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VIRUSES OF FUNGI

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INTRODUCTION

Fungi are an important part of the ecoworldsystem we live in. They cause diseases in man, animals, and plants.But some fungi also serve useful functions: they produce food and chemicals and assist in the decomposition of waste products.

Few kinds of organisms are immune to viruses. Even microorganisms like bacteria and fungi are subject to their attack. Viruses in fungi probably have existed for a long time but they were discovered only recently. The viruses that occur naturally and multiply in fungi are commonly called as mycoviruses. These are distinguished from other non-fungal viruses which are occasionally associated with certain fungi.

Only salient features of the mycoviruses are presented here to give an overview of this new discipline. Readers interested in additional details should refer to other reviews (BOZARTH, 1972; HOLLINGS and STONE, 1971; LEMKE and NASH, 1974; LEMKE, 1976; SAKSENA, 1977; SAKSENA and LEMKE, 1978).

DISCOVERY

Between 1936 to 1961, several observation were made on abnormal growth habit or unusual biological activity of certain fungi. These were: anomolous lysis in, and an infectious disorder of, yeast cultures; diseases of two mushrooms, *Agaricus bisporus* and *Laccaria laccata;* "stunted" colonies of *Helminthosporium victoriae;* symptoms of "vegetative death" in *Aspergillus glaucus;* the "senescence" symptoms in *Podospora anserina*, as well as the antiviral activity of the culture filterates from *Penicillium funiculosum* and *Penicillium stoloniferum.* Although some of these abnormalities were suspected to be viral in nature, it was only after viruses were found associated with the diseased mushrooms, *Agaricus bisporus* (HOLLINGS, 1962) that their existance in fungi was confirmed. Subsequently, viruses containing double-stranded RNA (dsRNA) were found in *Penicillium* species and, since then, viruses or virus-like particles (VLPs) have been recognized in numerous fungi in all taxonomic classes (SAKSENA and LEMKE, 1978).

GENERAL PROPERTIES

The list of mycoviruses or VLPs is already a long one but only a few of them have been well characteried. Detailed studies of mycoviruses have generally been hampered due to difficulties in their isolation and purification, complicated further by their multicomponent nature or presence of multiple species of virus in a host. Nevertheless, for certain mycorivuses ample information has accumulated, and it is apparent that they share several common features. They are generally isometric particles, 25-50 nm in diameter, and contain segmented dsRNA genome; consequently, they are often multicomponent systems exhibiting centrifugal and density heterogeneity.

Among the mycorivuses well characterized biophysically and biochemically are *Agaricus bisporus* viruses, *Aspergillys foetidus* viruses, *Penicillium chrysogenum* virus, *Penicillium brevicompactum* virus, and *Penicillium stoloniferum* viruses. Some others, although not as extensively characterized, also reveal properties typical of dsRNA mycoviruses. These include, *Gaeumannomyces graminis* virus, *Helminthosporium maydis* virus, and *Thielaviopsis basicola* viruses (SAKSENA and LEMKE, 1978). Still other mycoviruses or VLPs are recognized solely on the basis of electron microscopic observations of crude extracts from, or ultrathin sections of, host fungi. These particles, of course, need further confirmation as to their true identity or nature.

More than one tupe of virus particle can occur in certain fungi. For example, at least three morphologically distinct particle types (two sphericals, 25-27 nm and 34-36 nm and a bacilliform 19 x 50 nm) are commonly found in diseased mushrooms, *Agaricus bisporus* (DIELEMAN-VAN ZAAYEN, 1972; HOLLINGS and STONE, 1971; SAKSENA, 1975). Viruses in *Aspergillys foetidus* (BANKS et al, 1970) and in *Penicillium stoloniferum* (BUCK and KEMPSON-JONES, 1970) each represent a complex of two serologically distinct, but morphologically similar, isometric virus particles. In these cases, the two viruses can be distinguished as fast or slow form by their respective electrophoretic mobility. Evidently, the two forms can occur together and can replicate independently without any genotypic or phenotypic mixing.

BIOLOGICAL-SIGNIFICANCE

Natural transmission of mycoviruses from infected to normal cells commonly occurs by hyphal anastomosis (plasmogamy) between cells, or by heterokaryosis involving fusion of genetically compatible cell lines. Viruliferous spores aid in survival and dissemination of viruses in fungal populations.

Although virus infection in fungi occurs with relative ease in nature, artificial transmission of mycoviruses is rather difficult and inefficient. Infectivity of purified mycovirus particles has not been clearly demonstrated yet, probably because of their extreme sensitivity to inactivating agents or simply due to ineffective techniques. Use of isolated proto-

plasts for viral uptake and transmission is erratic but a protoplast fusion technique recently used to transmit *Pyricularia oryzae* virus from infected to healthy strains seems promising (BOISSONET-MENES and LECOQ, 1976). Viruses of *Aspergillus niger* and *Penicillium stoloniferum* have been transmitted to *Saccharomyces cerevisiae* by incubating compatible haploid yeast cells (BORDER, 1972; LHOAS, 1972).

Despite high concentration, most mycoviruses remain latent, and generally exhibit no overt affects on their hosts. There are few exceptions, however. Viruses of cultivated mushrooms, *Agaricus bisporus* are highly virulent and pathogenic and are clearly detrimental. Disease sumptoms include stunted, deformed mushrooms and slow degeneration of mycelium in compost (DIELEMAN-VAN ZAAYEN, 1972).

Several phytopathogenic fungi, including *Helminthosporium victoriae* (victoria blight of oats), *Helmintohosporium maydis* (southern corn leaf blight), *Puccinia graminis* (wheat rust), *Ustilago maydis* (corn smut), *Endothia parasitica* (chestnut canker), and *Gaeumannomyces graminis* (root rot of wheat) etc., contain viruses but their precise role in fungal pathogenecity is unclear. In some instances, viruses seem to have no effect at all; in others, still unconfirmed, some reduction of pathogenecity is indicated. Obviously, because of inconsistencies in correlation of virus with decreased pathogenecity, any possible use of mycoviruses in biological control of these pathogenic fungi seems unlikely at present.

The antiviral activity of the culture filterates from certain *Penicillium* molds has been associated with virus particles and identified to be due dsRNA (BANKS et al, 1968; KLEINSCHMIDT et al, 1968; LAMPSON et al, 1968). Virus particles, capable of inducing interferon in animals, have been recognized in several other fungi. Evidently, both intact particles and virus derived dsRNA are active inducers of interferon.

A cytoplasmically inherited "killer system" exists in *Saccharomyces cerevisiae* (MAKOWAR and BEVAN, 1963) and in *Ustilago maydis* (PUHALLA, 1968) and the three phenotypes e.g. killer, sensitive, and neutrals are recognized on the basis of their capability to produce, or to react with, the killer toxin. The killer strains release a protein toxic to sensitive strains but they themselves are immune to this toxin. Neutral strains do not produce toxin but are immune to killer toxin. Isometric virus particles containing dsRNA genome segments have been found both in *Saccharomyces cerevisiae* (ADLER et al, 1976; HERRING and BEVAN, 1974) and in *Ustilago maydis* (WOOD and BOZARTH, 1973; KOLTIN and DAY, 1976), and there seems to be a correlation between the presence of certain dsRNA species and the killer or immune responses.

Other biological implications of mycoviruses or viral dsRNA include their possible role in the production of secondary metabolites in their hosts or in conditional, asynchronous lysis in certain fungi. Many fungi that produce secondary metabolites like antibiotics (penicillin) and toxins (aflotoxin) also contain viruses but it is nor clear what, if any, role the viruses play in the production of these compounds. Unlike killer systems, where toxin production is apparently influenced by viruses or dsRNA species, production of

secondary metabolites in some fungi may indeed be inversely related to the virus titer. For example, strains of *Penicillium stoloniferum* and *Penicillium brevicompactum* that produce mycophenolic acid (an antiviral compound) apparently do not contain viruses. However, these interactions are not yet clearly defined and need further studies.

ULTRASTRUCTURAL FEATURES

The majority of mycoviruses are isometric but occassionally other particle types have also been encountered (SAKSENA, 1977). This morphological diversity is represented in bacilliform particles, rigid rods, flexuous rods, herpestype particles, and bacteriophage-like particles.

Only few ultrastructural studies on intracellular distribution of mycoviruses have been done but a general pattern seems to emerge. Viruses of *Agaricus bisporus* seem to accumulate in all parts: vegetative mycilium, fruiting bodies, and spores (DIELEMAN-VAN ZAAYEN, 1972). Likewise, viruses of *Penicillium chrysogenum* (YAMASHITA, et al, 1975) and *Penicillium stoloniferum* (HOOPER et al, 1972) occur in mycelium and in spores and often agregate in cytoplasm or in vacuoles, occasionally in linear crystalline arrays. Evidently, most mycoviruses can multiply, and can reach substantial concentration, in host cells without causing any obvious damage.

The ultra structural studies of a herpes-type virus described from *Thraustochytrium* species (KAZAMA and SCHORNSTEIN, 1973) reveal general features and replication cycle similar to that of known herpes viruses.

REPLICATION

Association of RNA-dependent RNA polymerase activity has been reported in *Aspergillys foetidus*, *Penicillium stoloniferum* and *Penicillium chrysogenum*. Detailed studies with *Penicillium stoloniferum* (slow) virus (PsV-S) show that product of PsV-S polymerase activity is a dsRNA that remains within virus particle. The RNA-polymerase of PsV-S can copy both strands of dsRNA and, thus, acts essentially as a replicase. Accordingly, it has been proposed that dsRNA mycovirus replication may simply involve a particle associated duplication of dsRNA which is then encapsidated in two separate particles; that is, a duplicate particle is produced for each originally presente (BUCK and RATTI, 1975). This novel concept is very different from the replication cycle of other known dsRNA viruses (WOOD, 1973) but seems ideal for mycoviruses which remain latent and replicate indefinately without cellular lysis. Alternative mechanisms might exist for other dsRNA mycoviruses and future studies of single-stranded RNA - and DNA mycoviruses would possibly reveal other models.

OTHER VIRUSES

In addition to mycoviruses - the *bona fide* viruses of fundi-association of other viru-

ses of plant, animal, or bacterial origin with some fungi is now well known. For example, several plant viruses are naturally transmitted through fungi that serve merely as vectors without supporting virus multiplication (GROGAN and CAMPBELL, 1966). Also, experimental infection of fungi with other plant or animal viruses suggest that alien viruses may persist and perhaps even multiply in their surrogate host. Presence of yet another group of viruses - the *Penicillium*-derived bacterial viruses (PBVs)-has been reported (TIKCHONENKO et al, 1974) but their exact relationship with host fungus is still unclear.

CONCLUSIONS

The subject of mycoviruses is relatively new. Fortunately, from the very beginning, fungal virology assumed an interdisciplinary approach; consequently, significant knowledge has been gained about some mycoviruses. However, so much more remains to be learned about others and scientists continue to seek answer to many puzzling questions as: How are mycoviruses transmitted and maintained in fungal populations? Why are majority of the mycoviruses latent and why, despite their heavy concentration, generally they do not lyse the cells? How do they replicate? Does virus infection favorably affect antibiotic production and if so, could this feature be experimentally exploited? Do mycoviruses, of phytopathogenic fungi play any role in development of plant diseases as well? Can mycoviruses be used in biological control of pathogenic fungi? Future research in mycovirus should elucidate not only these aspects but also give a better understanding of the complexity of interactions between mycoviruses and fungi. Indeed, mycoviruses, because of their many unique features, make an excellent system for experiments in cell biology of an eukaryotic microbial system.

SUMMARY

Viruses in fungi are widespread. They are generally latent and rarely cause overt affects; nevertheless, they profoundly influence many biological activities of their hosts.

Natural transmission of mycoviruses occurs through plasmogamy or heterokaryosis but mechanical transmission is very difficult and inefficient.

Most mycoviruses share some common features. They are commonly isometric particles and contain dsRNA genome that is segmentally encapsidated.

RESUMO

Virus são largamente distribuídos nos fungos. São geralmente latentes e raramente têm efeitos evidentes; todavia, influenciam produndamente muitas atividades biológicas dos seus hospedeiros.

A transmissão natural dos micovirus ocorre através da plasmogamia ou da heterocariose, mas a transmissão mecânica é muito difícil e ineficiente.

A maioria dos micovirus apresentam alguns caracteres comuns. São comumente partículas isométricas e contém o genoma dsRNA que é segmentalmente encapsulado.

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