Polyamines as modulators of microcycle conidiation in *Aspergillus flavus*

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Since polyamines (PAs) play a potential role in the regulation of growth and developmental processes in a wide variety of organisms, we have examined the influence of the PAs putrescine (Put) and spermidine (Spd) and the PA biosynthetic inhibitors α -difluoromethylornithine (DFMO), α -difluoromethylarginine (DFMA), methylglyoxal bis-(guanyldihydrazone) (MGBG) and cyclohexylamine (CHA), singly and in combinations on microcycle conidiation (MC) in *Aspergillus flavus*. The exogenous application of the diamine Put (concentrations ranging from 0.1 to 5 mM) caused a sharp decline of MC in a dose-dependent fashion, but induced vegetative growth. However, the triamine Spd (0.1–5 mM) had a minimal effect on MC and induced a shift from MC to normal conidiation. PA inhibitors, especially DFMO, MGBG and CHA, produced greater inhibition of MC and complete inhibition of MC was observed at 5 mM of these inhibitors. DFMA even at 5 mM had only a weak inhibitory effect on MC. DFMO also inhibited conidial germination and germ tube growth. MGBG and CHA, while having an inhibitory effect on MC, induced vegetative growth. The inhibitory effect of PA inhibitors was partially reversed by exogenous Put or Spd, with Spd being more effective than Put. The analysis of free PA levels during various phases of MC revealed that undifferentiated spores contained a high Put/Spd ratio and there was a dramatic decrease in Put/Spd ratio before and during microcycle conidiophore maturity. The change in spermine titres could not be detected. These observations imply that Put is essential for vegetative growth, while Spd is involved in MC, and that a low Put/Spd ratio seems to be important for spore differentiation to MC.

Keywords: *Aspergillus flavus*, development, microcycle conidiation, polyamines, polyamine biosynthesis inhibitors

INTRODUCTION

Microcycle conidiation (MC) (a condensed developmental process of conidiation without any intervening vegetative mycelium) has been reported in *Aspergillus niger* using temperature manipulation by Anderson & Smith (1971a, b). Since then attempts have been made to induce this phenomenon in other *Aspergillus* spp. and fungi by manipulating culture conditions and/or nutrient factors (Maheshwari, 1991). Earlier, we reported MC on a novel medium, D-glucose soluble starch (D-GSS) in *Aspergillus* spp. (Saxena *et al.*, 1992) and under light-independent

conditions in *Trichoderma* spp. (Khurana *et al.*, 1993). Although some work has been done on the induction and on the ultrastructure, the internal events regulating microcycle differentiation are still not fully understood (Maheshwari, 1991). However, our work on MC in D-GSS medium implied that the osmolarity of medium can regulate MC, at the transcriptional level (Khurana *et al.*, 1992).

Polyamines (PAs), spermidine (Spd) and spermine (Spm) and their precursor the diamine putrescine (Put) are involved in a variety of growth and developmental events in a wide range of organisms, including fungi (Rajam & Galston, 1985; Rajam *et al.*, 1985, 1986, 1989; Tabor & Tabor, 1985; Walters, 1987; Singhania, 1991; Rajam, 1993). No attempts so far have been made to demonstrate the possible role of PAs in MC. Thus, we have examined the influence of exogenous PAs Put and Spd, and specific

Abbreviations: CHA, cyclohexylamine; DFMA, α -difluoromethylarginine; DFMO, α -difluoromethylornithine; D-GSS, D-glucose soluble starch; MC, microcycle conidiation; MGBG, methylglyoxal bis-(guanyldihydrazone); PA(s), polyamine(s); Put, putrescine; Spd, spermidine; Spm, spermine.

PA biosynthesis inhibitors α -difluoromethylornithine (DFMO, a potent inhibitor of ornithine decarboxylase), α -difluoromethylarginine (DFMA, a potent inhibitor of arginine decarboxylase), methylglyoxal *bis*-(guanyldihydrazone) (MGBG, an inhibitor of adenosylmethionine decarboxylase, an enzyme which furnishes aminopropyl groups to Put for the formation of Spd and Spm) and cyclohexylamine (CHA, an inhibitor of Spd synthase) on MC in *A. flavus*. We have also measured the temporal changes in intracellular PA levels during various phases of MC.

METHODS

Growth conditions. *A. flavus* was maintained on a complete medium (Saxena & Sinha, 1973; Saxena, 1976) slants at 4 °C. For initiation of liquid cultures, conidia were harvested from 3-d-old agar cultures using normal saline and 0.01 % Tween-80 as a detergent. Conidial harvest was filtered through sterilized cotton, centrifuged for 15 min at 1000 g, washed twice with sterilized water, and resuspended in normal saline before being dispensed in culture flasks.

The normal developmental pattern was investigated in minimal medium (Saxena & Sinha, 1973; Saxena, 1976). Liquid shake cultures were grown in 50 ml medium in 250 ml Erlenmeyer flasks, seeded with about 1×10^7 conidia. Flasks were incubated at 37 °C at 250 r.p.m. in an incubation shaker (New Brunswick Scientific). Microscopic observations were recorded after a regular period of 4 h.

Induction of MC. MC was induced by using a new medium D-GSS previously described by Saxena *et al.* (1992). The medium contained 5 g D-glucose l⁻¹, 3.5 g soluble starch l⁻¹ and 130 mg KH₂PO₄ l⁻¹. Approximately 5×10^7 conidia were inoculated in 250 ml Erlenmeyer flasks containing D-GSS medium. Flasks were incubated in an incubation shaker at 37 °C and 250 r.p.m. MC was assessed by direct counting of conidia harvested at a regular period of 2 h. Conidiophore counts included both immature and mature structures. MC was highly synchronous and about 99 % conidia undergo MC in D-GSS medium.

PA extraction and quantification. Fungal spores and/or mycelial mats were obtained from liquid cultures grown at 37 ± 1 °C and 250 r.p.m. in 250 ml Erlenmeyer flasks containing 50 ml sterile D-GSS for various incubation times. Fungal biomass was allowed to air dry at room temperature and ground in a pre-chilled mortar with 5 % (v/v) pre-chilled HClO₄ at a ratio of 1 ml HClO₄ per 0.1 g fresh weight. The homogenates were centrifuged at 15000 g for 20 min at 4 °C. The free PAs in the supernatant fraction were dansylated along with standard PA mixture (Bajaj & Rajam, 1995) and extracted in 250 μ l benzene, and the clear supernatant was used for the determination of PAs using high-resolution TLC plates (silica gel 60 of 250 μ m thickness; E. Merk, Germany). The TLC plates were developed in cyclohexane:ethylacetate (5:4, v/v) solvent system. Dansylated PA bands marked under a UV lamp were scraped off, eluted in ethylacetate and quantified using a spectrophotofluorometer (Shimadzu RF540) with excitation and emission wavelengths at 350 and 495 nm, respectively.

Effect of PAs and PA biosynthesis inhibitors, singly and in combinations on MC. Filter-sterilized stock solutions of Put and Spd and their biosynthesis inhibitors (DFMO, DFMA, MGBG and CHA), were added to autoclaved D-GSS liquid medium that had been cooled to room temperature, to get final concentrations of 0.1, 0.5, 1 and 5 mM of these compounds. For experiments involving reversal of inhibition, we utilized 5 mM

MGBG and 1 mM DFMO inhibitor (which yielded 90–100 % MC inhibition) with four concentrations (0.01, 0.1, 0.5 and 1.0 mM) of Put and Spd. Control flasks contained only culture medium with no PA/PA inhibitor. The cultures (in duplicate) containing conidia were incubated as described above. Microscopic observations were made after 24 h of incubation, which included both mature as well as immature conidiophore counts along with observation on vegetative and ungerminated conidia. The data were scored as percentage MC.

RESULTS

Cellular PA levels during various phases of MC

The free pools of PAs estimated during various stages of MC of *A. flavus* are given in Table 1. The results show that all three common PAs were present, Put being predominant followed by Spd and Spm. Conidia and germinating spores contained a high Put/Spd ratio; however, as the germ tube committed to undergo MC (after 18 h of culture), there was a dramatic decrease in Put titres with a concomitant increase in Spd titres resulting in a reduction of Put/Spd ratio by about 50 %. The changes in Spm titre could not be detected.

Effect of PAs on MC

Conidia of *A. flavus* undergo a phase of vegetative growth (Fig. 2a) followed by normal asexual differentiation on 48 h of incubation in minimal medium (Fig. 1). However, on D-GSS medium, *A. flavus* shows four distinct developmental stages of MC: conidial germination stage which lasts for 2–3 h followed by germ-tube elongation (4–8 h); vesicle induction and phialodogenesis (10–16 h); and conidiogenesis (18–24 h; Fig. 2b) as shown in Fig. 1. On supplementation of D-GSS medium with various concentrations (0.1, 0.5 and 1.0 mM) of Put or Spd, a progressive decline in MC was observed; Put being more inhibitory than Spd. Put caused a greater inhibition of MC even at a very low concentration of Put (0.1 mM) where

Table 1. Temporal changes in cellular free PA levels during various phases of MC

Age of culture (h)	Developmental stage of MC*	Put	Spd	Put/Spd ratio
0	A	631.4 $\pm$ 17.2	91.5 $\pm$ 12.0	6.90
4	B	585.5 $\pm$ 21.9	82.5 $\pm$ 3.5	7.09
8	C	709.5 $\pm$ 27.6	99.5 $\pm$ 0.7	7.13
18	D	674.5 $\pm$ 20.5	172.5 $\pm$ 3.5	3.91
24	E	464.5 $\pm$ 21.9	180.8 $\pm$ 2.8	2.57

* Various developmental stages of MC: A, freshly inoculated conidia; B, germ-tube initiation; C, germ-tube elongation; D, phialodogenesis; E, conidiogenesis.

Fig. 1. Developmental pattern of *A. flavus* in (a) minimal medium and (b) D-GSS medium. A, conidium; B, conidial germination; C, germ-tube elongation; D, phialodogenesis; E, conidiogenesis.

only 10% microcycle conidiophores were formed. Put at 0.5 mM caused complete inhibition of MC. Increase in Put concentration also induced vegetative growth in a medium which otherwise supports MC (Fig. 2c). Sporadic normal conidiation was also observed on Put-amended medium but the conidiophores were immature without any vesicle induction (Table 2). Conversely, Spd had a weak inhibitory effect on MC as compared to Put, but resulted in a shift from MC to normal asexual differentiation (Table 2; Fig. 2d). Spd at 0.1 and 0.5 mM caused about 20 and 30% inhibition of MC, respectively. However, no MC could be detected in a medium fortified with 1 mM Spd.

Effect of PA synthesis inhibitors on MC

Data on the effect of DFMO, DFMA, MGBG and CHA on MC in *A. flavus* are summarized in Figs 3–5. DFMO, an inhibitor of Put biosynthesis, induced a sharp decline in percentage MC. Of the various concentrations of DFMO tested (0.01–5 mM), complete inhibition of MC was observed at 5 mM, and at 1 mM only 2–3% conidia underwent MC (Fig. 3). DFMO also caused an increase in the percentage ungerminated conidia with a concomitant decrease in MC. Some conidia germinated at higher concentrations of DFMO (1 and 5 mM) to form thin germ tubes whose further growth and development were restricted even after the increase in the incubation period (Fig. 2e). By contrast, DFMA had a weak inhibitory effect on MC as compared to DFMO (Fig. 5) and it caused only 20–30% inhibition MC even at 5 mM (Fig. 5). However, the microcycle conidiophores formed had a higher degree of intracellular vacuolation.

Spd biosynthesis inhibitors (MGBG and CHA) at various concentrations tested (0.01–5 mM) produced successively greater inhibition of MC (Figs 4 and 5) with MGBG being more effective than CHA. MGBG at a low concentration (0.5 mM) resulted in 50% inhibition of MC. However, complete inhibition of MC was observed only at 5 mM MGBG (Fig. 4), and at 1 mM around 20% MC was observed but the conidiophores were immature (Fig. 2h).

However, CHA was found to be less inhibitory, and 50% inhibition of MC was observed at 1 mM (Fig. 5).

Reversal of the inhibitory effects of PA inhibitors on MC by exogenously supplied PAs

Spd at 0.01 and 0.1 mM caused 85–90% restoration of inhibition of MC induced by 1 mM DFMO (Figs 3 and 2g). An increase in Spd concentration, however, was not that effective in reversing the inhibitory effect on MC, and a high degree of vegetative growth with sporadic normal conidiation was observed at 1 mM Spd. Put, however, caused only partial reversion of MC as compared to the reversion frequencies obtained with Spd (Figs 3 and 2f). A similar reversion pattern by Put and Spd was recorded for MC inhibition induced by 5 mM MGBG (Figs 2i, j, and 4). However, the microcycle conidiophores formed with 0.01 mM Spd plus 5 mM MGBG were immature (without vesicle induction) and complete maturity was attained only with 0.1 mM Spd (Fig. 2j); sporadic normal conidiation was also observed in the latter treatment (Table 2).

DISCUSSION

Put, Spd and Spm are widely distributed in many organisms. In general, Spd is frequently the most abundant PA in fungi, Put being present in lower concentrations and Spm, if present, usually only in small amounts (Stevens & Winther, 1979; Tabor & Tabor, 1985). However, the present results show that three common PAs were present in *A. flavus* and Put was present in the highest concentration followed by Spd and Spm. Put, Spd and Spm have also been shown to be widely distributed in thermophilic moulds representing zygomycetes, ascomycetes and deuteromycetes along with the appreciable amounts of cadaverine (Singhania *et al.* 1991). Similar results were reported in *Helminthosporium oryzae*, *Curvularia lunata*, *Pythium aphanidermatum* and *Colletotrichum capsici* (Bharti, 1995). Our results also imply that PA biosynthesis is active during germ-tube elongation and a

Fig. 2. Effect of PA and/or PA biosynthesis inhibitors on MC in *A. flavus*: (a) vegetative growth in minimal medium after 24 h; (b) MC in D-GSS medium; (c) induction of vegetative growth by 0.5 mM Put; (d) a shift from MC to normal conidiation by 0.5 mM Spd; (e) suppression of germ-tube growth by 1 mM DFMO; (f) reversal of MC by supplementation of 0.1 mM Put with 1 mM DFMO; (g) reversal of MC by 0.1 mM Spd in 1 mM DFMO; (h) suppression of MC by 1 mM MGBG; (i) absence of MC reversion by 0.1 mM Put in 5 mM MGBG; (j) reversal of MC by 0.1 mM Spd in 5 mM MGBG.

Table 2. Extent of asexual differentiation induced by exogenously applied PAs and/or PA inhibitors in MC in D-GSS medium

Treatment (mM)	Extent of asexual differentiation* induced by:					
	Put	Spd	MGBG	CHA	MGBG (5 mM) + Put	MGBG (5 mM) + Spd
0.01	NT	NT	NT	NT	—	—
0.1	++	++++	—	—	—	+
0.5	+	++++	—	—	—	++
1.0	+	+++	—	—	NT	NT
5.0	—	+	—	—	NT	NT

NT, Not tested.

*++++, Greater than 50%; +++, about 50%; ++, less than 50%, but greater than 10%; +, less than 10%; —, not detected.

Fig. 3. Effect of DFMO singly and in combination with PAs (Put or Spd) on MC: (a) control; (b) 0.1–5.0 mM DFMO; (c) 1 mM DFMO + 0.01–1.0 mM Put; (d) 1 mM DFMO + 0.01–1.0 mM Spd. ND, None detected. **Fig. 4.** Effect of MGBG singly and in combination with PAs (Put or Spd) on MC: (a) control; (b) 0.1–5.0 mM MGBG; (c) 5 mM MGBG + 0.01–0.5 mM Put; (d) 5 mM MGBG + 0.01–0.5 mM Spd. ND, None detected.

high Put/Spd ratio is important for fungal spore germination and germ-tube elongation, whereas a low Put/Spd ratio supports spore differentiation to MC. In other words, the drop in the Put/Spd ratio at the 18 h time point precedes with the onset of MC. PA biosynthesis has also been seen in early stages of germinating spores of wheat stem rust uredosporelings (Kim, 1971) and differentiation processes in *Mucor rouxii* (Calvo-Mendez *et al.*,

1987; Martinez-Pacheco *et al.*, 1989), and specific inhibitors of PA biosynthesis such as DFMO and/or DFMA (Rajam *et al.*, 1985, 1986, 1989; Birecka *et al.*, 1986; Machatsckke *et al.*, 1990) and D-arginine (Gaur *et al.*, 1989) can restrict spore germination/germ-tube growth/sporulation in a variety of fungi (Tabor & Tabor, 1985; Rajam, 1993). The role of PAs in the germination and outgrowth as ascospores of *Saccharomyces cerevisiae* has

Fig. 5. Effect of CHA and DFMA singly and in combination with DFMO on MC: (a) control; (b) 0.1–5.0 mM CHA; (c) 0.1–5.0 mM DFMA; (d) 1 mM DFMA + 1 mM DFMO.

also been studied using α -methylornithine and MGBG (Brawley & Ferro, 1979). Germinating spores were found to be capable of initiating and progressing through the first cell cycle in the presence of PA inhibitors, but subsequent cellular growth was retarded but not completely arrested. Data further indicate that PA synthesis may be needed for meiosis and sporulation (Brawley & Ferro, 1979). Besides this, the involvement of PAs, especially Spd for cell differentiation to embryoids (Feirer *et al.*, 1984) or flower buds (Kaur-Sawhney *et al.*, 1988) using cell suspensions or thin-layer cultures of carrot and tobacco, respectively, has been clearly demonstrated, and a low Put/Spd ratio was found to be essential for flower bud differentiation (Kaur-Sawhney *et al.*, 1988).

The present findings clearly show that exogenous PAs and their biosynthetic inhibitors had a profound effect on MC. Put, while inhibiting MC, induced vegetative growth at a higher concentration (1 mM) indicating its role in vegetative growth. In contrast, Spd had a weak inhibitory effect on MC but it resulted in a shift from MC to asexual differentiation. This indicates that higher cellular Spd levels then needed for MC are required for asexual differentiation. Further, optimum rather than higher Spd levels, and an optimal Put/Spd ratio, seem to be important for MC. Calvo-Mendez *et al.* (1987) and Martinez-Pacheco *et al.* (1989) have shown that elevated levels of PAs precede all differentiation processes in *M. rouxii*, including yeast–mycelium transition. These variations were ac-

companied by changes in ornithine decarboxylase (ODC, the key enzyme controlling PA biosynthesis in fungi) activity and inhibition of its activity by the reaction product analogue 1,4-diaminobutanone blocked spore germination at the transitions between the isodiametric and polarized growth stages, aerial mycelium formation, and the dimorphic yeast–mycelium transition (Martinez-Pacheco, 1989).

Our results showed a strong inhibition of MC by the Put synthesis inhibitor DFMO and this could be due to reduced levels of Spd as Put is the obligatory precursor of Spd formation or to defects in germ tube emergence. DFMA, however, was ineffective in suppressing MC, which implies that this fungus may possess only the ODC pathway for PA biogenesis like many other fungi (Walters, 1987; Rajam, 1993). Further, the weak effect of DFMA on MC may be due to the lack of the arginine decarboxylase (another key enzyme in PA synthesis) pathway in *Aspergillus*, or the lack of inhibitor uptake or a rapid metabolism of the inhibitor (Akhtar & Minocha, 1989; Rajam *et al.*, 1989; Bharti, 1995). The inhibition of MC by MGBG and CHA, which was associated with pronounced vegetative growth, was most likely due to the accumulation of inhibitor-induced Put as Put is not converted to Spd due to the blockage of adenosyl-methionine decarboxylase and Spd synthase, respectively (Bharti & Rajam, 1995). The induction of vegetative growth by MGBG or CHA could be because of availability of a minimal level of Spd and the high Put to Spd ratio as a result of inhibitor-induced Put accumulation (Bharti & Rajam, 1995). This further supports the involvement of Put in mycelial growth. CHA was less effective on MC and it required much higher concentrations to get similar effects as MGBG; this may be because of poor uptake and rapid metabolism of the inhibitor, or because of insensitivity of the target enzyme (Akhtar & Minocha, 1989; Rajam *et al.*, 1989; Bharti, 1995). The partial/greater reversion of inhibitory effect of DFMO and MGBG on MC by Put and Spd, respectively, also indicates that PAs, particularly Spd, may be required for MC. The inhibitory effect of DFMO and other PA inhibitors on fungal growth and spore germination can be completely reversed by addition of Put or Spd to the medium (Rajam & Galston, 1985; Rajam *et al.*, 1989; Rajam, 1993).

In conclusion, these observations provide the first evidence that Spd is intricately involved in MC of *A. flavus*, and optimum, rather than higher, Spd levels and a low Put/Spd ratio seems to be important for MC. Further, these results may have possible utility to biotechnology and fermentation industries for mass inoculum production of industrially useful micro-organisms.

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REFERENCES

- Akhtar, J. K. & Minocha, S. C. (1989).** Polyamine biosynthetic enzymes and the effect of their inhibition on the growth of some phytopathogenic fungi. *Plant Cell Physiol* **30**, 655–663.
- Anderson, J. G. & Smith, J. E. (1971a).** The production of conidiophores and conidia by newly germinated conidia of *Aspergillus niger* (microcycle conidiation). *J Gen Microbiol* **69**, 185–197.
- Anderson, J. G. & Smith, J. E. (1971b).** Synchronous initiation and maturation of *Aspergillus niger* conidiophores in culture. *Trans Br Mycol Soc* **56**, 9–29.
- Bajaj, S. & Rajam, M. V. (1995).** Efficient plant regeneration from long-term callus cultures of rice by spermidine. *Plant Cell Rep* **14**, 717–720.
- Bharti (1995).** *Inhibition of fungal polyamine biosynthesis and control of leaf rust of wheat (Triticum aestivum L.): physiological and cytogenetical studies in the host plant.* PhD thesis, University of Delhi, India.
- Bharti & Rajam, M. V. (1995).** Effects of the polyamine biosynthesis inhibitor difluoromethyl-ornithine on growth, polyamine levels, chromosome behaviour and polygenic traits in wheat (*Triticum aestivum* L.). *Ann Bot* **76**, 297–301.
- Birecka, H., Garraway, M. O., Baumann, R. I. & McCann, P. P. (1986).** Inhibition of ornithine decarboxylase and growth of the fungus *Helminthosporium maydis*. *Plant Physiol* **80**, 798–800.
- Brawley, J. V. & Ferro, A. J. (1979).** Polyamine biosynthesis during germination of yeast ascospores. *J Bacteriol* **140**, 649–654.
- Calvo-Mendez, C., Martinez-Pacheco, M. & Ruiz-Herrera, J. (1987).** Regulation of ornithine decarboxylase activity in *Mucor bacilliformis* and *Mucor rouxii*. *Exp Mycol* **11**, 270–277.
- Feirer, R., Mignon, G. & Litvay, J. D. (1984).** Arginine decarboxylase and polyamines required for embryogenesis in the wild carrot. *Science* **223**, 1433–1435.
- Gaur, S. C., Shekhawat, N. S. & Arya, H. C. (1989).** Growth and sporulation of millet leaf blast fungus *Pyricularia penicillata*: role of polyamines. *Curr Sci* **58**, 198–200.
- Kaur-Sawhney, R., Tiburcio, A. F. & Galston, A. W. (1988).** Spermidine and flower bud differentiation in thin-layer explants of tobacco. *Planta* **173**, 282–284.
- Khurana, N., Gupta, R., Kuhad, R. C. & Saxena, R. K. (1992).** Effect of protein synthesis and respiratory inhibitors on microcycle conidiation of *Aspergillus tamarii*. *J Gen Appl Microbiol* **38**, 617–621.
- Khurana, N., Gupta, R., Kuhad, R. C. & Saxena, R. K. (1993).** Light independent conidiation in *Trichoderma* spp. – a novel approach to microcycle conidiation. *World J Microbiol & Biotechnol* **9**, 353–356.
- Kim, W. K. (1971).** Folate and polyamine content of undifferentiated and differentiated wheat stem rust uredosporelings. *Can J Bot* **49**, 1119–1122.
- Machatsckke, S., Kamrowski, C., Moerschbacher, B. M. & Reisenger, H. J. (1990).** Polyamine levels in stem rust infected wheat leaves and effects of alpha-difluoromethylornithine on fungal infection. *Physiol Mol Plant Physiol* **36**, 451–459.
- Maheshwari, R. (1991).** Microcycle conidiation and its genetic basis in *Neurospora crassa*. *J Gen Microbiol* **137**, 2103–2115.
- Martinez-Pacheco, M., Rodriguez, G., Reyna, G., Calvo-Mendez, C. & Ruiz-Herrera, J. (1989).** Inhibition of the yeast mycelial transition and the phorogenesis of Mucorales by diaminoethanol. *Arch Microbiol* **151**, 10–14.
- Rajam, M. V. (1993).** Polyamine biosynthesis inhibitors: new protectants against fungal plant diseases. *Curr Sci* **65**, 461–469.
- Rajam, M. V. & Galston, A. W. (1985).** The effects of some polyamine biosynthetic inhibitors on growth and morphology of phytopathogenic fungi. *Plant Cell Physiol* **26**, 683–692.
- Rajam, M. V., Weinstein, L. H. & Galston, A. W. (1985).** Prevention of a plant disease by specific inhibition of fungal polyamine biosynthesis. *Proc Natl Acad Sci USA* **82**, 6874–6878.
- Rajam, M. V., Weinstein, L. H. & Galston, A. W. (1986).** Kinetic studies on the control of the bean rust fungus (*Uromyces phaseoli* L.) by an inhibitor of polyamine biosynthesis. *Plant Physiol* **82**, 485–487.
- Rajam, M. V., Weinstein, L. H. & Galston, A. W. (1989).** Inhibition of uredospore germination and germ tube growth by inhibitors of polyamine metabolism in *Uromyces phaseoli* L. *Plant Cell Physiol* **30**, 37–41.
- Saxena, R. K. (1976).** *Studies on the control of reproductive differentiation in Aspergillus nidulans under submerged culture conditions.* PhD thesis, University of Delhi.
- Saxena, R. K. & Sinha, U. (1973).** Conidiation of *Aspergillus nidulans* in submerged liquid culture. *J Gen Appl Microbiol* **19**, 141–146.
- Saxena, R. K., Khurana, N., Kuhad, R. C. & Gupta, R. (1992).** D-Glucose soluble starch, a novel medium for inducing microcycle conidiation in the genus *Aspergillus*. *Mycol Res* **96**, 490–494.
- Singhania, S., Satyanarayana, T. & Rajam, M. V. (1991).** Polyamines of thermophilic moulds: distribution and effect of polyamines biosynthesis inhibitors on growth. *Mycol Res* **95**, 915–917.
- Stevens, L. & Winther, M. D. (1979).** Spermine, spermidine and putrescine in fungal development. *Adv Microb Physiol* **19**, 63–148.
- Tabor, C. W. & Tabor, H. (1985).** Polyamines in microorganisms. *Microbiol Rev* **49**, 81–99.
- Walters, D. R. (1987).** Polyamines: the Cinderellas of cell biology. *Biologist* **34**, 73–76.

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