt diet for fungi have additionally been reported to induce MC in some fungi [23,24]. Lastly, the aeration also played a good role in inducing the MC in fungi [25].

Morphology and Ultrastructure of MC

Most forms of microcycle conidiation show an initial increase in conidial diameter, associated with a period of supraoptimal temperature[14]. This extensive increase in size is a growth process accompanied by large increase in macromolecular synthesis and a net increase in dry weight. Ultrastructural studies have also been carried out during the enlargement of the conidium [16,26].

Aspergillus niger is one of the most earliest and intensively studied fungus for microcycle synchronous conidiation achieved via alteration in nutrition and temperature. This is type I conidiation characterized by the conidial swelling followed by the direct conidiophore production [4,19]. Morphologically, varying expressions are observed in conidia during the course of microcycle conidiation. For example, the conidia of *Aspergillus niger* swell almost double in size before the germ tube emerges. The conidiophores are formed with thicker and broader walls having vesicles and phialides. However, the metulae are often absent as observed in normal conidiation. The conidia of *Fusarium solani* and *Cercospora zeae-maydis* swell almost equal in size to that of normal germination. Conidia are often produced by intercalary phialides, unlike normal conidiation where conidia are formed on specialized phialides at the tips of conidiophores [3].

The very simplest form of microcycle conidiation is seen in entomopathogenic fungi, where not only the elongation of germ tube is arrested but the development of conidiophores was also

absent. New conidia are developed from the old one appearing as the budding yeast. This could be an adaptation for survival of a pathogen in either devoid of nutrition or where quick dissemination is a paramount. In *Neurospora crassa* the germ tube undergoes multiple septation behaving each section as conidia leading to fragmentation in a chain of conidia. Similarly, the two genes responsible to control MC in *Neurospora crassa* are mcb and mcm [3,16,27].

Nucleic Acid Metabolism During the Course of MC

Some of the genes responsible for MC during the course of Nucleic acid metabolism in fungi are as mcb and mcm for *Neurospora crassa* [27], mmc for Metarhizium anisopliae [3], MaCreA for *M.acridum* [28] and deletion of veA gene mediated MC in Fusarium [3,28].

Microcycle conidiation is an active process regulating the *de novo* biosynthesis of nucleic acid and protein [7,23]. A common insect pathogen, *Metarhizium anisopliae* is used for biocontrol applications. mmc has been responsible for the same regulation of MC. MaCreA and MaCts1 genes are linked with conidiation of endochitinase and arginine metabolism [12,28].

Currently on nucleic acid metabolism during microcycle conidiation have attracted the focus on how genetic factors are regulating the phenomenon by the specific genes involved. The most preferred experimental fungi on which the nucleic acid synthesis studies conducted are *Metarhizium acridum, Aspergillus* and the *Fusarium* species [3,20,28]. It includes the DNA and RNA synthesis, involvement of FluG pathway and the regulation of conidiation patterns via nitrogen assimilation and nitric oxide (NO) production. The FluG pathway involves a gene meant for the autolysis of the fungus *Aspergillus nidulans*. The FluG gene is closely linked with the br1A gene. They are jointly responsible for the development and sporulation[3,29].

Further, nucleic acid metabolism, normal conidiation FluG pathway were up-regulated during microcycle conidiation including SnaD, GNAT, pkaA, fadA, and gasA [24]. The C2H2 zinc finger protein MaNCP1 contributes to conidiation through governing the nitrate assimilation pathway in the *Metarhizium acridum* [30]. It governs the conidiation pattern shift via regulating the reductive pathway for nitric oxide (NO) synthesis in the same fungus [31]. The gene is a regulator of the carbon catabolite repression (CCR) pathway, influencing both the types of conidiation [28]. MaNmrA gene is involved in arginine metabolism in the reductive pathway for NO synthesis for microcycle conidiation [32].

Protein and Amino Acids Metabolism During the Course of MC

Control of spore germination have considered the increasing attention of self inhibitors and stimulants of protein and nucleic acid biosynthesis during the course of microcycle conidiation in fungi. A plethora of good informations are now available on the identity of several inhibitors and chemical regulators of protein and lipid biosynthesis and mitochondrial functioning on conidial germination and microcycle conidiation of several fungi [10].

Recent studies have documented the role of amino acids and proteins metabolism in microcycle conidiation (MC). Most specifically, an entomopathogenic fungi like *Metarhizium acridum* suppress the shifts in conidiation pattern by specific amino acid pathways involving arginine metabolism, including nitric oxide levels and nitric oxide synthase activity during the process 10]. Other amino acids and proteins have also taken part in MC. Although fungi often prefer ammonium, glutamine and glutamate as nitrogen sources but utilize other amino acids as well. However, the utilization is often regulated by the nitrogen catabolic repression (NCR) mediated by the transcription factors like GATA [33]. Fungi have evolved the mechanism of utilizing amino acids via nitrogen catabolite repression (NCR) and target of rapamycin [24]. The MaCreA gene regulates the normal condition and microcycle conidiation in *Metarhizium acridum* [28].

Some Specific Pathways During the Course of MC

Although the researchers are engaged in identifying the responsible genes for MC in different fungi they have also traced some of the biochemical pathways as under:

- **Chitin Deacetylase (CDA)**
  CDA played a good role in MC in *Beauveria bassiana*. CDA with chitosonase activity to soften the insect cuticle for penetration. Chitin deacetylase has played a key role in microcycle conidiation. Ma Eng1 in *M.acridum* has been found involved in cell separation and conidiation [34]. Similarly, an enzyme dipeptidase gene MapepdA in *Metarhizium acridum* is also found associated with a shift of conidiation pattern [35]. Studies have shown that various pathogenic fungi like *Aspergillus nidulans, A. fumigatus* and *A. cerevisiae* have harbor numerous amino acids transporters for which N-acetylcystine may inhibit the germination of conidia by disrupting fungal amino acid transport mechanisms as potential antifungal targets [36].
- **Arginine Metabolism and Nitric Oxide**
  The concentration between arginine metabolism and nitric oxide synthase activity has been established in *Metarhizium acridum* suggesting the role of nitrogen metabolism and signalling molecules in microcycle conidiation [32].
- **Endochitinase**
  Ma Cts1 is an enzyme involved in the chitinolysis of fungal cell walls. It plays a role in conidial germination, MC and yield of conidia, stress tolerance from high temperature and UV light. [12,28,34].

Lipid Metabolism During the Course of MC

Lipid metabolism also plays a crucial role in fungal sporulation and role in microcycle conidiation in fungi. Fatty acids, phospholipids, cholesterol and ergosterol synthesis and lipid droplet dynamics, OLE pathway involved in oleic acid synthesis are some of the links associated with the microcycle conidiation and are regulated by various genes and signaling pathways in fungi [37,38]. Targeting fungal lipid synthetic pathways have been emerging as a promising strategy for developing new antifungal drugs in future [39]. Steryl Acetyl Hydrolase1 (BbSay1) links with lipid homeostasis to conidiogenesis and virulence in the entomopathogenic fungus, *Beauveria bassiana* [40].

Conclusion

Microcycle conidiation is a kind of asexual reproduction where spores germinate directly into new conidia without intervening the

typical mycelial growth phase. Might be this is due to the survival of fungi when faced with unfavorable conditions allowing them to quickly withstand stress. Normally, the spores germinate to form a mycelial network that produces conidiophores and conidia but in microcycle conidiation, it bypasses the mycelial phase. Further, microcycle conidiation is usually influenced by the temperature, pH of the medium, lack of nutrition, stress and overcrowding. Moreover, it could be used as a tool for the production of fungal spores by a kind of life cycle following a model of short cut allowing rapid spore production in a large scale under specific conditions. Similarly, the very process of microcycle conidiation is used for the study of conidiation and the development of conidia in fungi providing very convenient and rapid systems to study the developmental stages. The paper explores microcycle conidiation (MC) in fungi covering its discovery and occurrence, the factors inducing MC, and the morphological and ultrastructural characteristics associated with it. It also examines the metabolism of nucleic acids, proteins, and amino acids, and discusses the specific genes and molecular pathways involved in the process of microcycle conidiation in fungi.

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Ethical Clearance

As this is purely a review article, it does not require any ethical clearance.

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