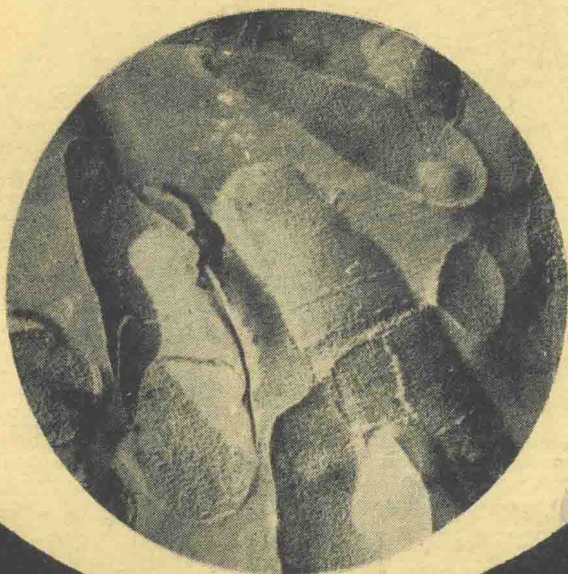


**METHODS
IN
MICROBIOLOGY**



VOLUME 9

Edited by
J.R. Norris

METHODS in MICROBIOLOGY

Edited by

J. R. NORRIS

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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 *Bdellovibrios*, 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 Tom 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

CONTENTS OF PUBLISHED VOLUMES

Volume 1

- E. C. ELLIOTT AND D. L. GEORGALA. Sources, Handling and Storage of Media and Equipment.
- R. BROOKES. Properties of Materials Suitable for the Cultivation and Handling of Micro-organisms
- G. SYKES. Methods and Equipment for Sterilization of Laboratory Apparatus and Media
- R. ELSWORTH. Treatment of Process Air for Deep Culture
- J. J. McDADE, G. B. PHILLIPS, H. D. SIVINSKI AND W. J. WHITFIELD. Principles and Applications of Laminar-flow Devices
- H. M. DARLOW. Safety in the Microbiological Laboratory
- J. G. MULVANY. Membrane Filter Techniques in Microbiology
- C. T. CALAM. The Culture of Micro-organisms in Liquid Medium
- CHARLES E. HELMSTETTER. Methods for Studying the Microbial Division Cycle
- LOUIS B. QUESNEL. Methods of Microculture
- R. C. CODNER. Solid and Solidified Growth Media in Microbiology
- K. I. JOHNSTONE. The Isolation and Cultivation of Single Organisms
- N. BLAKEBROUGH. Design of Laboratory Fermenters
- K. SARGEANT. The Deep Culture of Bacteriophage
- M. F. MALLETT. Evaluation of Growth by Physical and Chemical Means
- C. T. CALAM. The Evaluation of Mycelial Growth
- H. E. KUBITSCHKE. Counting and Sizing Micro-organisms with the Coulter Counter
- J. R. POSTGATE. Viable Counts and Viability
- A. H. STOUTHAMER. Determination and Significance of Molar Growth Yields

Volume 2

- D. G. MACLENNAN. Principles of Automatic Measurement and Control of Fermentation Growth Parameters
- J. W. PATCHING AND A. H. ROSE. The Effects and Control of Temperature
- A. L. S. MUNRO. Measurement and Control of pH Values
- H.-E. JACOB. Redox Potential
- D. E. BROWN. Aeration in the Submerged Culture of Micro-organisms
- D. FREEDMAN. The Shaker in Bioengineering
- J. BRYANT. Anti-foam Agents
- N. G. CARR. Production and Measurement of Photosynthetically Usable Light
- R. ELSWORTH. The Measurement of Oxygen Absorption and Carbon Dioxide Evolution in Stirred Deep Cultures
- G. A. PLATON. Flow Measurement and Control
- RICHARD Y. MORITA. Application of Hydrostatic Pressure to Microbial Cultures
- D. W. TEMPEST. The Continuous Cultivation of Micro-organisms: 1. Theory of the Chemostat
- C. G. T. EVANS, D. HERBERT AND D. W. TEMPEST. The Continuous Cultivation of Micro-organisms: 2. Construction of a Chemostat

J. ŘÍČKA. Multi-stage Systems

R. J. MUNSON. Turbidostats

R. O. THOMSON AND W. H. FOSTER. Harvesting and Clarification of Cultures—
Storage of Harvest

Volume 3A

S. P. LAPAGE, JEAN E. SHELTON AND T. G. MITCHELL. Media for the Maintenance and Preservation of Bacteria

S. P. LAPAGE, JEAN E. SHELTON, T. G. MITCHELL AND A. R. MACKENZIE. Culture Collections and the Preservation of Bacteria

E. Y. BRIDSON AND A. BRECKER. Design and Formulation of Microbial Culture Media

D. W. RIBBONS. Quantitative Relationships Between Growth Media Constituents and Cellular Yields and Composition

H. VELDKAMP. Enrichment Cultures of Prokaryotic Organisms

DAVID A. HOPWOOD. The Isolation of Mutants

C. T. CALAM. Improvement of Micro-organisms by Mutation, Hybridization and Selection

Volume 3B

VERA G. COLLINS. Isolation, Cultivation and Maintenance of Autotrophs

N. G. CARR. Growth of Phototrophic Bacteria and Blue-Green Algae

A. T. WILLIS. Techniques for the Study of Anaerobic, Spore-forming Bacteria

R. E. HUNGATE. A Roll Tube Method for Cultivation of Strict Anaerobes

P. N. HOBSON. Rumen Bacteria

ELLA M. BARNES. Methods for the Gram-negative Non-sporing Anaerobes

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N. E. GIBBONS. Isolation, Growth and Requirements of Halophilic Bacteria

JOHN E. PETERSON. Isolation, Cultivation and Maintenance of the Myxobacteria

R. J. FALLON AND P. WHITTLESTONE. Isolation, Cultivation and Maintenance of Mycoplasmas

M. R. DROOP. Algae

EVE BILLING. Isolation, Growth and Preservation of Bacteriophages

Volume 4

C. BOOTH. Introduction to General Methods

C. BOOTH. Fungal Culture Media

D. M. DRING. Techniques for Microscopic Preparation

AGNES H. S. ONIONS. Preservation of Fungi

F. W. BEECH AND R. R. DAVENPORT. Isolation, Purification and Maintenance of Yeasts

MISS G. M. WATERHOUSE. Phycomycetes

E. PUNITHALINGHAM. Basidiomycetes : Heterobasidiomycetidae

ROY WATLING. Basidiomycetes : Homobasidiomycetidae

M. J. CARLILE. Myxomycetes and other Slime Moulds

D. H. S. RICHARDSON. Lichens

- S. T. WILLIAMS AND T. CROSS. Actinomycetes
E. B. GARETH JONES. Aquatic Fungi
R. R. DAVIES. Air Sampling for Fungi, Pollens and Bacteria
GEORGE L. BARRON. Soil Fungi
PHYLLIS M. STOCKDALE. Fungi Pathogenic for Man and Animals: 1. Diseases of the Keratinized Tissues
HELEN R. BUCKLEY. Fungi Pathogenic for Man and Animals: 2. The Subcutaneous and Deep-seated Mycoses
J. L. JINKS AND J. CROFT. Methods Used for Genetical Studies in Mycology
R. L. LUCAS. Autoradiographic Techniques in Mycology
T. F. PREECE. Fluorescent Techniques in Mycology
G. N. GREENHALGH AND L. V. EVANS. Electron Microscopy
ROY WATLING. Chemical Tests in Agaricology
T. F. PREECE. Immunological Techniques in Mycology
CHARLES M. LEACH. A Practical Guide to the Effects of Visible and Ultraviolet Light on Fungi
JULIO R. VILLANUEVA AND ISABEL GARCIA ACHA. Production and Use of Fungal Protoplasts

Volume 5A

- L. B. QUESNEL. Microscopy and Micrometry
J. R. NORRIS AND HELEN SWAIN. Staining Bacteria
A. M. PATON AND SUSAN M. JONES. Techniques Involving Optical Brightening Agents
T. IINO AND M. ENOMOTO. Motility
R. W. SMITH AND H. KOFFLER. Production and Isolation of Flagella
C. L. OAKLEY. Antigen-antibody Reactions in Microbiology
P. D. WALKER, IRENE BATTY AND R. O. THOMSON. The Localization of Bacterial Antigens by the use of the Fluorescent and Ferritin Labelled Antibody Techniques
IRENE BATTY. Toxin-antitoxin Assay
W. H. KINGHAM. Techniques for Handling Animals
J. DE LEY. The Determination of the Molecular Weight of DNA Per Bacterial Nucleoid
J. DE LEY. Hybridization of DNA
J. E. M. MIDGLEY. Hybridization of Microbial RNA and DNA
ELIZABETH WORK. Cell Walls

Volume 5B

- D. E. HUGHES, J. W. T. WIMPENNY AND D. LLOYD. The Disintegration of Microorganisms
J. SYKES. Centrifugal Techniques for the Isolation and Characterization of Sub-Cellular Components from Bacteria
D. HERBERT, P. J. PHIPPS AND R. E. STRANGE. Chemical Analysis of Microbial Cells
I. W. SUTHERLAND AND J. F. WILKINSON. Chemical Extraction Methods of Microbial Cells
PER-ÅKE ALBERTSSON. Biphaseic Separation of Microbial Particles

- MITSUHIRO NOZAKI AND OSAMU HAYAISHI. Separation and Purification of Proteins
 J. R. SARGENT. Zone Electrophoresis for the Separation of Microbial Cell Components
 K. HANNIG. Free-flow Electrophoresis
 W. MANSON. Preparative Zonal Electrophoresis
 K. E. COOKSEY. Disc Electrophoresis
 O. VESTERBERG. Isoelectric Focusing and Separation of Proteins
 F. J. MOSS, PAMELA A. D. RICKARD AND G. H. ROPER. Reflectance Spectrophotometry
 W. D. SKIDMORE AND E. L. DUGGAN. Base Composition of Nucleic Acids

Volume 6A

- A. J. HOLDING AND J. G. COLLEE. Routine Biochemical Tests
 K. KERSTERS AND J. DE LEY. Enzymic Tests with Resting Cells and Cell-free Extracts
 E. A. DAWES, D. J. MCGILL AND M. MIDGLEY. Analysis of Fermentation Products
 S. DAGLEY AND P. J. CHAPMAN. Evaluation of Methods to Determine Metabolic Pathways
 PATRICIA H. CLARKE. Methods for Studying Enzyme Regulation
 G. W. GOULD. Methods for Studying Bacterial Spores
 W. HEINEN. Inhibitors of Electron Transport and Oxidative Phosphorylation
 ELIZABETH WORK. Some Applications and Uses of Metabolite Analogues in Microbiology
 W. A. WOOD. Assay of Enzymes Representative of Metabolic Pathways
 H. C. REEVES, R. RABIN, W. S. WEGENER AND S. J. AJL. Assays of Enzymes of the Tricarboxylic Acid and Glyoxylate Cycles
 D. T. GIBSON. Assay of Enzymes of Aromatic Metabolism
 MICHAEL C. SCRUTTON. Assay of Enzymes of CO₂ Metabolism

Volume 6B

- J. L. PEEL. The Use of Electron Acceptors, Donors and Carriers
 R. B. BEECHY AND D. W. RIBBONS. Oxygen Electrode Measurements
 D. G. NICHOLLS AND P. B. GARLAND. Electrode Measurements of Carbon Dioxide
 G. W. CROSBIE. Ionization Methods of Counting Radio-Isotopes
 J. H. HASH. Liquid Scintillation Counting in Microbiology
 J. R. QUAYLE. The Use of Isotopes in Tracing Metabolic Pathways
 C. H. WANG. Radiorespirometric Methods
 N. R. EATON. Pulse Labelling of Micro-organisms
 M. J. ALLEN. Cellular Electrophysiology
 W. W. FORREST. Microcalorimetry
 J. MARTEN. Automatic and Continuous Assessment of Fermentation Parameters
 A. FERRARI AND J. MARTEN. Automated Microbiological Assay
 J. R. POSTGATE. The Acetylene Reduction Test for Nitrogen Fixation

Volume 7A

- G. C. WARE. Computer Use in Microbiology
 P. H. A. SNEATH. Computer Taxonomy

- H. F. DAMMERS. Data Handling and Information Retrieval by Computer
M. ROBERTS AND C. B. C. BOYCE. Principles of Biological Assay
D. KAY. Methods for Studying the Infectious Properties and Multiplication of Bacteriophage
D. KAY. Methods for the Determination of the Chemical and Physical Structure of Bacteriophages
ANNA MAYR-HARTING, A. J. HEDGES AND R. C. W. BERKELEY. Methods for Studying Bacteriocins
W. R. MAXTED. Specific Procedures and Requirements for the Isolation, Growth and Maintenance of the L-Phase of Some Microbial Groups

Volume 7B

- M. T. PARKER. Phage-Typing of *Staphylococcus aureus*
D. A. HOPWOOD. Genetic Analysis in Micro-organisms
J. MEYRATH AND GERDA SUCHANEK. Inoculation Techniques—Effects Due to Quality and Quantity of Inoculum
D. F. SPOONER AND G. SYKES. Laboratory Assessment of Antibacterial Activity
L. B. QUESNEL. Photomicrography and Macrophotography

Volume 8

- L. A. BULLA JR., G. ST. JULIAN, C. W. HESSELTINE AND F. L. BAKER. Scanning Electron Microscopy
H. H. TOPIWALA. Mathematical Models in Microbiology
E. CANALE-PAROLA. Isolation, Growth and Maintenance of Anaerobic Free-living Spirochetes
O. FELSENFELD. Borrelia
A. D. RUSSELL, A. MORRIS AND M. C. ALLWOOD. Methods for Assessing Damage to Bacteria Induced by Chemical and Physical Agents
P. J. WYATT. Differential Light Scattering Techniques for Microbiology.

CONTENTS

LIST OF CONTRIBUTORS	v
ACKNOWLEDGMENTS	vi
PREFACE	vii
CONTENTS OF PUBLISHED VOLUMES	xi
Chapter I. Substrate Specificities of Aminopeptidases: A Specific Method for Microbial Differentiation—R. R. WATSON	1
Chapter II. Mechanized Identification of Micro-organisms—C-G. HEDEN, T. ILLENI AND I. KUHN	15
Chapter III. Gas-Liquid Chromatographic Chemotaxonomy— D. B. DRUCKER	51
Chapter IV. Transmission Electron Microscopy of Bacteria—K. G. LICKFELD	127
Chapter V. Electron Microscopy of Small Particles, Macromolecular Structures and Nucleic Acids—P. KAY	177
Chapter VI. <i>Bdellovibrio</i> Methodology—M. P. STARR AND H. STOLP	217
<i>Subject Index</i>	245

CHAPTER I

Substrate Specificities of Aminopeptidases: A Specific Method for Microbial Differentiation

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Indiana University School of Medicine, Indianapolis, Indiana 46202, U.S.A.

I. Differentiation by Assay of Microbial Aminopeptidases	1
II. Aminopeptidase Substrate Specificities Measured in Whole Cells	8
A. Preparation of yeast cell suspension for aminopeptidase assay	8
B. Preparation of amino-acid- β -naphthylamide substrates	9
C. Profile determination with whole yeast cells	10
III. Aminopeptidase Substrate Specificities Measured with Soluble Enzymes	10
A. Preparation of cell-free aminopeptidases from <i>Enterobacteriaceae</i>	10
B. Profile determination with cell-free aminopeptidases	11
References	12

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 (β NA) 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- β NA's 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 *Neolichinohynchus 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 (Ruitenbergh 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, Reza, 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- β NAs. 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- β NA and L-hydroxyprolyl- β NA. The other two fungi differed less, with quantitative differences in hydrolysis

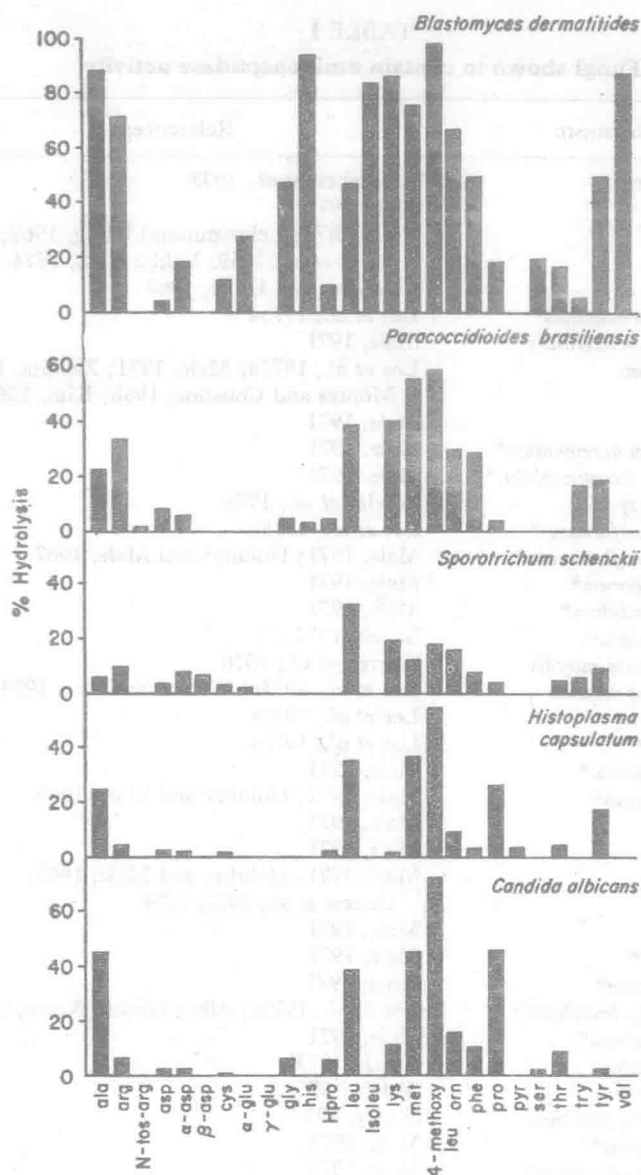


FIG. 1. Amino peptidase profiles of five yeasts, *Histoplasma capsulatum*, *Sporotrichum schenckii*, *Candida albicans*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis*. These profiles were obtained as described above for the whole cell technique.

TABLE I
Fungi shown to contain aminopeptidase activity

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

TABLE I—cont.

Organism	References
<i>T. elegans</i>	Stout and Shaw, 1973
<i>Torulopsis famata</i> *	Male, 1971
<i>Trichophyton guinekeanum</i> *	Holubar and Male, 1967
<i>T. granulorum</i> *	Male, 1971
<i>T. interdigitale</i> *	Male, 1971
<i>T. mentagrophytes</i> *	Holubar and Male, 1967
<i>T. persicolor</i> *	Male, 1971
<i>T. rubrum</i>	Male, 1971; Holubar and Male, 1967; Danew <i>et al.</i> , 1971
<i>T. schonlenii</i> *	Holubar and Male, 1967
<i>T. terrestre</i>	Male, 1971
<i>T. tonsurans</i>	Male, 1971
<i>T. verrucosum</i> *	Male, 1971; Holubar and Male, 1967
<i>T. violaceum</i> *	Holubar and Male, 1967
<i>Trichosporon cutaneum</i> *	Male, 1971
<i>Trichothecium roseum</i> *	Male, 1971
<i>Trigonopsis variabilis</i> *	Male, 1971
<i>Tritirachium album</i>	Hennrich <i>et al.</i> , 1973
<i>Verticillium cinnabarium</i> *	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- β NA, L-alanyl- β NA, L-methionyl- β NA, L-phenylalanyl- β NA and L-threonyl- β NA 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 albicans*, *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,