APPLICATIONS OF BIOTECHNOLOGY TO THE STUDY OF NEMATODES

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Abstract

A brief review is given of the application of some of the newer biotechnological methods to the study of nematodes. Within the area of biochemistry, the application of nuclear magnetic resonance spectroscopy to the studies of parasite metabolism, and the continued use of isoenzyme analysis for understanding intraspecific variation in parasites are discussed. Aspects of the molecular characterization of nematodes are described, including methodologies for extraction of DNA, and the identification of specific parasite antigens. The use of in vitro DNA amplification by the polymerase chain reaction for diagnostic purposes is considered.

INTRODUCTION

The development and implementation of newer biotechnological procedures for the study of parasitic infections will undoubtedly have a substantial impact on the ways that parasitology research is conducted in the future. Signals of concern for the need to apply newer developments in biotechnology have come from several sources, but two of the major sources are the World Health Organization and the European Federation of Parasitologists. As part of the Special Programme for Research and Training in Tropical Diseases (TDR), a new undertaking is the TDR Initiative for Biotechnology Implementation. As with most TDR-sponsored programs this was to reflect a partnership between scientists and institutions in developing and developed countries.

The European Federation of Parasitologists developed a series of resolutions at the Fifth European Multicolloquium of Parasitology held in Budapest, Hungary in September 1988, that pertain to directions of parasitology research. It was pointed out that parasites and parasitic infections can serve as good examples to test fundamental principles in immunology, ecology, and other areas, and encouragement should be provided to non-parasitologists to apply developments in molecular biology, biochemistry and immunology, to the study of parasites. Specific areas suggested that need continued development include immunodiagnosis of parasitic infections, the role of parasites in human nutrition, zoonotic infections, and the increasing problems of opportunistic parasitic infections.

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There is some concern that fundamental parasitologists per se are disappearing and being replaced by molecular biologists, immunologists, biochemists, and others who use parasites as tools and know little about them, how they exist in nature, what their morphological and biological features are, and often what is their relevance to animal and human populations. However, as those of us who have worked with parasites for many years well know, these are truly fascinating organisms to work with and what is needed now is the application of these new and powerful molecular tools to their study. There is ample room for parasitologists with more classical training to work with those trained in areas of biochemistry, immunology, and molecular biology and the synergy of these two can do nothing but enhance our knowledge. In fact, without the integrated efforts of basic parasitologists who have a thorough understanding of the biology of parasites, and molecular biologists who have the technical expertise and understanding of the new methodology, it will be difficult to translate new findings into useful knowledge for application in the field to control both animal and human parasites.

It is my task to summarize, if only briefly, some of the developments in the application of biotechnology to the study of nematodes. In doing so, two things should be recognized. First, work with nematodes, other than the filarial worms, has lagged behind what has been accomplished with the protozoa and other helminths. Secondly, the application of newer methodologies has led to significant advances in many areas, including diagnosis, genetics, biochemistry, physiology, immunology, and molecular biology. As a consequence, I will be selective in discussing some of these developments and what is presented must be restrictive both in breadth and depth of the topics covered.

DEVELOPMENTS IN BIOCHEMISTRY

To better understand and subsequently to deal with nematodes parasitizing animals or humans it is important to have information on metabolic regulation in these organisms. Studies that involve nutrient uptake, biosynthesis, osmoregulation, cell multiplication and differentiation are all important aspects in the life and reproduction of nematodes. Many aspects of metabolic regulation in nematodes, as well as other parasites, revolve around enzyme activity including types of enzymes, the chemical messengers utilized, and their concentration. Although considerable information in these areas has been obtained for many parasites there is a need yet for such studies with many important roundworm parasites of animals and humans.

Among the newer applications to the study of parasite metabolism is the use of nuclear magnetic resonance spectroscopy. Thus far, applications of this technology have been directed mainly at cultured forms of intestinal protozoa. This enables the investigator to 1) monitor changes in culture medium in which the parasites are grown, 2) use parasite extracts, and 3) make observations on whole parasites. In the procedure, a spectrum is taken of the medium prior to use and then subsequently at different stages of parasite growth the supernatant can be examined for peaks of major metabolites. As pointed out by these authors, the utility of this procedure is that it provides direct information on major fuel metabolism, what precisely is used and what products are produced. Utilization of this procedure for the study of
nematodes, relatively large organisms, requires adaptation of the methodology and to this point this has not occurred widely. However, in work done with Angiostrongylus cantonensis, studies on young adult worms provided evidence that carbohydrate metabolism is not dependent upon the alternate pathway but upon glycolysis. These workers could also demonstrate that the end products of metabolism did not differ from young parasites living in the brain to those adults living in the pulmonary artery.

Isoenzyme analysis has proven to be a useful biochemical technique for understanding intraspecific variation in parasites. This procedure has proven useful with protozoan organisms (Entamoeba histolytica and Leishmania spp are excellent examples) in delineating strains or isolates in terms of infectivity and pathogenesis. Application to the study of nematodes has been less widely used but its usefulness has already been demonstrated for several species.

Speciation within the genus Trichinella has been a controversial topic since the early 1960’s when the first indications were given that there appeared to be distinct geographic populations of T. spiralis. Subsequent studies presented evidence for a domestic "spiralis" form, a "nativa" form in circumpolar areas of North America, Europe and Asia, a "nelsoni" form in Africa and parts of Europe, and the "pseudospiralis" form. Analysis of these strains by using twelve enzyme systems indicated that all four forms could be differentiated from one another. In addition to the genus Trichinella, taxonomic problems with closely related ascaridoids (eg. Ascaris lumbricoides and A. suum, dog and cat ascarids), anisakids, and filarial worms have been studied with enzyme electrophoresis. Such studies have shown that at least by enzyme analysis that A. lumbricoides and A. suum merit being considered as distinct species.

Trichostrongylid nematodes are not only important parasites of ruminant animals but they represent a group of nematodes that appear to have a rapid response to new selection pressures resulting in differing epidemiological patterns within strains of some members of the group. Haemonchus contortus, an important parasite of sheep, has received considerable study because of its apparently extensive strain variation in nature. Metabolic strain variation has been demonstrated in sheep treated with benzimidazole anthelmintics. These studies have shown that worms susceptible to the anthelmintic had 2-10 times as much fumarate reductase activity as did an anthelmintic-resistant strain, although other studies have failed to demonstrate a significant difference in the two. A similar kind of phenomenon has been demonstrated in Trichostrongylus colubriformis, another sheep parasite, in which two strains of the parasite, one resistant and one not resistant to the anthelmintic thiabendazole, had different metabolic patterns. The resistant strain was found to take up methyl glucose more rapidly and its carbohydrate reserves became depleted in comparison to the susceptible strain.

The importance of this information on metabolism has obvious ramifications on chemotherapeutic approaches to trichostrongylid infections and it also demonstrates the difficulties encountered in extrapolating data from one species to the next, or even between strains of the same species.

DEVELOPMENT IN MOLECULAR CHARACTERIZATION OF NEMATODES

Nematodes are complex organisms as is evidenced by the fact that their genome size
is larger than that of viruses, bacteria and protozoa and only somewhat smaller than that in humans. Separation of nematode strains by morphological and biochemical means has some inherent weaknesses that can be resolved by direct characterization of the genome. At the molecular level, genetic characterization of organisms is of the utmost importance and is accomplished by analysis of DNA occurring in individual cells. Among the factors of concern in DNA analysis are: structure, size and density, sequencing (only this provides a direct measure of genotypic variation), DNA-DNA hybridization, restriction site analysis, and base pair composition.

In nematode speciation, some workers have made use of genomic DNA restriction fragment length difference to identify a number of different free-living and parasitic nematode species. A cloned element from Trichinella spiralis was used as a diagnostic probe for the identification of species of Trichinella and dot blot analyses using the probe did distinguish the spiralis, nativa and nelsoni forms, but not the pseudospiralis form, from seven other nematode species. As pointed out by these latter authors, the use of DNA technology for the identification of helminths in general has the distinct advantage of allowing for direct examination of genetic material without the problems associated with gene expression or phenotypic variation. Added advantages are that the technique can be utilized with all stages of the parasite’s life cycle and the DNA material is chemically stable and can be stored for long periods of time.

The isolation of DNA material from nematodes poses some unique problems to the molecular biologist. In achieving this in nematodes the first barrier to such studies is the external covering of nematodes, the cuticle. Electron microscopy has revealed that the cuticle is a complex structure consisting of an outer epicuticle, an underlying cortex, and three basal layers; all of these overlie the hypodermis. The cuticle not only gives form and substance to the organism as a flexible exoskeleton but it serves the organism well as a barrier to procedures that would chemically, physically or enzymatically disrupt cells. The epicuticle is considered to be an inert, polyanionic, trilaminar structure. The cortical layer contains a collagen-like material that is referred to as pseudocollagen. In its composition, the subunit chains of polypeptides of the pseudocollagen fold back on themselves and when this is combined with the unique amino acid composition of the pseudocollagen this gives considerable strength to the cuticle. Thus, the disruption of the cuticle is an overriding consideration in attempting to extract DNA from nematodes.

Dawkins and Spencer have described the procedures needed to prepare nucleic acids from filariform larvae of the sheep parasites Trichostrongylus colubriformis and Ostertagia circumcincta. Work with larvae such as these is further complicated by the fact that infective larvae of these species retain the molted sheath about them. The authors first had to use exsheathment techniques on these larvae and they discovered that if this was followed immediately by enzymatic digestion the larvae were susceptible to the enzyme activity. The techniques described resulted in the preparation of both genomic and chromosomal DNA. The application of electrophoretic separation of intact chromosomes and large DNA fragments allows for species karyotyping and analysis of individual chromosomes.
DEVELOPMENTS IN THE STUDY OF IMMUNOLOGICAL RESPONSES OF THE HOST

Considerable attention has been focused on nematode antigens. These antigens may be presented to the host in several ways - 1) surface antigens on the cuticle, 2) antigens present in excretory-secretory products, and 3) somatic antigens which usually are presented only after the parasite has died or been killed.

It is well recognized that there may be active shedding of parasite surface antigens. Some of these antigens may elicit protective immune responses but others may not, and in fact, it has been suggested that some of these surface antigens may "divert the attention" of the host immune response from other parasite antigens\textsuperscript{14}. This serves to protect the parasite.

To identify these various surface antigens metabolic and enzyme radiolabelling procedures have been utilized. Through use then of chromatography, electrophoresis, immunopurification, and hybridoma technology we identify and purify specific parasite antigens. Other recent papers that contribute to these topics include those by Lightowlers and Rickard,\textsuperscript{15} and Pritchard et al. \textsuperscript{16}.

DEVELOPMENTS IN DIAGNOSIS OF NEMATODE INFECTIONS

For a number of years now specific DNA probes for the identification of parasites have been employed. In the nematodes, filarial worms have been one of the prime targets for such studies. The basic premise of specific DNA probes is to detect diseases by hybridization to DNA in the patient. There have been a number of limitations. One is that production of the DNA probe may be limited by the amount of parasite antigen needed, a difficulty with organisms that cannot be readily cultured in the laboratory. A second difficulty has been the limited sensitivity of the hybridization technique used since the target sequence in the DNA of the suspected case may be at too low a level or simply the amount of DNA material available is too small.

A recent development that may prove to be a powerful tool to detect parasitic infections in which only a few parasites can be detected is through use of \textit{in vitro} DNA amplification by the polymerase chain reaction\textsuperscript{17}. It has been shown that the polymerase chain reaction can detect a DNA sequence that is only a minor component of a more complex mixture and this makes the procedure several orders of magnitude more sensitive than conventional hybridization procedures. However, although the polymerase chain reaction will probably play an even greater role in parasitology in the future it does have some disadvantages that should be considered before it is generally applied\textsuperscript{18}.

SUMMARY

Without question, we are in a new era of parasitologic research. In the summary of some of the biotechnological developments that have been applied to the study of nematodes I have touched on some of the highlights but certainly not all have been touched. Time could have been spent equally on many other topics. Cryopreservation techniques have great relevance for many studies on all parasites, including nematodes. The development of vaccines and newer approaches to the development of effective chemotherapeutic agents dovetail with advances in immunology, biochemistry, and parasite physiology. In Southeast Asia, with the presence of the wide range of animal and
human parasites, the opportunities for basic research and the application of new methodologies to parasites is probably still unlimited. It should prove to be an exciting time as scientists delve more deeply into the mysteries of these fascinating organisms and how scientists can outwit them to lead to their control.

REFERENCES

QUESTIONS AND ANSWERS:

1. Question: Isoenzyme analysis has proven to be successful for understanding intraspecific variation in parasites.
   What do you mean by intraspecific variation?
   Can you give an example for nematodes?

   Answer: Intraspecific variation: epidemiology, morphology.
   Example: Haemonchus, Trichostrongylus.

2. Question: 1. Which one of the 3 kinds of antigens which is much influenced by the nematode differentiation stage?
   2. For the purpose of controlling by biotech product do we focus only on the two antigens, namely the surface and the secretory/excretory ones?

   Answer: 1. Excretory and secretory antigens
   2. The ones listed are the most important for biotechnological products.

3. Question: I have the opinion that species of parasites (taxonomy) in Indonesia need to be revised (greater part). How far antigen (surface Ag./somatic Ag. etc), enzyme electrophoresis, DNA probe, etc. can be used in the taxonomy of parasites?
   What are their limitations?

   Answer: Yes, the application of newer biotechnology will be useful in resolving speciation in nematodes especially veterinary parasites such as Haemonchus, Trichostrongylus, Trichinella.