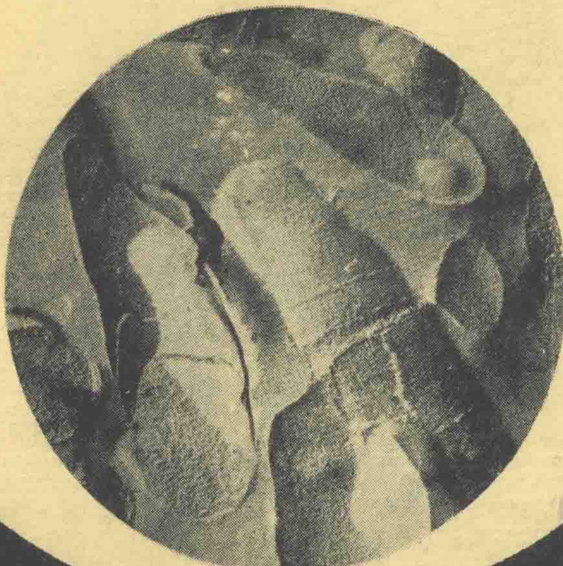
METHODS IN MICROBIOLOGY



VOLUME 9

Edited by

J. R. Norris

METHODS in MICROBIOLOGY

Edited by

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PREFACE

With the publication of Volume 8 of "Methods in Microbiology" the editors decided that they should not proceed immediately to gather material for further Volumes but that it was appropriate to wait a little and re-assess the developing field of microbial technology from time to time. During the intervening period, we have been approached by authors offering contributions and we have received a number of comments concerning areas which have not so far been adequately covered in the Series. These persuaded us that a further Volume should be produced but Professor Ribbons, because of developing interests elsewhere, decided that he could not devote the time to the production of a further Volume. I therefore decided to go ahead with a short Volume consisting of six contributions covering a number of fields selected partly on a basis of general interest and partly on the ground that they had not so far been covered.

The importance of electron microscopy in microbiology need hardly be emphasized and two Chapters concern this subject. The Chapter by Professor Lickfield is a general treatment of transmission electron microscopy with particular emphasis on the handling of micro-organisms and is suitable for the orientation of a newcomer to the field; Dr Kay's Chapter, on the other hand, deals with an application of electron microscopy of particular interest to the specialist.

Three Chapters concern rapid or novel methods for the characterization of micro-organisms which are finding growing application in the areas of microbial ecology and diagnosis. The remaining Chapter deals with the unusual parasitic Bdellevibrios, again a subject of general interest.

It is a pleasure for me to record the friendly co-operation that I have received from authors during the preparation of this Volume and my appreciation to Academic Press for their help throughout.

Several communicants have pointed out the need for a text detailing the methods of typing applied to various groups of bacteria and, after careful consideration of the merits of a text dealing with this important subject, I have embarked on a collaborative venture with Professor Tøm Bergan, of the Microbiological Department of the Institute of Pharmacy in the University of Oslo, aimed at producing several further Volumes in the Series which will deal with specific groups of bacteria and the typing methods used for studying them. Manuscript material is at present coming in very well and I hope that a number of useful Volumes will appear in fairly rapid succession.

July, 1976 J. R. NORRIS

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CHAPTER I

Substrate Specificities of Aminopeptidases: A Specific Method for Microbial Differentiation

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I. DIFFERENTIATION BY ASSAY OF MICROBIAL AMINOPEPTIDASES

During the past few years the substrate specificities of aminopeptidases in various species of bacteria, parasites and fungi have been shown to be radically different. These differences have been used to distinguish closely related species and even strains of the same species (Huber *et al.*, 1970; Huber and Mullanax, 1969; Huber and Warren, 1975; Krawczyk and Huber, 1976; Mulczyk and Szewczyk, 1970; Peterson *et al.*, 1975; Uglem and Beck, 1972; Westley *et al.*, 1967; Lee *et al.*, 1975a; Perrine and Watson, 1975; Watson and Lee, 1974; Peterson and Hsu, 1974; Male, 1971; Muftic, 1967; Muftic, 1971). The aminopeptidase (arylamidase) procedure is based upon the enzymatic liberation of fluorescent β-naphthylamine (βNA) from a non-fluorescent L-amino-acid-β-naphthylamide. This allows quantitative and qualitative measurements as fluorescence is linear. Bacteria, fungi and parasites are differentiated on their ability to enzymatically hydrolyse a series of L-amino-acid-βNAs producing a specific, characteristic profile (see Fig. 1). Differentiation by substrate specificity of the aminopeptidases complements and sometimes supercedes morphological and cultural identification techniques. It does not suffer from data interpretational difficulties which are sometimes caused by interfering chemical constituents in electrophoretic, chromatographic, serological or phosphorescent techniques (Krawczyk and Huber, 1976). The biochemical basis of aminopeptidase identification avoids much of the time consuming growth in selective media which is a feature of identification by subjective morphological, cultural, physiological and pathogenic characteristics.

Recently aminopeptidases or aminopeptidase activity have been observed in multinucleated, pathogenic micro-organisms. Uglem and Beck (1972) were able to differentiate *Neolchinohynchus crassus* and *N. cristatus* which inhabit different parts of a fish intestine by the distinctive substrate specificities of their aminopeptidases. An interesting inverse relationship was found between the parasites' aminopeptidase activity, which is related to their pathogenicity, and that of the corresponding tissue they inhabit. Thus the strain of parasite with the lowest aminopeptidase activity inhabits the portion of the intestine which has the highest. It appears that they survive best where the combination of their own and host aminopeptidases produce an adequate supply of amino-acids (Uglem and Beck, 1972). Aminopeptidases have been observed in other parasites: *Paramecium caudatum* (Hunter, 1967), *Anisakis* species (Ruitenberg and Loendersloot, 1971) and *Ascaris suum* (Rhodes *et al.*, 1966).

Aminopeptidase activity has been routinely found in smaller, nucleated organisms. Many fungi contain aminopeptidases as shown in Table I. Aminopeptidases may well be found in all fungi, certainly they are found in fungi pathogenic for man (Lee *et al.*, 1975a), non-pathogenic fungi (Male, 1971), in the mycelium form (Lee *et al.*, 1975a), in the yeast form (Lee *et al.*, 1975a; Male, 1971; Reiss, 1971) and in basidiomycetes (Blaich, 1973a, b). In *Histoplasma capsulatum* Watson and Lee (1974) found that aminopeptidases were exclusively intracellular while Labbe, Rebeyrotte and Turpin (1974) showed the presence of extracellular aminopeptidases in *Aspergillus oryzae*. Lee, Reca, Watson, and Campbell (1975) differentiated 8 human pathogenic yeasts based upon their aminopeptidase substrate specificities. Earlier Huber and Mullanax (1969) showed the presence of aminopeptidase activity in fungi pathogenic for plants by their ability to hydrolyse various L-amino-acid-βNAs. These fungi, *Fusarium oxysporum*, *F. solani* and *F. roseum* were differentiated on the basis of the substrate specificities of their aminopeptidases. *F. solani* was the only one to hydrolyse significantly L-prolyl-βNA and L-hydroxyprolyl-βNA. The other two fungi differed less, with quantitative differences in hydrolysis

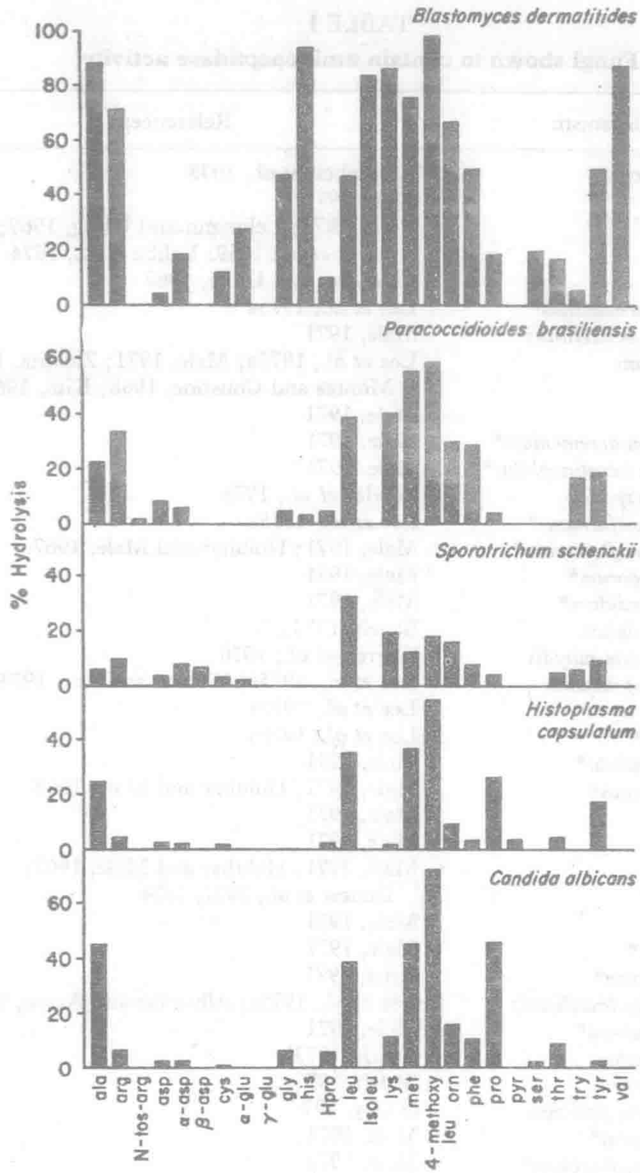

FIG. 1. Aminopeptidase profiles of five yeasts, *Histoplasma capsulatum*, *Sporotrichum schenckii*, *Candida albicans*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis*. These profiles were obtained as described above for the whole cell technique.

TABLE I

Fungi shown to contain aminopeptidase activity

Organism	References
Aspergillus flavus	Konoplich *et al.*, 1973
*A. niger**	Male, 1971
A. oryzae	Reiss, 1971; Lehmann and Uhlig, 1969; Jolles *et al.*, 1969; Labbe *et al.*, 1974
A. parasiticus	Lehmann and Uhlig, 1969
*Blastomyces dermatitidis**	Lee *et al.*, 1975a
*Brettanomyces bruxellinsis**	Male, 1971
Candida albicans	Lee *et al.*, 1975a; Male, 1971; Zaikina, 1969; Montes and Constine, 1968; Kim, 1962
*C. tropicalis**	Male, 1971
*Cephalosporium acremonium**	Male, 1971
*Chrysosporium keratinophilus**	Male, 1971
Colletotrichum species	Bender *et al.*, 1975
*Cryptococcus neoformans**	Lee *et al.*, 1975a
*Epidermophyton floccosum**	Male, 1971; Holubar and Male, 1967
*Fusarium oxysporum**	Male, 1971
*Geotrichum candidum**	Male, 1971
Hapalopilus nidulans	Blaich, 1973a, b
Helminthosporium maydis	Warren *et al.*, 1976
Histoplasma capsulatum	Lee *et al.*, 1975a; Watson and Lee, 1974
H. dubosii	Lee *et al.*, 1975a
*H. farcinosum**	Lee *et al.*, 1975a
*Kloeckera apiculata**	Male, 1971
*Microsporum canis**	Male, 1971; Holubar and Male, 1967
M. cookei	Male, 1971
*M. distortum**	Male, 1971
M. gypseum	Male, 1971; Holubar and Male, 1967; Danew *et al.*, 1971, 1974
*M. nanum**	Male, 1971
*Mucor pusillus**	Male, 1971
*Neurospora crassa**	Reiss, 1971
Paracoccidioides brasiliensis	Lee *et al.*, 1975a; Albornoz and Aasen, 1971
*Penicillium glaucum**	Male, 1971
Pleurotus ostreatus	Blaich, 1973b
Podospora anserina	Blaich, 1973c
Polysphondylium pallidum	O'Day, 1974
*Rhizopus nigricans**	Male, 1971
*Rhodotorula mucilaginosa**	Male, 1971
*Saccharomyces cerevisiae**	Reiss, 1971
S. lactis	Desmazeaud and Devoyod, 1974
Schizosaccharomyces pombe	Rock and Johnson, 1970
*Scopulariopsis brevicaulis**	Male, 1971
*Stremphylium sarcinaeforme**	Male, 1971
Thamnidium anomalum	Stout and Shaw, 1973

TABLE I—*cont.*

Organism	References
T. elegans	Stout and Shaw, 1973
*Torulopsis famata**	Male, 1971
*Trichophyton guinekeanum**	Holubar and Male, 1967
*T. granulosum**	Male, 1971
*T. interdigitale**	Male, 1971
*T. mentagrophytes**	Holubar and Male, 1967
*T. persicolor**	Male, 1971
T. rubrum	Male, 1971; Holubar and Male, 1967; Danew *et al.*, 1971
*T. schonlenii**	Holubar and Male, 1967
T. terrestre	Male, 1971
T. tonsurans	Male, 1971
*T. verrucosum**	Male, 1971; Holubar and Male, 1967
*T. violaceum**	Holubar and Male, 1967
*Trichosporon cutaneum**	Male, 1971
*Trichothecium roseum**	Male, 1971
*Trigonopsis variabilis**	Male, 1971
Tritirachium album	Hennrich *et al.*, 1973
*Verticillium cinnabarium**	Male, 1971

* Organism whose aminopeptidase activity was measured without isolation or characterization of their aminopeptidase(s). Organisms not starred are those from which aminopeptidases have been isolated.

of L-γ glutamyl-βNA, L-alanyl-βNA, L-methionyl-βNA, L-phenylalanyl-βNA and L-threonyl-βNA making their differentiation rapid and unequivocal. The substrate specificities of the aminopeptidases in some fungi are clearly related to their amino-acid growth requirement (Lee *et al.*, 1975b, c). The distinctive aminopeptidase profiles of some yeasts *Candida albiçans, Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidioides brasiliensis* and *Cryptococcus neoformans* have been successfully used to determine the N-terminal L-amino acids most rapidly liberated by the aminopeptidases of these organisms (Lee *et al.*, 1975b, c). The most rapidly liberated amino-acids are required for growth or differentiation.

As shown in the Table II aminopeptidases have been found often in bacteria. Their distinctive profiles have been used to distinguish closely related bacteria such as the *Neisseria* (Perrine and Watson, 1975), *Leptospira* (Burton *et al.*, 1970) and some *Enterobacteriaceae* (Petersen *et al.*, 1975; Petersen and Hsu, 1974). Some of this distinctive aminopeptidase activity may be related to pathogenicity or survival of the pathogen in the special environment of the host. Hence the aminopeptidase specificities may be quite distinctive in pathogens (Huber *et al.*, 1970; Perrine and Watson,