
Contents

| | |
|---------------------------|-----|
| Contributors | vii |
| Preface to first edition | ix |
| Preface to second edition | xi |

CHAPTER I

Initial handling and diagnosis of diseased invertebrates

| | |
|--|----|
| <i>L.A. Lacey and L.F. Solter</i> | 1 |
| 1 Introduction | 1 |
| 2 Collection of diseased invertebrates and entomopathogens | 2 |
| 3 Examination in the laboratory | 6 |
| 4 Safety considerations | 13 |
| Acknowledgments | 13 |
| References | 13 |

CHAPTER II

Basic techniques in insect virology

| | |
|--|----|
| <i>Karolin E. Eberle, Jörg T. Wennmann, Regina G. Kleespies, and Johannes A. Jehle</i> | 15 |
| 1 Introduction | 15 |
| 2 Brief overview of the different groups of insect viruses | 17 |
| 3 Identification | 29 |
| 4 Virus propagation | 41 |
| 5 Virus quantification | 51 |
| 6 Quantification of virus activity (virulence) | 55 |
| 7 Cell culture techniques | 58 |
| 8 Reverse genetics | 61 |
| References | 67 |
| Glossary | 72 |
| Appendix: Recipes for stains | 74 |

Contents

CHAPTER III

Isolation, culture, preservation, and identification of entomopathogenic bacteria of the Bacilli

T.W. Fisher and S.F. Garczynski

| | | |
|---|--------------------|----|
| 1 | Introduction | 75 |
| 2 | Isolation | 75 |
| 3 | Cultivation | 78 |
| 4 | Preservation | 80 |
| 5 | Identification | 81 |
| 6 | Concluding remarks | 94 |
| | Acknowledgments | 94 |
| | Disclaimer | 94 |
| | References | 94 |
| | Appendix | 96 |

CHAPTER IV

Bioassay of bacterial entomopathogens against insect larvae

Maureen O'Callaghan, Travis R. Glare, and Lawrence A. Lacey

| | | |
|---|--|-----|
| 1 | Introduction | 101 |
| 2 | Laboratory bioassay of <i>Bacillus</i> pathogens against lepidopteran larvae | 102 |
| 3 | Bioassay of <i>Bacillus thuringiensis</i> against dipteran larvae | 111 |
| 4 | Bioassay of <i>Bacillus thuringiensis</i> against other orders | 120 |
| 5 | Bioassay of other entomopathogens | 121 |
| 6 | Conclusions | 122 |
| | Acknowledgments | 123 |
| | References | 123 |
| | Appendix: artificial diet recipes | 126 |

CHAPTER V

Bacteria for use against soil-inhabiting insects

Albrecht M. Koppenhöfer, Trevor A. Jackson, and Michael G. Klein

| | | |
|---|----------------------------|-----|
| 1 | Introduction | 129 |
| 2 | Isolation | 129 |
| 3 | Identification | 131 |
| 4 | Propagation and production | 134 |
| 5 | Bioassay | 136 |
| 6 | Preservation | 140 |
| | References | 144 |
| | Appendix 1 | 145 |
| | | 147 |

CHAPTER VI

Identification of entomopathogenic fungi

Richard A. Humber

| | | |
|---|---|-----|
| 1 | Introduction | 151 |
| 2 | Quick guide to light microscopy | 151 |
| 3 | Key to major genera of fungal entomopathogens | 154 |
| 4 | Major genera of fungal entomopathogens | 158 |
| | References | 182 |

| | |
|---|-----|
| Brief glossary of mycological terms | 188 |
| Appendix: recipes of stains and reagents | 186 |
| | |
| CHAPTER VII | |
| Laboratory techniques used for entomopathogenic fungi: Hypocreales | |
| <i>G. Douglas Inglis, Juerg Enkerli, and Mark S. Goettel</i> | |
| 1 Introduction | 189 |
| 2 Detection and characterization | 190 |
| 3 Inoculum propagation and preparation | 211 |
| 4 Quantification | 214 |
| 5 Infection and bioassay | 228 |
| 6 Assessment of environmental parameters | 237 |
| Acknowledgments | 243 |
| References | 243 |
| Appendix | 251 |
| | |
| CHAPTER VIII | |
| Mass production of entomopathogenic Hypocreales | |
| <i>Stefan T. Jaronski and Mark A. Jackson</i> | |
| 1 Introduction | 255 |
| 2 Culture maintenance | 255 |
| 3 Solid substrate production of aerial conidia | 256 |
| 4 Liquid fermentation production | 272 |
| Disclaimer | 281 |
| Acknowledgments | 281 |
| References | 281 |
| Appendix | 282 |
| | |
| CHAPTER IX | |
| Methods for study of the Entomophthorales | |
| <i>Ann E. Hajek, Bernard Papierok, and Jørgen Eilenberg</i> | |
| 1 Introduction | 285 |
| 2 Collecting and preparing specimens | 286 |
| 3 Species identification | 287 |
| 4 Isolation | 289 |
| 5 <i>In vitro</i> culture and production | 296 |
| 6 <i>In vivo</i> culture and production | 301 |
| 7 Bioassays | 302 |
| Acknowledgments | 309 |
| References | 310 |
| Appendix: media | 315 |
| | |
| CHAPTER X | |
| Preservation of entomopathogenic fungal cultures | |
| <i>Richard A. Humber</i> | |
| 1 Introduction | 317 |
| 2 Preservation at temperatures above freezing | 319 |

Contents

| | |
|--|-----|
| 3 Preservation by freeze-drying (lyophilization) | 321 |
| 4 Preservation in a frozen state | 322 |
| 5 Record keeping for storage facilities | 326 |
| 6 Other less common preservation techniques | 326 |
| References | 327 |

CHAPTER XI

Research methods for entomopathogenic microsporidia and other protists

| | |
|---|-----|
| <i>Leellen F. Solter, James J. Becnel, and Jiri Vávra</i> | 329 |
| 1 Introduction | 329 |
| 2 Identification | 331 |
| 3 Techniques for managing spores | 350 |
| 4 Molecular and proteomic techniques | 357 |
| 5 Propagation and production | 360 |
| 6 Controlling infections in insect colonies | 363 |
| 7 Bioassays and experimental infections | 364 |
| Acknowledgments | 367 |
| References | 367 |

CHAPTER XII

Nematode parasites, pathogens and associates of insects and invertebrates of economic importance

| | |
|--|-----|
| <i>S. Patricia Stock and Heidi Goodrich-Blair</i> | 373 |
| 1 Introduction | 373 |
| 2 Major groups of nematodes associated with insects and other invertebrates of economic importance | 374 |
| 3 Sampling techniques | 381 |
| 4 Diagnostic methods | 387 |
| 5 Staining methods | 399 |
| 6 Culturing methods | 400 |
| 7 Nematode quantification methods | 405 |
| 8 Preservation methods | 406 |
| 9 Shipping and handling of samples | 408 |
| 10 Bioassays with entomopathogenic nematodes and symbiotic bacteria | 409 |
| Acknowledgments | 418 |
| References | 418 |
| Appendix I | 422 |
| Appendix II | 423 |

CHAPTER XIII

Pathogens and parasites of terrestrial molluscs

| | |
|--|-----|
| <i>Michael J. Wilson</i> | 427 |
| 1 Introduction | 427 |
| 2 General techniques for working with terrestrial molluscs | 428 |
| 3 Major groups of pathogens and parasites associated with terrestrial molluscs | 429 |
| Acknowledgments | 437 |
| References | 437 |
| Appendix | 439 |

CHAPTER XIV

Testing the pathogenicity and infectivity of entomopathogens to mammals

Joel P. Siegel

| | | |
|---|---------------------------------------|-----|
| 1 | Introduction | 441 |
| 2 | Definition of infection | 442 |
| 3 | Definition of persistence | 443 |
| 4 | Mammalian safety tests and philosophy | 443 |
| 5 | Animal procurement and housing | 445 |
| 6 | Modified tier scheme | 445 |
| 7 | Conclusion | 448 |
| | Acknowledgments | 448 |
| | References | 448 |

CHAPTER XV

**Complementary techniques: preparations of entomopathogens and diseased specimens
for more detailed study using microscopy**

James J. Becnel

| | | |
|---|---------------------|-----|
| 1 | Introduction | 451 |
| 2 | Light microscopy | 451 |
| 3 | Electron microscopy | 461 |
| | Disclaimer | 465 |
| | Acknowledgments | 465 |
| | References | 466 |
| | Appendix | 466 |