METHODS in MICROBIOLOGY

Edited by

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METHODS in MICROBIOLOGY

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PREFACE

Microbiology involves or impinges upon the study of all micro-organisms although for many years it has become, at least in practice, almost a pseudonym for bacteriology. Yeasts, of course, have been included but the fact that they are fungi has generally been ignored. As a mycologist therefore it gives me considerable pleasure to present a book on Methods in Mycology in a general microbiology Series.

To many, fungi has meant toadstools or things growing on wood whether trees or structural timbers. A specialized few have been involved with their economic importance as pathogens of plants and animals including man, and others in mould deterioration and biodegradation problems. With the onset of the use of fungi for antibiotic production interest has continued to increase and they are now used as a source of protein, of growth promoting substances and enzymes. Their use in bioassay work and for various biochemical syntheses has also expanded. All this has introduced, to a much wider spectrum of workers from other disciplines, an interest in the moulds, their use and cultivation.

The aims and outlines of this Series on Methods in Microbiology has been given in the preface to Volume one and there is no need to reiterate them here. This Volume is intended both as a reference manual and also as an introduction to workers from other fields to the methods used in mycological studies. The work has been planned to cover general methods and media, examination techniques and preservation. Fungi from certain natural groups and the specialized techniques for collecting, isolation and cultivation of these are outlined in specific Chapters. These special Sections also include lichens, slime moulds and the Actinomycetes. These three are not true fungi, the lichens being an association of algae and fungi, but because the methods of investigation are similar to those used for the true fungi they are included here for convenience.

Some of the groups dealt with are based on ecological rather than taxonomic considerations: Thus the special methods required for handling aquatic fungi, soil fungi, dermatophytes and air spora are described in specialized Chapters. The final Section is an introduction to the methods involved in a study of the physical, biochemical and genetic aspects.

Throughout this book where some methods are similar to those used in bacteriology it is the mycological aspect of the method that has been stressed.

My thanks are due to the contributors for their helpful collaboration in producing the Volume and to the staff of Academic Press for their thoroughness and care in preparing the material for press.

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CHAPTER I

Introduction to General Methods

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I. INTRODUCTION

Fungi have a part in the cycle of degeneration of almost all organic matter. They cause spoilage of food-stuffs and occur as human, animal and plant pathogens. Under humid conditions, they can derive sufficient subsistence from inorganic matter or from surface algae to grow over or through many manufactured products. Thus they often cause damage by short-circuiting electronic equipment, by the etching of glass in optical equipment, and damage pictures or decorated surfaces by mould growth. Various chemicals such as standard hypo and the paraffins used as fuel in jet aircraft support fungal growth. Industrial material such as wood pulp is spoilt by the presence of blue-staining fungi and all timber subjected to damp has to be preserved against fungal attack or frequently replaced.

Before studying the effects of fungal attack it is usually necessary to isolate the fungus both for the purpose of identification and in order to determine its growth requirements and the by-products of its metabolism. The methods used for the isolation of fungi and for their cultivation depend largely upon their environment in nature. Methods which are applicable to the cultivation of the common moulds and other fungi imperfecti, which are for the most part conidial states of ascomycetes, are described in this Chapter. Details of methods of isolation and cultivation from more specific groups and from habitats such as soil, water, human and animal tissues, are described in later Chapters under the respective headings.

For the purposes of isolation, fungi can be roughly divided into the parasitic and saprophytic species, although it should be fully understood that this distinction is by no means clear-cut. There is a close relationship between the genetic complement, the health and environment of the host, and the parasitism of many fungi. This may be a question of merely a mechanical barrier in the hosts' protective mechanism preventing access of the fungus. Such protection may be greatly reduced or destroyed following mechanical injury of the host.

In general it is easier to isolate the saprophytic moulds than specific animal or plant pathogens. In either case, isolation is made easier if the fungus is producing either sexual or asexual fructifications so that the isolation can be made from single spores. This makes purification easier and simplifies later handling and identification of the fungus.

II. ISOLATION

A. Isolation from plant tissue

In the case of fungi which cause cankers or necrotic lesions in plants, the problems of isolation are chiefly concerned with separating the disease-

causing organism from the many saprophytic species which frequently invade necrotic tissue behind the advancing front of the parasitic mycelium. Similarly fungi invading the vascular tissue of the host plant have to be isolated from the mass of saprophytic species which cover the surface of the host. Other fungi which produce fructifications on or below the surface of the host can be cultured by isolating the spores or part of the fructification such as conidiophores or sometimes from part of the perithecial wall.

Isolation from diseased or infected tissue, therefore, may be considered under two headings: those based on plating out infected host tissue and those based on obtaining spores, mycelium or other fungal tissue from the host or substratum as a starting point for their cultivation.

B. Host tissue transplants

Basically these depend upon the elimination of surface contamination. The general procedure is to remove small pieces of infected tissue from the host. In the case of parasitic species these should be taken from the growing front and not from the necrotic area behind. That is, from the edge of the apparently sound host tissue where it meets the obviously diseased tissue of the lesion. Surface contaminants are removed and the tissue is placed on an agar plate under sterile conditions. With some material where the pathogen is deep-seated, it is possible to remove surface contaminants by slicing off a thin layer of host tissue. With other material such as potatoes it is possible to use the infected tissue as a graft by placing it in a cut made in a healthy tuber. The healthy tissue is often more quickly invaded by the pathogen than by the secondary organisms and it can be isolated from the fresh host tissue after surface sterilization.

The most common means of eliminating surface contamination is by surface sterilization. One of the simplest non-toxic means of reducing surface contamination is by prolonged washing coupled with some form of agitation. It is preferable to use a flask or suitably-sized jar for this washing process so that the material is violently agitated by the inflowing water (Fig. 1). The large outlet is covered with a strainer.

Although this method does not produce surface sterility it does readily remove most surface contaminants and is particularly useful when dealing with species parasitizing the surface tissue and with Phycomycetes and other species which are particularly susceptible to toxic chemicals. Harley and Waid (1955) described their method for the serial washing of roots in their study of root surface fungi. They used distilled water in a series of sterile boiling tubes. Williams (1963) used Perspex boxes fitted with stainless steel sieves of graded sizes for the serial washing of soil. For a controlled method of both washing and surface sterilization one is directed to the somewhat elaborate apparatus used by Slankis (1958). With fungal species