TAXONOMY AND DISTRIBUTION OF FUNGI ISOLATED FROM BEACH RIDGE AND MARSH SOIL AT DELTA, MANITOBA

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by

Joseph William Pearn

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JOSEPH WILLIAM PEARN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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For Gloria

Friend
Lover
Mother

ABSTRACT

Fungi were isolated from the 0-30 cm profile of beach ridge soil and the 0-10 cm profile of marsh soil, Delta Marsh, Manitoba. A soil washing technique followed by dilution plating on four different culture media each at four different incubation temperatures, was employed to maximize the range of fungi recovered.

A combined total of 109 species from 43 genera of fungi, were isolated. Thirty-nine of 81 beach ridge fungi and 28 of 68 marsh species were site specific; the remaining 42 species were common to both soils.

A large proportion of the fungi isolated belong within eight predominant genera; Acremonium (Cephalosporium), Chrysosporium, Cylindrocarpon, Fusarium, Paecilomyces, Penicillium, Trichoderma, and Verticillium or are representatives of the Phycomycetes or Sphaeropsidales.

Sixty-five of the species isolated have not been previously reported from Manitoba soil. Eleven previously undescribed species, including five placed within the genus Cylindrocarpon, and eight variants of known species, were among the fungi isolated.

Soil depth, culture media, and incubation temperature influenced the number and species of fungi recovered. The propagule number for beach ridge soil was greatest in the 10-20 cm profile, followed by the 20-30 cm and the 0-10 cm profiles. The most frequently occurring species were recovered throughout the 0-30 cm profile. Seventy-seven percent of all beach ridge fungi were isolated from the 10-20 cm profile; 72% and 62% of all fungi were isolated from the 0-10 cm and 20-30 cm profiles respectively. This atypical distribution pattern was thought to be a result of differences in organic content, moisture, and temperature within the undeveloped 0-30 cm beach ridge soil profile.

OAES culture medium recovered the largest number of propagules from

both beach ridge and marsh soils. The greatest diversity of species also resulted from the use of OAES medium. This was attributed to its effect on the nutrition and growth of fungi; discrete and slower growing mycelium on OAES allowed for more complete isolation of fungi compared to other culture media.

The overall effect of temperature on the number of propagules and diversity of fungi isolated was not consistent for beach ridge and marsh soils. The largest number of propagules from beach ridge soil was recovered on all media at lower incubation temperatures (10° C and 15° C). No such effect was observed for marsh soil. Species diversity was greatest at high incubation temperatures (20° C and 25° C) for beach ridge soil, but was greatest at a lower incubation temperature (15° C) for marsh soil where all but the lowest temperature produced similar results. The lowest incubation temperature (10° C) recovered the smallest complement of species from both soils.

Both incubation temperature and culture media were observed to affect the recovery of specific fungi from both soils. While the most frequently occurring fungi were recovered on all culture media at all incubation temperatures, some species were restricted in occurrence by culture media or incubation temperature. Six species appeared to be restricted by low incubation temperatures (10° C or 15° C) and four species by higher temperatures (20° C or 25° C), but they were not restricted by media. Ten species were restricted in appearance by culture media, but not incubation temperatures.

Variations exist in the composition and frequency of species from beach ridge and marsh soils, compared to similar soil types. While other dune-type soils contain the same dominant genera as beach ridge

soil, major differences exist in the composition and frequency of species within the genera *Trichoderma*, *Fusarium* and *Penicillium*.

Eight of the 12 most frequent marsh soil species are uncommon to other highly organic near-neutral soils. Most of the dominant marsh soil fungi are known cellulose decomposers. Two groups of fungi commonly present in organic soils, *Gliocladium* spp. and sterile mycelial forms, were absent in marsh soil. It is possible that a greater disruption of marsh soil before preparation of dilution plates is required for the isolation of sterile mycelial forms.

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INTRODUCTION

AND

LITERATURE REVIEW

I. Microfungi in Canadian Soils

The occurrence of fungi on all manner of substrates is well known. While the role of fungi in soil has received considerable attention, only limited research has been conducted on the ecology of fungi from Canadian soils with just sixteen papers published. Bisby et al (1933, 1935), Kendrick (1962), Reddy and Knowles (1965), Morrall and Vanterpool (1968), Vaartaja (1968), Bhatt (1970), Widden and Parkinson (1973), and Widden (1979), surveyed a variety of agricultural and undisturbed natural soils from the temperate regions of Canada. Ivarsen (1965) and Widden and Parkinson (1979) surveyed Arctic tundra soils; Bissett (1975) and Bissett and Parkinson (1979 a, 1979 b, 1979 c) examined Alberta alpine soils. Bissett and Parkinson (1980) also investigated a subalpine soil.

Only two of these studies (Bisby et al 1933, 1935) deal with microfungi from Manitoba soils. The present study was designed to expand our knowledge of microfungi in two unique and previously uninvestigated Manitoba soils. The site chosen for this research was the University of Manitoba Field Station, Delta Marsh, Manitoba. Located at the southern tip of Lake Manitoba, the Field Station serves as a research facility for a wide range of studies in marsh ecology. The marsh ecosystem, with its varied and productive communities, contains soils which are likely to contain unique and diverse fungal populations.

The soils selected were from two distinct and different habitats: (1) a sandy loam from the beach ridge separating the marsh from Lake Manitoba; and (2) an organic muck from the *Phragmites* habitat of the marsh. Although the mycoflora of soils from these types of communities has not been investigated in Canada, surveys of the microfungi of soils

of a similar type, i.e. neutral to alkaline organic or sandy soils, have been conducted in a variety of other countries. Organic soils surveyed include those from a British (England) fen (Stenton, 1953), an Iowa (U.S.A.) forest (Taber, 1951), a Wisconsin (U.S.A.) cattail marsh (Tews, 1970, 1971), a British (England) salt marsh (Turner and Pugh, 1961; Pugh, 1972; Pugh, 1963), and the Florida Everglades, U.S.A. (Wallace and Dickinson, 1978). Sandy soils are represented by British (England) coastal dunes (Brown, 1958; Pugh, 1963; Pugh et al, 1963), Lake Michigan (U.S.A.) sand dunes (Wohlrab et al, 1963; Wohlrab and Tuveson, 1965), and Wisconsin (U.S.A.) willow-cottonwood lowland soil (Gochenaur and Whittingham, 1967).

A variety of approaches has been used to examine the mycoflora of soils depending on the type of information desired, i.e. taxonomic survey, seasonal variation, decomposition of litter, isolation and identification of fungi from distinct soil horizons, etc.. A common approach is to isolate the soil fungi and determine the species composition and frequency at various soil depths. This study takes such a survey approach and includes additional information on the numbers of fungi, taxonomy of undescribed or unusual species, and new reports of fungi from Manitoba soils.

2. Isolation Technique

Over the past eighty years, successive methods have been developed to overcome problems related to accurate and representative isolation of fungi from soil. Such problems are directly related to the fact soil is a complex heterogenous environment containing a mixed population of fungi in a number of forms (either mycelial or reproductive propagules); these can be active or inactive and have a variety of roles and

nutritional requirements (Garrett, 1951). Garrett (1955) noted that isolation techniques are selective; each has its own limitations and biases. Since no one method can yield a reliable picture of the total fungal activity in soil (Watson, 1970), workers must design and interpret their research to reflect this fact. Accurate and comparative information can be obtained, but only within the limits of the survey parameters and method(s) employed (Parkinson et al, 1971).

In a preliminary survey, a major objective should be to isolate as many different and representative species as possible from the total spectrum of soil microfungi. Hopefully this should in turn produce representative "population" data.

This objective can be accomplished by two general approaches. The first employs several different isolation methods simultaneously, each with its own bias and limitations, and compares the results. This approach is most often used when specific information about the role or activity of soil fungi is desired (Warcup, 1957; Chesters and Thornton, 1956; Parkinson and Thomas, 1965). The time, labour and laboratory facilities required by this approach generally makes it impractical for survey studies, although it has been employed by a number of workers (Brown, 1958; Sewell, 1959 c; Chou and Stephen, 1968).

A more common approach uses the isolation method judged to have the least degree of bias regarding the survey parameters of isolating and enumerating soil fungi. The reliability of this approach can be increased by controlling variables which select against the isolation of certain fungi, and by reducing the inherent bias in the methodology wherever possible.

A modified soil wash technique (Watson, 1960), combined with soil dilution plating or soil plating of washed soil (Warcup, 1950), utilizing four different media at four different incubation temperatures, was adopted for this study. This approach was adopted because the method's bias will not likely detract from the results, therefore yielding reliable and comparable qualitative and quantitative data while still remaining manageable.

(a) Soil Wash Technique

The soil dilution plate method originally designed for the isolation and study of soil bacteria and modified for fungi (Waksman, 1927) was, until recently, the most common method employed in studying the nature and number of soil fungi (Parkinson et al, 1971). The defects and sources of error of this method were reviewed by Brierly et al (1927) and consequently it has been modified by subsequent workers in an effort to obtain the best possible results.

A major defect is the significant advantage given to abundantly sporing species. These species, which predominate on soil dilution plates, are usually overrepresented in population estimates (Warcup, 1950; Parkinson et al, 1971). This likely bears little resemblance to the mycelial density of a particular species in soil because differences often exist in conditions required for the development of spores and mycelium (Hawker, 1950). In contrast, Warcup (1955 a) demonstrated that the mycelial component on soil dilution plates represented a group of often slow-growing sterile species. These mycelial species are underrepresented on soil dilution plates because of their inability to compete with faster growing species, and the smaller number of propagules

relative to sporulating species (Warcup, 1955 b, 1957).

Warcup (1950) introduced the soil plate technique for the direct isolation of fungi from soil. A larger number of species was isolated by this technique than from soil dilution plates from the same soil. Warcup attributed this to the loss of mycelial species attached to soil particles during successive soil dilutions; a conclusion supported by Cohen (1950). An examination of the lack of representation among soil isolates of species originating from mycelial propagules was conducted by Warcup (1955 b, 1957). In an effort to separate the active mycelial component from dormant or inactive spores, he plated hyphae or washed soil particles containing hyphae, directly onto an enriched agar medium. While the direct isolation method removed a large group of mostly sterile species missed by soil dilution plates, or soil plates, it suffers from a practical disadvantage in that it fails to isolate heavily sporing species found on soil dilution plates or soil plates (Williams et al, 1965). This feature limits the usefulness of this technique in surveys of soil fungi.

A number of other methods for direct isolation or observation of soil fungi has also been developed, including direct inoculation (Waksman, 1916), buried slide (Rossi, 1928; Cholodny, 1930; Ziemiecka, 1935; Isakova, 1938), immersion tube (Chesters, 1940, 1948), agar film (Jones and Mollison, 1948), and soil sectioning (Burges and Nicholas, 1961). However, all have a particular bias or selectivity which makes them unsuitable for soil surveys using a single method for the isolation, identification and enumeration of fungi.

The development of washing techniques, first applied to roots and organic particles (Simmonds, 1930; Kurbis, 1937; Glynne, 1939; Chesters, 1948; Robertson, 1954; Harley and Waid, 1955) and, later to

soil (Watson, 1960), proved to be an effective means of separating the vegetative and reproductive phases of soil fungi prior to plating on enriched culture media. The distinction and separation of these forms has been stressed by many researchers as important to our understanding of soil ecology (Chesters, 1949; Garrett, 1955; Harley and Waid, 1955; Chesters and Thornton, 1956; Sewell, 1959 c).

The soil wash method uses serial washings to remove most of the spores from soil and permits separate dilution plating of spore-laden wash-water and washed soil. This has the effect of reducing competition, resulting in a more realistic and complete picture of soil fungi compared to other methods. Watson (1960) and others (Parkinson and Williams, 1961; Williams et al, 1965; Parkinson and Thomas, 1965) report that serial washing of soil yields more genera of fungi (especially those originating from mycelium), a larger number of rare fungi, and more soil-borne pathogens than the soil plate or soil dilution methods when applied to the same soil. These advantages make this method a good choice for studies of a survey type.

The modifications of Watson's soil wash method for this study include an increase in the quantity of soil washed; fewer washings but with longer washing and settling times; and the use of a round-bottomed rather than a flat-bottomed, washing flask. Washing of larger volumes of soil than those employed by Watson, has been demonstrated to produce a greater revelation of the fungal composition of soil (Lisina-Kulik and Moiseeva, 1971). The washing and settling times employed fall within the acceptable limits for spore removal and still yield good species diversity for plated washed soil (Watson, 1960; Lisina-Kulik and Moiseeva, 1971). It was observed in preliminary tests that superior

washing action was generated by use of round-bottomed, as opposed to flat-bottomed, flasks.

(b) Dilution Technique

The wash water from both the sandy-ridge soil and organic-muck was diluted and plated in the manner described by Watson (1970). However the washed soil received differential treatments. The organic-muck received the standard soil dilution plate treatment while soil plates (Warcup, 1950) were prepared from the washed sandy-ridge soil. It is recognized that highly mineral soils are best prepared as soil plates because of excessive particle settling during serial dilutions (Brown, 1958; Montégut, 1960; Wohlrab et al, 1963).

Replicate dilution plates were prepared from a number of dilution sets rather than a large number of replicates from a single dilution set. This technique was demonstrated to improve the accuracy of the dilution plate method (James and Sutherland, 1939). Use of the Menzies' (1951) "dipper" helped prevent settling of soil particles during serial dilution preparation and sample removal. Soil particle suspension was further aided by the use of a 1% carboxy methyl cellulose solution as a diluent.

The final dilution for all plate counts was chosen to produce an approximate average of 25 colonies per plate as suggested by Bisby et al (1933) and recommended as statistically valid by James and Sutherland (1939). This produces plates which are relatively easy to count and should reduce competition and antagonism created by the use of higher density plates (Garrett, 1951).

(c) Culture Media

One of the most significant problems for the mycologist studying soil mycoflora is the selective growth and development of fungi after plating on a nutrient medium. Martin (1950) pointed out that culture media must be altered to promote the growth of the greatest possible number and variety of soil fungi; the latter being relatively less numerous in soil than bacteria and actinomycetes. The specific nature of the alterations falls into two broad categories: the addition of inhibitory chemicals which suppress either the development of bacteria and actinomycetes or the growth rate of certain fungi; and the addition of materials, usually specific nutrients, which promote the growth of fungi.

Acidification of culture media was the earliest modification attempted to suppress the growth of bacteria in mixed cultures (Waksman, 1922; Jensen, 1931; Tyner, 1944). The resulting reduction in numbers of pathogenic fungi led to the investigation of other possible suppressant chemicals. Smith and Dawson (1944) introduced the use of rose bengal, a bacteriostatic agent which reduced fungal spread and prevented actinomycete growth in culture. Streptomycin and crystal violet were subsequently shown to be effective inhibitors of bacterial growth (Littman, 1947). Culture media containing these two agents, combined with oxgall, were bacteria free and capable of supporting a full range of discrete nonspreading colonies of saprophytic and pathogenic fungi (Littman, 1947). The colonies failed to spread because of the suppression of growth by oxgall. More recently, sodium propionate (Crook et al, 1959) and synthetic detergents (Steiner and Watson, 1965) have been used to reduce the spread of fast growing soil fungi. Martin (1950), in a review of inhibitors, recommended the use of peptone-dextrose agar

containing rose bengal and streptomycin for the bacteria-free isolation of large numbers and kinds of fungi from soil.

All culture media employed in this study incorporated oxgall to suppress the spread of fungal colonies. Crystal violet and sodium propionate were also added to two of the media. Streptomycin sulfate and chloramphenicol were used to inhibit the development and growth of bacteria and actinomycetes.

The choice of a culture medium for the isolation and growth of fungi from a mixed population such as that in soil can produce biased results unless carefully considered. It is accepted that any culture medium is selective since fungi have a variety of nutritional requirements (Martin, 1950). However, because of differential growth rates, not all fungi capable of growth on a particular medium are isolated (Smith and Dawson, 1944; Chesters and Thornton, 1956). Some genera are therefore recorded in greater numbers than their actual mycelial concentration warrants (Watson, 1960).

Sewell (1959 c) demonstrated that the isolation of particular species of soil fungi from soil plates was affected by their growth rates on the isolation medium. The isolation medium of modified Rossi-Cholodny buried slides and immersion tubes also influences the mycoflora isolated from soil (Chesters, 1948; Chesters and Thornton, 1956; Sewell, 1959 c). This phenomenon may, in part, be due to the production of growth-inhibiting metabolic byproducts by some strains of soil fungi on enriched media (Chesters, 1948; Nicot and Chevaugeon, 1949). The concentration and type of carbohydrate in the isolation medium is also known to have a strong selective effect (Chesters, 1948; Cohen, 1950; Garrett, 1951).

Despite these problems most studies use a single culture medium for the isolation of soil fungi. The most commonly employed medium is Czapek-Dox or Czapek-Dox with yeast extract, a "broad spectrum" medium. In selecting a culture medium Parkinson et al (1971) suggest three broad groups be considered: soil extract based media; media containing peptone or a similar nitrogen source; and synthetic media. Since it was desirable to reduce the selective effect of culture media, four culture media were selected. Three of these are in the categories suggested by Parkinson et al, i.e. soil extract agar, Litmans crystal violet agar (Litman, 1947), and Ohio Agricultural Experimental Station agar (Williams and Schmitthenner, 1958). The fourth medium is a common, much used medium, potato dextrose agar. Williams and Schmitthenner (1958) have demonstrated these media are effective in the isolation of a broad range of soil fungi.

(d) Incubation Temperature

The incubation temperature of isolation plates has received only rare consideration as an operative variable in the isolation of fungi from soil (Dickinson and Kent, 1972). The incubation temperature selected is usually at or around room temperature (20° C - 25° C) and is rarely related to environmental temperatures. Panasenko (1967) notes that most soil fungi are mesothermotolerant and have a developmental temperature range of 5° C to 35° C with an optimum of 20° C to 25° C. This suggests that studies using this 20° C to 25° C incubation range are within the temperature requirement for development of most soil fungi. However, it is recognized that not all soil fungi have the ability to grow at the same rate at the same temperature on the same medium (Dickinson and Kent, 1972).