Ornithine Decarboxylase of Stagonospora (Septoria) nodorum Is Required for Virulence toward Wheat*

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Polyamines are a class of positively charged molecules found in all organisms (1–3). They are required for cell growth and are reported to have various functions in stabilizing DNA or membrane structure, and in resistance to oxidative stress. Ornithine decarboxylase (ODC) catalyzes the conversion of ornithine to putrescine and is the first and rate-limiting step in polyamine biosynthesis in most organisms (4). ODC is an effective therapeutic target in the treatment of human protozoal pathogens (5–9) and has been proposed as a target in treatment of certain cancers and in the eradication of economically damaging plant pathogens (10). Inhibition of the polyamine biosynthetic enzyme ODC using the specific and highly potent inhibitor, di-fluoromethylornithine (DFMO) (11), is an effective therapy in treatment of Trypanosomiasis and other diseases caused by Plasmodia, Giardia, and Leishmania (5–9).

Control of fungal disease in humans and plants has relied on compounds that specifically interdict fungal metabolic pathways, in particular, ergosterol biosynthesis. Resistance to such fungicides generally arises within a few years either as a result of mutation in the native population or because of the introduction of resistant strains from other areas. Many effective fungicides are being withdrawn from use due to public concern about their toxicity. The search for new classes of fungicide is becoming increasingly urgent due to the limited number of targets for fungicide action so far identified. Most fungi (including the economically important plant pathogens, Rhizoctonia solani, Botrytis cinerea, Fusarium oxysporum f.sp. lycopersici, Verticillium dahliae, Cochliobolus carbonum, and Phytophthora infestans) are prevented from growing by low concentrations of DFMO (10, 12, 13). The same concentration of DFMO does not inhibit plant growth, because plants can synthesize polyamines from arginine (14). For example, DFMO applied to R. solani-infected tomato plants markedly reduced disease severity without adversely affecting plant growth. Polyamine biosynthesis (and in particular ODCase) has therefore been proposed as a good candidate target for developing a new type of fungicidal chemical.

Although DFMO is not toxic to plants per se, its toxicity to animals and high cost make it unsuitable for development as a fungicide. Moreover, certain important fungal plant pathogens, such as Septoria tritici, Stagonospora nodorum, Pyrenophora avenae, and Ophiostoma ulmi, are reportedly insensitive to DFMO (10, 15), suggesting the presence of divergent ODCase enzymes or an alternative route to polyamine biosynthesis in these organisms. Beneficial soil fungi, such as mycorrhiza, are reportedly insensitive to DFMO due to the presence of the agmatine route to polyamine biosynthesis (10) but other ODCase inhibitors have not been tested.

In theory, plants contain sufficient polyamines to support fungal growth. It is possible that, even after inhibition of ODC, pathogenic fungi may be able to obtain enough polyamines from the plant to support invasion of plant tissues. The ability to disrupt chromosomal gene loci in haploid fungi is well established and allows a direct test of gene function in growth and pathogenicity. ODC genes have been isolated and disrupted in yeast (16), Neurospora crassa (17), and Ustilago maydis (18). Yeast and N. crassa are non-pathogens and disruption in U. maydis prevents formation of the diploid state, which is a pre-requisite for pathogenicity. A recent paper describes an ODC gene knockout in Leishmania (19) but does not describe effects of the lesion on pathogenicity, hence it is not yet known whether ODC is required for virulence of any pathogen. We have now isolated ODC gene sequences from the ascomycete fungus, S. nodorum, which is one of the major causes

A knockout strain of Stagonospora (Septoria) nodorum lacking the single ornithine decarboxylase (ODC) allele has been created by targeted gene replacement. A central region of the S. nodorum ODC gene was isolated by polymerase chain reaction using degenerate oligonucleotides and used to probe a λ genomic library. The gene was sequenced and the encoded ODC protein sequence was shown to be similar to those from other fungi. The functionality of the S. nodorum ODC was confirmed by complementation of an Aspergillus nidulans mutant (puA) strain devoid of ODC activity, restoring growth in the absence of exogenous polyamines. Sporulation of the transformants was reduced suggesting abberant regulation of the S. nodorum gene in A. nidulans. Transformation-mediated gene replacement was used to create strains which were auxotrophic for putrescine and lack ODC coding sequences. Pathogenicity studies on these mutants showed that they are greatly reduced in virulence compared with non-disrupted transformants. This confirms that the strains carrying an ODC disruption cannot obtain sufficient polyamines from the host plant for normal growth and, thus, that fungal ODC may be a suitable target for chemical intervention.

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The abbreviations used are: ODC, ornithine decarboxylase; DFMO, difluoromethylornithine; PCR, polymerase chain reaction; kb, kilobase(s); bp, base pair(s); PIPES, 1,4-piperazinediethanesulfonic acid; PDA, potato dextrose agar; CMA, Czapek Malt agar; MM, minimal medium.

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of cereal crop loss throughout the world. To define the role of ODC in polyamine biosynthesis and pathogenicity in this pathogen, gene function was tested genetically by gene disruption. Disruptants demonstrated the essential function of ODC in polyamine metabolism, growth, and pathogenesis. This provides the first demonstration for the requirement for ODC in disease development.

**EXPERIMENTAL PROCEDURES**

*Strains and Media—*The following fungal isolates were used in this work: *S. nodorum* BS171, and *Aspergillus nidulans* G0171. *S. nodorum* was maintained as a *StuI*-BamHI fragment into *pBluescript* SK- to form plasmid pFRONT2. A 1062-bp fragment containing 3'-non-coding sequences was amplified by PCR from *pXmn* using primers 5GCGCGATCCGCCACAGCCCAGCT and a standard *pBluescript* SK- flanking primer. The resulting PCR product was subcloned as a *SacI*-HindIII fragment into *pBluescript* SK- to form plasmid pFBS. A 1.4-kb fragment from *pST28* was constructed by ligating a 1.4-kb HindIII fragment of *ODC* from *S. nodorum* into *pBluescript* SK- to form plasmid pFRONT2. The *SaltIII*-HindIII fragment from pFRONT2 was then inserted into *pSaltIII*-HindIII and cloned into BamIII-HindIII digested pDIS3 to form plasmid pSNODCDIS. This plasmid was linearized with *KpnI* prior to transformation into *S. nodorum*. Plasmid DNA was isolated by a modification of the alkaline lysis method (27).

**DNA Isolation and Southern Blotting—*Genomic DNA was purified by a modification of the method by Specht et al. (28). Liquid cultures were collected on Miracloth in a funnel and washed with TSE (100 mM NaCl, 100 mM EDTA, 50 mM Tris-HCl, pH 8). The washed mycelia were subsequently freeze-dried. Mycelium (1 g dry weight) was ground to a powder and suspended in 0.6 volume of lysis buffer (TSE + 2% SDS) and 0.2 volumes of toluene was added. The flask was shaken very slowly on a rotary shaker for 3 days. The DNA was separated from the toluene by spinning at 15,000 × g for 15 min in a SS34 rotor. The DNA is concentrated in the interphase between the water and the toluene phase. The DNA was removed and spun again to remove the toluene. 0.25 volumes of 7.5 M NH4Ac was added and the polysaccharides were precipitated overnight at 4 °C. Poly saccharides were pelleted at 39,000 × g for 30 min in a SS34 rotor. The DNA was precipitated with 0.7 volumes of isopro pyl alcohol. The precipitate was removed with a sealed-off Pasteur pipette and resuspended in 750 μl of TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) and 50 μg/ml RNase. The RNase digestion was performed at 37 °C for 30 min. Proteinase K was added to 100 μg/ml and left to incubate for another 2 h at 37 °C. The DNA solution was extracted with phenol/CHCl3. The DNA was once more precipitated and resuspended in TE. For Southern analysis, 5 μg of genomic fungal DNA was digested with appropriate restriction enzymes overnight at the appropriate temperature. The DNA was separated on a 0.8% agarose gel and standard techniques (29) were employed for treating and blotting the DNA onto a Nyl on membrane (Boehringer Mannheim). Pre-hybridization and hybridization solutions were 5 × Denhardt's (2% gelatin, 2% Ficoll 400, 2% polyvinylpyrrolidone 3600, 10% SDS), 100 μg/ml denatured calf thymus DNA, 0.2% SDS, and 1 × HIB (3 mM NaCl, 0.1 mM PIPES, 20 mM EDTA, pH 6.8). Hybridization was performed overnight at 65 °C. All washes were performed at high stringency: 1 × SSC and 0.5% SDS at 65 °C (twice for 30 min).

Transformation of *S. nodorum*—*S. nodorum* was transformed essentially as described by Cooley et al. (25). After transformation, the plates were incubated overnight at 18 °C and the next day overlaid with Cz-V8 agar containing 1 μM putrescine and 100 μg/ml hygromycin B or 500 μg/ml zeacin (Invitrogen), as appropriate. The plates were incubated at 18 °C under near UV light until the first emerging colonies were visible; these were transferred weekly to new selective media.

**Transformation of *A. nidulans*—**The method was essentially as reported by De Graaff (30). Plates of transformed protoplasts were incubated at 37 °C in the dark until the first emerging colonies were visible. Spores from these were removed and streaked out to single colonies on MM plates (MM, 1.2% agar and appropriate supplements). Spores from a single colony were transferred into 100 μl of water and subsequently propagated and tested for complementation of putrescine auxotrophy.

**ODC Enzyme Assay—**Fungal ODC activity was measured by following the release of 14CO₂ from [1-14C]ornithine in a protocol adapted from Stevens et al. (31). Spores were harvested from confluent sporulating Cz-V8 plates and washed extensively with sterile distilled water. They were suspended in Cz-Dox liquid media (100 ml) at 1 × 10⁷/ml and incubated at 25 °C with shaking. Material was harvested by filtration through 0.45 μm filters and cold washed with PBS and kept in liquid nitrogen with glass beads, then resuspended in 2–3 ml of extraction buffer (10 mM potassium phosphate, 2 ml dithiothreitol, 1 mM MgCl₂, 0.1 mM EDTA, 0.1 mM pyridoxal phosphate, pH 7.6) and briefly vortexed. The resulting extract was centrifuged at 25,000 × g to remove cell debris then desalted to remove other decarboxylase substrates by buffer exchange in a Nap 10 column (Amersham Pharmacia Biotech).
RESULTS

ODC Gene Isolation—Degenerate oligonucleotides designed against conserved amino acids within the ODC proteins of human (32), yeast (16), N. crassa (17), and Datura stramonium (33) were used as PCR primers to amplify an internal fragment of the S. nodorum ODC gene. A product of 609 bp was obtained using S. nodorum DNA as a template and this was cloned into pBluescript SK (Stratagene) and sequenced to confirm its identity. The fragment was purified, labeled with [32P]dCTP and then used as a hybridization probe to screen a genomic DNA library of S. nodorum in the vector λGEM11. 20 Positive plaques were identified, purified by a further two rounds of screening and DNA purified. Digested DNA was electrophoresed, blotted onto nylon membranes, and probed to identify those fragments corresponding to the ODC gene. These were subcloned into pUC18 and sequenced. A 4.8-kb region was fully sequenced (GenBank accession number AJ249387). This contained an open reading frame that encoded a protein of 462 amino acids and was interrupted by a single intron of 51 bp. The intron is bounded by sequences typical of fungal introns (34). The predicted amino acid sequence of this protein is shown in Fig. 1. The protein encoded by this gene is typical of ornithine decarboxylase proteins and contains all of the motifs conserved in those of other species. However, analysis of the sequence failed to reveal the PEST sequences which are believed to be important for the rapid protein degradation (35) observed in ODCs from some other species. The mRNA of ODC from N. crassa has a long 450-bp 5′-leader sequence and it has been suggested that this may allow for post-transcriptional control of ODC by enabling regulation of mRNA stability (36). Although we have not precisely determined the transcription start site for S. nodorum ODC, reverse transcriptase-PCR using primer pairs from the ODC promoter region has shown that this occurs within a region 470–600 bp upstream of the predicted methionine initiation codon, so it is possible that the S. nodorum ODC is also regulated in the same manner (data not shown).

Expression of S. nodorum ODC in A. nidulans—The identity of the cloned gene was confirmed by co-transforming the 4.8-kb genomic fragment containing the proposed S. nodorum ODC gene, pXmnI, with pGM32 a plasmid containing pyr-4 into an ODC minus pyrG mutant (p4, pyrG) of A. nidulans that is auxotrophic for putrescine and uridine (37). Transformants were recovered on the basis of their ability to grow in the absence of uridine. A number of these transformants were able to grow in the absence of exogenous polyamine demonstrating that the S. nodorum ODC gene was capable of complementing the puA mutation.

Although the transformed colonies had the same radial growth rate as wild-type A. nidulans, they exhibited delayed and reduced conidiation. It is known that high levels of polyamines are needed for conidiation in A. parasiticus and that conidiation can be prevented by the addition of diamino butanone (a polyamine biosynthesis inhibitor) at concentrations that do not inhibit mycelial growth (38). This suggested that although sufficient putrescine was being produced for mycelial growth, there was not enough polyamine synthesized to fully support sporulation and so presumably this heterologous gene was not properly regulated or fully functional.

ODC Gene Disruption—In order to examine the effect of ODC mutation in S. nodorum, a two-step gene disruption strategy was devised. Having cloned the gene, it was necessary to make a construct suitable for gene disruption. A 1-kb region from upstream of the N-terminal methionine and 1 kb from downstream of the stop codon were isolated, and ligated into pUC19 along with the hygromycin resistance cassette so that they flanked the hygromycin gene (Fig. 2A). This construct was transformed into S. nodorum using standard protocols (21) and transformants recovered on media containing both 100 μg/ml hygromycin and 100 μM putrescine. The transformants were purified and tested for putrescine-dependant growth. Of 150 transformants tested, numbers 26, 128, 132, and 143 were unable to grow in the absence of exogenous putrescine and so were presumably devoid of ODC activity. DNA was isolated from these transformants and analyzed to confirm that the ODC gene had indeed been deleted from these isolates. Fig. 2B shows a Southern blot of DNA from wild-type and candidate disruptant colonies probed with the ODC encoding region. The ODC coding region has been deleted from three of the candidate disruptants, 26, 128, and 135; these strains have been renamed mutants Δ26, Δ128, and Δ135, respectively. Transformant 143 has a complex rearrangement at this locus. This confirms that the colonies that were unable to grow without putrescine have indeed lost the ODC coding sequence, and that a functional ODC gene is required for growth when no exogenous polyamines are available. Since deletion of a single copy of the ODC gene from this haploid organism removes all hybridizing bands on this Southern blot, this result also confirms the presence of a single copy of the ODC gene in the S. nodorum genome.

ODC Enzyme Activity in Wild-type and Mutant Fungus—ODC enzyme activity was determined in wild-type and ODC− S. nodorum. As ODC activity was difficult to detect in mycelia we determined activity in germinating spores where activity was expected to be high. The highest level of ODC activity is observed at the same time as germination occurs (Fig. 3). No ODC activity could be detected in any ODC− mutant strain. This observation agrees well with a role for polyamines in the differentiation of fungi.

Nutritional Requirements of ODC− Pathogens—To test the ability of different polyamines to restore growth of the ODC− mutant, strains were grown on minimal agar medium containing 60 μM putrescine, spermine, and spermidine, with or without the addition of DFMO. We note that DFMO has been reported to be a poor inhibitor of growth for a number of fungal species, among them S. nodorum. These inhibition experiments relied on measurements of growth rate on rich media, such as potato dextrose agar (PDA) or Czapek Malt agar (CMA). Therefore, growth rates for wild-type and ODC− S. nodorum were determined using PDA, CMA, and a defined minimal medium (MM) in combination with added polyamines and DFMO to determine the effects of ODC inhibition. DFMO is a potent inhibitor of wild-type S. nodorum growth on defined minimal medium but not on rich medium such as PDA or CMA (Fig. 4A). However, the ODC− mutant is restored to wild-type growth...
rate on MM with the addition of 60 μM putrescine, spermine, or spermidine (Fig. 4B). The antagonistic effects of DFMO on growth on defined minimal medium can be completely reversed by addition of 60 μM putrescine, spermine, or spermidine, which suggests that the PDA and CMA media contain sufficient amounts of polyamines to counteract the effects of DFMO. This may explain the poor inhibition of fungal growth previously reported on these media. It would be interesting to re-examine the inhibitory effects of DFMO on “insensitive” fungi using defined minimal media. Furthermore, interesting questions are raised concerning polyamine uptake and ODC turnover in those fungi which were DFMO-sensitive on rich media.

**DISCUSSION**

We have cloned and sequenced the ODC gene from *S. nodorum* and shown that it is functional in *A. nidulans* by restoring putrescine prototrophy to a putrescine auxotroph. Sporulation of the *A. nidulans* transformants was reduced compared with the wild-type; this may indicate that the *S. nodorum* gene cannot provide sufficient putrescine to support the normal levels of conidiation of *A. nidulans*. It may be because the heterologous enzyme is expressed at a reduced rate, is not assembled correctly, cannot be fully induced in the heterologous host, or that some control regions were not present in the construct used for complementation. It does, however, confirm that a supply of polyamines is essential for the sporulation process and that even if normal vegetative growth can proceed, sporulation and, hence, disease dissemination may be impaired by inhibition of the polyamine pathway in a fungus. Targeted gene
replacement of ODC in *S. nodorum* demonstrates the essential housekeeping function of this enzyme in this species. These mutants are strict auxotrophs, showing germination but almost no growth on minimal medium in the absence of exogenous polyamines. These ODC disruptants grow at a rate similar to the wild-type when supplemented with sufficient exogenous putrescine. Putrescine, spermine, and spermidine were equally able to complement the polyamine auxotrophy in the ODC null mutant cells, suggesting that there is no strict requirement for putrescine in growth. ODC-deficient mutants of *N. crassa*, *A. nidulans*, and mammals also require polyamines for growth suggesting a similarly vital role for ODC in these organisms. This result also confirms that ODC is the sole route to polyamine biosynthesis in *S. nodorum*. Together with the observation that previous reports of ODC inhibitor insensitivity may well have arisen from unintentional contamination of media with polyamines, this suggests that inhibition of polyamine biosynthesis may be a useful therapeutic target for this class of pathogens. It is not known whether the fungus has a strict requirement for spermidine in growth and disease development. A *S. nodorum* spermidine synthase genomic clone has been isolated and work is underway to create spermidine synthase knockout mutants to test the validity of this enzyme as a therapeutic target.
Deletion of the ODC genes from *Leishmania donovani* (19) results in a requirement for either putrescine or spermidine but not for spermine, hence, this protozoan seems to lack a catabolic pathway for spermine. Spermine is the major polyamine in mammals and, thus, ODC null mutants of this parasite are unlikely to be able to scavenge usable polyamines from their host. Plants contain significant amounts of spermidine and putrescine. ODC-deficient *S. nodorum* and other fungi can utilize all three polyamines to maintain growth suggesting that ODC mutants could scavenge sufficient polyamine from the plant host to maintain growth during disease development. Pathogenicity assays of the mutant strains using spore inoculum resulted in greatly reduced lesions compared with the wild-type. This shows that although the strains are still pathogenic, their virulence is greatly reduced. We believe this to be the first demonstration of a role for ODC in pathogenesis of any organism. The limited growth of the ODC disruptants in * planta* is presumably sustained by utilizing plant-derived polyamines. The possibility that spores used in the assay have reserves of polyamines that allow limited growth within the plant cannot be disregarded. Attempts to produce polyamine-starved spores were unsuccessful, because continued growth of ODC mutants in the absence of polyamines did not occur. Progressive reduction of polyamine levels in the medium also resulted in greatly reduced sporulation, preventing reliable pathogenicity tests. If it were possible to find a compound that completely inhibited fungal ODC activity during plant infection, mimicking the effects of the mutation, then presumably the same degree of reduction in lesion development would be observed. It would be interesting to investigate whether ODC+ strains are more sensitive to toxic polyamine analogues (39–41) than the wild-type, since they must be utilizing a polyamine uptake system to supply their requirement, raising the possibility of a dual control approach. It is also noteworthy that a number of plant defense compounds are polyamine conjugates. Due to the need for uptake of exogenous polyamines, it is possible that the mutants would be more sensitive to such compounds. Our work has demonstrated that ornithine decarboxylase is essential for virulence of a fungal pathogen and that inhibition of ODC may be a valid approach to the design of new fungicides. This disease is a prime target for chemical or biological control in winter cereals and the demonstration of a requirement for ODC in virulence and growth has important implications in the consideration of polyamine biosynthesis as a fungicide target.

REFERENCES

1. Pegg, A. E., and Williams-Ashman, H. G. (1981) in *Polyamines in Biology and Medicine* (Morris, D. R., and Marton, L. J., eds) pp. 3–42, Marcel Dekker, Inc., New York
2. Tabor, C. W., and Tabor, H. (1985) *Microbiol. Rev.* 49, 81–99
3. Zappia, V., and Pegg, A. E. (1988) *Adv. Exp. Med. Biol.* 250, 1–34
4. Pegg, A. E. (1986) *Biochem. J.* 234, 249–262
5. Bach, C. J., and McCann, P. P. (1987) in *Inhibition of Polyamine Metabolism* (McCann, P. P., Pegg, A. E., and Sjoerdema, A., eds) pp. 317–344, Academic Press, New York
6. Bittoni, A. J., McCann, P. P., and Sjoerdema, A. (1987) *Exp. Parasitol.* 64, 257–243
7. Gillin, F. D., Reiner, D., and McCann, P. P. (1983) *J. Protozool.* 31, 161–163
8. Kaur, K, Emmett, K., McCann, P. P., Sjoerdema, A., and Ullman, B. (1986) *J. Protozool.* 33, 518–521
9. Giffin, F. D., McCann, P. P., Bittoni, A. J., and Bachci, C. J. (1986) *J. Protozool.* 33, 238–243
10. Walters, D. R. (1995) *Mycol. Res.* 99, 129–139
11. Metcalf, B. W., Hey, P., Danzin, C., Casera, P., and Vevert, J. P. (1978) *J. Am. Chem. Soc.* 100, 2551–2553
12. West, H. M., and Walters, D. R. (1989) *Mycol. Res.* 92, 453–457
13. Davis, R. H. (1990) in *The Mycota III: Biochemistry and Molecular Biology* (Brambl, R., and Marzluff, J., eds) pp. 347–356, Springer Verlag, Berlin
14. Kumar, A., Altabella, T., Taylor, M. A., and Tiburcio, A. Y. (1997) *Trends Plant Sci.* 2, 124–130
15. Smith, T. A., Barker, L. K. A., and Owen, W. J. (1992) *J. Biol. Chem.* 267, 3781–3788
16. Fonzi, W. A., and Sypherd, P. S. (1987) *J. Biol. Chem.* 262, 10127–10133
17. Williams, L. J., Barnett, G. R., Ristow, L. L., Pitkin, J., Perriere, M., and Davis, R. H. (1992) *Mol. Cell BioL* 12, 347–359
18. Guevara-Olvera, L., Xeomostille-Casares, B., and Ruiz-Herrera, J. (1997) *Microbiology* 143, 2237–2245
19. Jiang, Y., Roberts, C., Jardim, A., Carter, N. S., Shih, S., Ariyanayagam, M., Fairlamb, A. H., and Ullmann, U. (1999) *J. Biol. Chem.* 274, 667–669
20. Mattern, I. E., and Punt, P. J. (1988) *Fungal Genet. Newsl.* 25, 158–163
21. Beneditz, P. W., Mappledoram, C. J., and Scott, P. R. (1981) *Trans. Br. Mycol. Soc.* 77, 667–669
22. Specht, C. A., DiRusso, C. C., Novotny, C. P., and Ullrich, R. C. (1982) *Methods Enzymol.* 81, 179–187
23. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. De Graaff, L. H. (1989) *The Structure and Expression of the Pyruvate Kinase Gene of Aspergillus nidulans and Aspergillus niger*. PhD thesis, Agricultural University Wageningen, Netherlands
25. Stevens, L., McKinnon, I. M., and Winther, M. (1976) *Biochem. J.* 158, 235–241
26. Hickok, N. J., Seppanen, P. L., Gunsalus, G. L., and Janne, O. A. (1987) *Biochem. J.* 235, 241–245
27. Michael, A. L., Purse, J. M., Rhodes, M. L. C., and Burtin, D. (1996) *Biochem. J.* 314, 241–248
28. Rogers, S., Wells, R., and Rechsteiner, M. (1986) *Science* 234, 364–368
29. Pitkin, J., Perriere, M., Kanae, I. A., Ristow, J. L., and Davis, R. H. (1994) *Arch. Biochem. Biophys.* 315, 153–160
30. Sneath, P. H. A. (1955) *Nature* 175, 818
31. Guzman-de-Pena, D., and Ruiz-Herrera, J. (1999) *Fungal Genet. Cell Biol.* 21, 198–205
32. Havis, N. D., Walters, D. R., Foster, S. A., Martin, W. P., Cook, F. M., and Robins, D. J. (1994) *Pestic. Sci.* 41, 61–69
33. Havis, N. D., Walters, D. R., Martin, W. P., Cook, F. M., and Robins, D. J. (1994) *Pestic. Sci.* 41, 71–76
34. Mackintosh, C. A., and Walters, D. R. (1997) *J. Phytopathol.* 145, 43–45

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