MPMI Vol. 9, No. 6, 1996, pp. 443-449. Publication no. M-1996-0620-10O.

Current Review

Recent Advances in the Genetics of Oomycete Plant Pathogens

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Received 31 January 1996. Accepted 15 April 1996.

The oomycetes are a group of diverse organisms that include significant pathogens of plants, insects, and animals, as well as saprophytic species. Oomycetes infecting plants include obligate pathogens, such as the downy mildews and the white rusts, and facultative pathogens within the genera *Phytophthora* and *Pythium*. The need to learn more about the biology and pathology of the oomycetes has been long recognized, and it is likely that such studies will yield novel biological findings as oomycetes do not share a close taxonomic affinity with more intensely studied organisms. For example, contemporary assessments of phylogenetic relationships based on rRNA sequence comparisons indicate that oomycetes are more closely related to chrysophytes, diatoms, and heterokont algae than to ascomycetes and basidiomycetes, despite the linkage of oomycetes to other fungi in traditional classification schemes (Förster et al. 1990; Gunderson et al. 1987).

Until recently, research studies of oomycetes were hampered by the unavailability or inefficiency of methods for genetic investigation. Oomycetes are consequently less well characterized as a group than are other fungi such as ascomycetes and basidiomycetes, for which sophisticated procedures for molecular and classical genetic analyses have been available for many years. Due to improvements to the repertoire of genetic techniques available for the oomycetes, the potential now exists to make rapid advances in our understanding of these important organisms. This review provides an update on the current status of oomycete genetics and its application to the study of plant-microbe interactions.

CLASSICAL GENETICS

The availability of tractable techniques for performing controlled laboratory crosses is a prerequisite for dissecting the genetic basis of host-species and cultivar specificity and other important issues in plant pathology. Such methods are now available for several plant pathogenic oomycetes, employing a variety of approaches depending on whether the species being studied is heterothallic or homothallic, or whether it is culturable on artificial media or is an obligate pathogen. A key difference between the genetics of oomycetes and that of fungi such as most ascomycetes and basidiomycetes is that in the oomycetes vegetative structures are diploid, with meiosis occurring in well-differentiated gametangia. This concept of diploidy in oomycetes was initially highly controversial and did not become fully accepted until the 1970s (rev. in Shaw 1983).

Matings in heterothallic species.

Among the heterothallic oomycetes that are easily cultured in the laboratory, most genetic analyses have been performed with *P. infestans* (Shaw 1991), although controlled crosses have been made with other species including *P. drechsleri* (Galindo and Zentmeyer 1967), *P. parasitica* (Förster and Coffey 1990), *Pythium sylvaticum* (Martin 1995), and the saprophyte *Achlya bisexualis* (Lasure and Griffin 1975). In *Phytophthora* and *Pythium*, progeny are generally recovered by pairing isolates of the opposite mating type, extracting oospores in bulk, and then selecting germinating oospores by microdissection (Förster and Coffey 1990; Martin 1995; Shattock et al. 1986). Studies using DNA and isozyme markers have indicated that most oospores recovered from mating mixtures are hybrids (Förster and Coffey 1990; Shattock et al. 1986). However, most heterothallic oomycetes are bisexual to varying degrees. Therefore, selfing could occur within a single isolate in a culture stimulated by the opposite mating type. Although selfing is infrequent in practice, outcrossing is generally confirmed using markers such as isozymes (Shattock et al. 1986), random amplified polymorphic DNAs (RAPDs; Judelson et al. 1995), or restriction fragment length polymorphisms (RFLPs; Drenth et al. 1995; Förster and Coffey 1990; Goodwin et al. 1992). The microdissection step for obtaining germinated oospores is considered by many to be important to ensure that the colonies are derived from a single oospore and not residual parental mycelium. This time-consuming step can pose a practical barrier to the establishment of large numbers of offspring, particularly in pairings in which the rate of germination of oospores is low. As a consequence of the development of transformation procedures for several species of *Phytophthora* (described below), it may be possible to more readily isolate progeny by performing crosses between transformants that are resistant to different agents such as G418 and hygromycin, and isolating sexual hybrids by plating oospores on media containing both drugs. This would be similar to procedures used with some ascomycetes, where prototrophic diploids are selected from auxotrophic parents. Although a double-selection method would bias the inheritance of regions of the genome containing the transgenes, it would facilitate the isolation of progeny from pairings which inefficiently produce viable oospores and the development of very large mapping populations.

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Controlled crosses have also been described for obligately pathogenic oomycetes including *Bremia lactucae* (Ilott et al. 1989; Michelmore and Ingram 1981) and heterothallic isolates of *Peronospora parasitica* (Moss et al. 1994). These were performed using strategies similar to those used for nonobligate pathogens, although most steps were performed in planta. In *B. lactucae,* for example, oospores were produced in cotyledons infected with both parents, aged, and then inoculated at low density onto seedlings to obtain progeny presumably derived from single oospores. Although the need to perform these steps in planta presents certain practical limitations, this has not posed a insurmountable obstacle to genetic analysis.

Matings in homothallic species.

Until recently it was generally assumed that the predominance of inbreeding in homothallic oomycetes posed an insurmountable barrier to the development of versatile systems for genetic analysis. Genetic studies were consequently limited to the analysis of somatic hybrids or heterokaryons resulting from protoplast fusion or anastomosis (Layton and Kuhn 1988; Long and Keen 1977). However, several groups have now demonstrated that outcrossing occurs at low but detectable frequencies in mixed cultures of homothallic strains. Two approaches have been used to obtain these infrequent hybrids. Bhat and Schmitthenner (1993) showed that in 17 of 23 crosses attempted in *P. sojae,* hybrid lines could be selected from parents carrying different drug-resistance mutations using double-inhibitor media. Other groups identified hybrids of *P. ultimum* or *P. sojae* by screening single-oospore derived cultures for the inheritance of RAPD markers from both parents (Francis and St. Clair 1993; Tyler et al. 1995; Whisson et al. 1994). Although a small fraction of oospores were hybrids (2 to 20%, depending on the cross), once identified their subsequent self-fertilization yielded useful F_2 populations. The ability to perform crosses has also been described for *Albugo candida*, an obligately pathogenic white rust fungus (Rimmer et al. 1995).

Inheritance of molecular markers.

Most of what is known about the segregation of loci in oomycetes has come relatively recently from the analysis of DNA or isozyme markers. This contrasts with the history of research in the haploid ascomycetes or basidiomycetes, where many morphological and biochemical mutants were available for inheritance studies long before DNA technologies were accessible. Early genetic studies in oomycetes were generally limited to the analysis of naturally varying traits such as mating type or host specificity, plus a few dominant mutations conferring drug resistance (Layton and Kuhn 1988; Michelmore and Ingram 1981; rev. in Shaw 1988).

Studies of the inheritance of DNA markers in both homothallic and heterothallic species of oomycetes have provided reassurance that nuclear loci generally segregate in a Mendelian fashion. The most extensive support for this can be drawn from studies in which AFLP, RAPD, or RFLP markers were used to construct genetic maps. For example, in *B. lactucae* (Legg 1991; Hulbert et al. 1988), *P. infestans* (Theo van der Lee and Francine Govers, personal communication) and *P. sojae* (Whisson et al. 1995), normal segregation was observed for most of the 114, 130, and 250 DNA markers, respectively, that were used to construct genetic maps for each species.

A few deviations from Mendelian genetics have been observed. For example, in a cross in *P. sojae* two of 28 RFLP loci showed aberrant segregation consistent with trisomy (Tyler et al. 1995). A second cross in *P. sojae* displayed a very biased assortment of most RAPD and RFLP markers; in this case, chromosomal rearrangements or lethal mutations were invoked as possible causes of the distorted segregation (Tyler et al. 1995). Several DNA markers were also not inherited at normal frequencies in *Py. sylvaticum* (Martin 1995). A possible system of balanced lethals has been observed at the mating type locus in *P. infestans*, although it is not yet clear if this should be regarded as an aberration or an important feature of that locus (Judelson et al. 1995).

Electrophoretic karyotypes have been developed for *B. lactucae* (Francis and Michelmore 1993), several species of *Phytophthora* (Howlett 1989; Judelson et al. 1993a; Tooley and Carras 1992), and *Py. sylvaticum* (Martin 1995); some of the resulting data may indicate possible causes of the genetic abnormalities observed in particular crosses. For example, substantial polymorphism was observed between isolates of *B. lactucae* (Francis and Michelmore 1993), as well as between isolates of *Py. sylvaticum* (Martin 1995), which might lead to aberrant segregation. Moreover, most isolates of *B. lactucae* were observed to contain B chromosomes or large linear plasmids which did not segregate in a Mendelian fashion, and in *Py. sylvaticum* several chromosomes were meiotically unstable.

It is important to point out that deviations from normal chromosome behavior are not the rule and are not unique to the oomycetes. Distorted segregation, chromosomal polymorphisms, deletions, inversions, and lethal loci are common features of many diploid organisms (Rhoades 1942) as well as haploid fungi (Perkins 1974). However, it is likely that some isolates within oomycete species might be more amenable to genetic analysis than others.

METHODS FOR GENE TRANSFER

Reliable techniques for the DNA-mediated transformation of oomycetes were first reported by Bailey et al. and Judelson et al. in 1991, more than a decade after such methods were developed for other filamentous fungi (Case et al. 1979). This delay was due to several factors, including difficulties in producing large numbers of viable protoplasts from the highly vacuolated and coenocytic hyphae typical of the majority of oomycetes, and to a lack of experimental data indicating which vectors would be suitable for selecting transformants. Some approaches that were used to select transformants in other fungi, such as using cloned genes to complement auxotrophic mutations, were not feasible for oomycetes due to the challenge of obtaining such mutations in diploids. Many of these limitations have now been overcome and effective procedures for transformation are available for several oomycetes.

Two general strategies have been employed to identify vectors useful for selecting transformants. In one approach, which culminated in the stable transformation of *P. infestans* and *P. megasperma* f. sp. *glycinea (P. sojae),* vectors were constructed that contained fusions between marker genes for drug resistance and promoters from the *ham34* and *hsp70* genes of *B. lactucae* (Judelson et al. 1991, 1993a). The use of these oomycete promoters was based on the assumption that

promoters on vectors used for ascomycetes and basidiomycetes would not function in oomycetes because there was no taxonomic affinity between these groups of fungi (Förster et al. 1990). This premise was supported by transient expression assays in *P. infestans, P. sojae,* and *Achlya ambisexualis* that indicated that oomycete promoters displayed much higher levels of activity than promoters from ascomycetes, basidiomycetes, plants, and other groups (Judelson et al. 1993b). Stable integrative transformants were obtained in *P. infestans* using vectors harboring fusions between the *ham34* and *hsp70* sequences and genes for resistance to G418, hygromycin, and streptomycin (Judelson et al. 1991); in *P. sojae* the *ham34*, but not the *hsp70* promoter, enabled the recovery of hygromycin-resistant strains (Judelson et al. 1993a).

Other groups pursued a different approach for identifying transformation vectors, which involved testing vectors developed for gene transfer in ascomycetes and basidiomycetes for activity in *Phytophthora.* Although this approach was generally unsuccessful (Kinghorn et al. 1991), Bailey et al. (1991) reported that plasmids containing the *Ustilago maydis hsp70* promoter, fused to a hygromycin phosphotransferase gene, enabled the selection of drug-resistant transformants from protoplasts of *P. capsici* and *P. parasitica.* It was not determined if transcription relied on the normal *U. maydis* promoter or on cryptic sequences in the vector. Bailey et al. (1993) also demonstrated that transformants could be generated by microprojectile bombardment of mycelia, which could be useful for species from which it is difficult to obtain large amounts of viable protoplasts.

The reliable transformation of oomycetes outside of the genus *Phytophthora* has not yet been described. The uptake of foreign DNA in *Achlya ambisexualis* was once reported (Manavathu et al. 1988), although the vector was not shown to be stably integrated and appeared to contain an inactive promoter (from SV40). Since promoters from the downy mildew, *B. lactucae,* function in *Phytophthora* and *Achlya*, there is reason to expect that vectors expressed in one oomycete might function in other members of the class. However, there is indirect evidence for heterogeneity in promoter recognition within the oomycetes, which resembles observations previously made in ascomycetes and basidiomycetes where strong promoters from one species can exhibit little activity in others. The *hsp70* promoter of *B. lactucae,* for example, functioned well in *P. infestans* but appeared less active in *P. sojae.* Similarly, *U. maydis* promoter and autonomously replicating sequences that were reported to function in *P. capsici* and *P. parasitica* (Bailey et al. 1991) showed no activity in *P. infestans* or *P. sojae* (Judelson et al. 1992, 1993a). The components of oomycete promoters that mediate transcription are not yet well understood, outside of the observation that of the more than 12 oomycete genes that have been cloned to date, each contain a 16-bp consensus sequence within the first 100 nt upstream of the ATG start codon (Pieterse et al. 1994b). The major transcription start point was found within this motif in seven out of eight genes for which transcription was studied. Not all of the genes contained traditional 'TATA'-like motifs.

A range of technologies based on transformation have been developed that will aid the future analysis of genes relevant to the pathology and biology of the oomycetes. For example, the ability to express nonselected genes was demonstrated through the use of genes such as β-glucuronidase, β-galactosidase, and luciferase (Judelson 1993; Judelson et al. 1993b; F. Govers, P. van West, and A. J. de Jong, personal communication). Curiously, while the expression of transgenes was usually mitotically and meiotically stable, they occasionally became silenced without undergoing any obvious mutation, deletion, or methylation (Judelson and Whittaker 1995). The inactivation of introduced genes, which is not unique to the oomycetes (Flavell 1994; Selker et al. 1993), will need to be considered in experiments that evaluate the function of transgenes encoding, for example, putative pathogenicity or avirulence factors. Additional transformation-based technologies that have been developed include methods for high-efficiency cotransformation and for inhibiting gene expression using antisense RNA (Judelson et al. 1993b). Techniques for gene disruption, which have been very useful for studying putative pathogenicity genes in ascomycetes and basidiomycetes (Rogers et al. 1994) have not been demonstrated in an oomycete. Doing so may be a challenge in the oomycetes since the recombination of transforming plasmids with homologous chromosomal sequences is not frequent (H. Judelson, unpublished). Also, since oomycetes are diploid, two rounds of disruption will be required to inactivate loci exclusive of avirulence genes and other loci which can be obtained in a heterozygous state.

GENETICS OF OOMYCETE-PLANT INTERACTIONS

Race-cultivar specificity.

The determination of race-cultivar specificity by gene-for-gene interactions has been proposed for several diseases caused by oomycetes. This has usually been based on genetic studies of the host alone, although analyses of the genetics of the pathogen have been performed only in a few systems. Attempts to isolate specificity genes from oomycetes are underway in several systems, generally by positional cloning strategies. This tactic has been favored since transformation rates for oomycetes are generally considered too low to make the isolation of the loci by shotgun cloning practical.

Within the oomycetes, some of the best and earliest studies of race-cultivar specificity examined the interaction between lettuce and *B. lactucae* (lettuce downy mildew). Simultaneous genetic analyses of host and pathogen identified 13 loci at which dominant alleles determined avirulence to 13 resistance genes *(Dm)* in lettuce (Ilott et al. 1989). The avirulence loci segregated independently, as observed for avirulence genes in other fungi that had been characterized by that date. One isolate was found to apparently contain an inhibitor locus which suppressed the expression of avirulence gene *Avr5/8.* As part of a strategy to isolate the gene by chromosome walking, five of the loci (*Avr4, Avr5/8, Avr6, Avr7,* and *Avr15*) were placed on a genetic map of *B. lactucae* constructed using RFLP markers (Hulbert et al. 1988).

Cultivar specificity in the late blight diseases of potato and tomato caused by *P. infestans* has been investigated by several groups. Genetic studies of the pathogen loci interacting with nine of the 11 known resistance genes in potato and one of two resistance genes in tomato have been performed using F_1, F_2, and backcross populations. In most cases, specificity appeared to be determined by single loci with dominant alleles for avirulence (Al-Kherb et al. 1995; Spielman et al. 1989, 1990). However, some contradictory results were obtained

from the analysis of different isolates. For example, in some isolates avirulence against potato genes *R2* and *R4* was dominant (Al-Kherb et al. 1995) while in others avirulence appeared to be determined by a recessive allele (Spielman et al. 1989). Whether this indicates the presence of inhibitor loci in those isolates or whether avirulence was determined by more than one locus in the different strains remains to be established. The presence of an inhibitor gene controlling compatibility with *R10* was inferred by several crosses studied by Al-Kherb et al. (1995). Several projects to place the avirulence genes onto molecular maps are under way.

The soybean pathogen, *P. sojae,* is another oomycete in which the genetics of race-cultivar specificity is being studied. Thirteen resistance *(Rps)* genes have been identified in soybean; these are heavily utilized to control the disease and consequently there is interest in understanding the corresponding genetics of the pathogen. This issue was first addressed by Layton and Kuhn (1988) through their studies of laboratory-synthesized heterokaryons, and more recently and definitively by several laboratories using F_1 and F_2 populations. These analyses usually supported the determination of avirulence by single dominant (or semidominant) alleles of independent loci. However, for some loci contradictory results were obtained by different laboratories in studies of different isolates. For example, genetic studies by Tyler et al. (1995) indicated that reaction to *Rps3a* was determined by a single gene, with a dominant allele for avirulence, and this was in agreement with the results obtained by Whisson et al. (1995) in a cross between isolates of race 7 and 25. However, a second cross by Whisson et al. (1994), between races 1 and 7, yielded data more consistent with the presence of two independently segregating, complementary genes. Crosses by Bhat et al. (1993) also provided possible evidence for cases of epistasis between specificity loci, and of avirulence being either dominant or recessive. Although there has been a tendency in the literature to ascribe deviation from simple one-gene models to aberrant segregation, the possibility of more complex modes of inheritance needs to be examined further. As part of a genome mapping project in *P. sojae* by Whisson et al. (1994, 1995), seven of the avirulence loci were linked to RAPD or RFLP markers, including four genes *(Avr1b, Avr1k, Avr4,* and *Avr6)* that were less than 1 cM away from a DNA locus. Tyler et al. (1995) also demonstrated linkage of *Avr1b* to a separate RFLP marker at a distance of 11 cM.

A fascinating observation from genetic studies of avirulence in *P. infestans* and *P. sojae* is the apparent linkage of some avirulence loci, which had not previously been described in other fungi. Genes determining avirulence to potato resistance loci *R3* and *R7* appeared 15 cM apart in *P. infestans* (Al-Kherb et al. 1995). More strikingly, in *P. sojae* absolute linkage was demonstrated between *Avr4* and *Avr6* (Gijzen et al. 1995; Whisson et al. 1995) and between *Avr1b* and *Avr1k;* while *Avr3a* and *Avr5* were 4.5 cM apart (Whisson et al. 1995). In plants, the clustering of resistance genes has been taken to indicate an evolutionary relationship between the loci (Pryor 1987). It will be exciting to determine whether a similar relationship exists between certain avirulence genes in the oomycetes.

Host-species specificity.

The basis of specificity at the host-species level is also a significant area of activity in which genetic studies are being employed. Goodwin and Fry (1994) examined the basis of host-species specificity in *P. infestans* (a pathogen of tomato and potato), and its close relative, *P. mirabilis* (a pathogen of *Mirabilis jalapa).* Since these two species are interfertile, it was possible to produce F_1 hybrids. Most of these were nonpathogenic on either host. Further analysis is required to test whether this indicates the presence of dominant incompatibility loci in each parent, or a general defect in pathogenicity resulting from, for example, chromosomal aberrations induced by the cross. Another interesting issue which requires genetic analysis is the apparent ability of some isolates of *P. infestans* to colonize potato, but not tomato (Legard et al. 1995).

Host-species specialization has also been examined in the downy mildew, *Peronospora parasitica.* This species grows on multiple hosts including *Brassica napus, B. oleracea,* and *B. napa,* but each isolate is generally pathogenic only on the plant species from which it was isolated. By making crosses between isolates obtained from different hosts, preliminary evidence was obtained for the determination of host range by major specificity loci (Moss et al. 1994). Determining the precise nature of these loci may be difficult since genotypes conferring incompatibility on multiple hosts will be lethal for this obligate pathogen.

A novel approach towards characterizing determinants of host range was pursued by Érsek et al. (1995), who created by zoospore fusion hybrids between *P. capsici* (a pathogen of radish, tomato, and several other hosts but not lemon) and *P. nicotianae* (a pathogen of lemon, tomato, and other hosts but not radish). Of three somatic hybrids characterized, two could infect radish and lemon, as well as tomato; one infected neither radish or lemon, but could still infect tomato. Further study is required to determine if the changes in specificity were a consequence of chromosome breakage, recombination, or exchange. The underlying genetic events could be similar to those observed in chemically mutagenized *P. capsici* by Mena et al. (1994), where nonpathogenicity was associated with chromosome breakage.

Elicitors and pathogenicity factors.

Over the past decade biochemical studies have identified several elicitor proteins produced by oomycetes, and recently some of the corresponding genes have been cloned by PCR using primers based on the amino acid sequence of the purified proteins. One of these genes encodes a 42-kDa (529 aa) glycoprotein from *P. megasperma* (Sacks et al. 1995) that induces defense responses in cultured cells or protoplasts of parsley, a nonhost species, although its effect on intact plants has not been demonstrated. Deletion mutants were used to localize the elicitor activity to a 47 amino acid region, which should assist future studies of the interaction of the glycoprotein and its receptor in parsley. Homologous sequences were shown to be present in other species of *Phytophthora,* although whether they also act as elicitors is not known.

Genes encoding a second group of elicitor proteins, collectively called "elicitins," have been isolated from *P. cryptogea* (Panabières et al. 1995) and *P. parasitica* (Kamoun et al. 1993a). Elicitins are small (10 kDa) extracellular proteins that are produced by most species of *Phytophthora* and at least some species of *Pythium* (Huet et al. 1995; Kamoun et al. 1993; Pernollet et al. 1993; Ricci et al. 1989). The proteins induce necrosis and defense reactions in certain plant species

such as tobacco and are encoded by multigene families, some of which are clustered within the genome (K. Klucher, S. Kamoun, E. Doyle, and B. Tyler, personal communication; Panabières et al. 1995). Distinct acidic (α) and basic (β) forms of these proteins exist, with the latter class having higher biological activity. Structure-function studies have examined more than ten naturally occurring α and β-elicitin isoforms and several variants formed by in vitro mutagenesis to identify residues important for host recognition (O'Donohue et al. 1995).

The story of the elicitins is intriguing since it is not yet clear if they serve as host-specific, necrosis-inducing determinants which limit the host range of potential pathogens, as pathogenicity factors which aid the colonization by causing the death of plant tissue in advance of the pathogen, or both roles. Elicitins were initially proposed as inducers of incompatibility between tobacco and *Phytophthora* species other than *P. parasitica* var. *nicotianae*. This was because the proteins induced necrosis and systemic acquired resistance in tobacco, and at least initially appeared to be produced by all *Phytophthora* species except *P. parasitica* var. *nicotianae* (Ricci et al. 1989). However, more recently Pernollet et al. (1993) reported that plants that serve as hosts for elicitin-producing species of *Phytophthora* also responded to elicitins. Although this contradicted the observations of Kamoun et al. (1993b), this led to the suggestion that these proteins might also act as pathogenicity factors. Further uncertainty as to the function of elicitins resulted when strains of *P. parasitica* var. *nicotianae* that produced the protein were isolated from tobacco (Bonnet et al. 1994; Kamoun et al. 1994). To address the role of the protein, crosses were made between isolates of *P. parasitica* that secreted elicitins and nonproducer strains (Kamoun et al. 1994). While there was some correlation between elicitin production and pathogenicity in the progeny, other loci were clearly important as well. Hopefully the molecular tools now available for studying the oomycetes will be able to clarify the role of elicitin by, for example, inhibiting its production using antisense RNA or by introducing elicitin genes into nonproducers.

In the interaction between *P. infestans* and potato, a differential cloning approach is being used to identify genes encoding putative pathogenicity factors. Pieterse et al. (1993; 1994a,b) identified several families of genes that are preferentially expressed during infection by hybridizing cDNA probes from infected leaves and from in vitro cultures to genomic libraries of *P. infestans*. Several of the in planta-induced genes were shown to encode known products, such as polyubiquitin and calmodulin, while the function of others remains to be determined. This latter class included a family of genes *(ipiB)* encoding glycine-rich proteins and a second family *(ipiO)* encoding a small, possibly secreted protein with a putative cell attachment sequence motif (arg-gly-asp). The roles of these gene products are currently being examined using biochemical and molecular genetic methods.

EPILOGUE

Oomycete fungi were among the first recognized plant pathogens. It is satisfying to see that the pace of progress towards understanding these important and destructive species is now accelerating, aided by the development of new and improved methods for genetic analysis. Undoubtedly, many genes important in the determination of host specificity and pathogenicity will be isolated from oomycetes in the very near future. At the same time, advances are being made in complementary studies in the plant hosts of oomycetes of resistance genes, defense responses, and other key components of plant-microbe interactions. As our knowledge of both host and pathogen improves, it will be exciting to learn if oomycetes have evolved strategies for colonizing their hosts that differ from those used by other fungi which have little taxonomic affinity with oomycetes.

Many areas of current investigation in oomycete biology were not covered by this review, which focused on issues and technologies directly related to plant-microbe interactions. However, several of these other topics, such as those involving the analysis of mating, differentiation, and fundamental aspects of cell biology such as protein secretion, are relevant to the ability of oomycetes to excel as pathogens. An improved comprehension of the basic biology of oomycetes will certainly contribute to our understanding of their pathology, and ultimately to the development of strategies for controlling the many economically important and devastating diseases of plants that they cause.

ACKNOWLEDGMENTS

I am grateful to my colleagues in the oomycete world for supplying helpful comments and information that aided the preparation of this review. This work was supported by USDA grant 93-37303-9654 and NSF grant MCB-9405179-001.

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