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The role of molecular genetics on parasite control

Abstract

Parasitic diseases are a common problem on communities in the tropics and subtropics, but parasitic infections could also affect people in developed countries. Recently there are a lot of approaches to control parasites, from the simplest application of traditional medicinal plants to some complex molecular genetics applications such as DNA sequencing and genetic engineering. Despite their positive features compared to older applications, molecular genetics also had some restriction factors that make it infrequent to apply. Hence, this paper tries to emphasize on molecular genetics aspect to give readers more information related with the use of this approach in parasites control.

Peran genetika molekular dalam pengendalian parasit

Abstrak

Penyakit yang disebabkan oleh parasit umumnya menjadi permasalahan pada masyarakat daerah tropis dan subtropis, akan tetapi infeksi parasit dapat pula terjadi pada negara maju. Pada masa ini ada banyak teknik pendekatan untuk mengontrol parasit, mulai dari aplikasi sederhana tanaman obat tradisional sampai dengan beberapa teknik yang lebih kompleks seperti sekuen DNA atau bahkan rekayasa genetik. Teknik genetika molekular memiliki beberapa keunggulan dibandingkan dengan aplikasi yang lebih lama, namun teknik ini juga memiliki keterbatasan yang membuatnya jarang diaplikasikan. Untuk itu penulis mencoba menggaris bawahi bahasan tentang kontrol parasit dengan teknik genetika molekular untuk memberi informasi kepada pembaca mengenai pendekatan ini.

Introduction

Parasites are organisms that lives on or in a host and gets their food from or at the expense of its host. Parasites can cause disease in humans. Some parasitic diseases are easily treated and some are not. The burden of these diseases often rests on communities in the tropics and subtropics, but parasitic infections also affect people in developed countries.¹ Protozoan, nematode, cestode and trematode are members of this group.

Humans parasites are classified in six major divisions. These include the Protozoa (amebae, flagellates, ciliates, sporozoans, coccidia, microsporidia), the Nematoda or roundworms, the Platyhelminthes or flatworms (cestodes, trematodes), the Pentastomids or tongue worms (may be grouped with the arthropods), the Acanthocephala or thorny-headed worms, and the Arthropoda (e.g., insects, spiders, mites, ticks).² Some general characteristics of parasites are: (i) hermaphroditic, parthenogenetic and asexual reproduction are common; (ii) the generation time is usually short; and (iii) parasite populations are highly fragmented, with many populations also experiencing strong seasonal fluctuations in size.³

Disease caused by parasite still becomes a problem in most countries. Study of lymphatic filariasis in children in India suggested that asymptomatic infection and acute form of disease were common occurrence among the children and more than half of the children population were either infected or having clinical manifestations of the diseased by pre-adult stage (11-15 years of age) in the endemic area.⁴

To date, there are several key ways to control parasites: the use of drugs (either chemical synthetic or natural product), ecological control (using parasite's natural enemy and inhibiting the expansion of parasites by making unsuitable environment for them to growth), and molecular genetics approach (genetic variation identification and genetic engineering). Molecular genetics properties have several advantage compared to other choices. In the future, this approach will hold an important role on parasite control. Therefore, this paper emphasize on molecular genetics

aspect to give readers more information related with the use of this approach in parasites control.

Why choosing molecular gen

Molecular genetics is a choice between several studies that could be use in controlling parasites. Although require more cost, the rapidness and the ability to analyze large number of samples become make it more preferable to study. Diagnosis on patients from Thailand have shown that Real-Time Polymerase Chain Reaction (RT-PCR) assay is a rapid, accurate, and efficient method for the specific diagnosis of malaria compared to conventional microscopy using Giemsa-staining thick and thin blood film, having to finish the process in just one hour.⁵ PCR assay also proven to be successful for *Brugia malayi* detection in Malaysian students using small amount of blood sample.⁶

Generally, studying molecular genetics of parasites would aim for these reasons:^{7,8}

1. Determination of complete sequence of the chromosomal (and plastid) genome of the parasites.
2. Identification of the coding genes (both protein and RNA) on the sequence (gene discovery).
3. Prediction of function of each of the genes, and the prediction of function of operator/promoter/control regions in the non-coding DNA.
4. Integration of functional, sequence and architectural information into biological models of the structure of the chromosomes and of the interaction between the expressed parts of the genome.
5. Investigating natural variation in the genome in the context of the host, population structure, drug treatment and other selective forces.

Additionally, studying genetic of parasite could give us different viewpoint in controlling parasites. Knowing the sequences within parasite DNA will help us to detect mutation, it is related with the case that a population of parasite is resistant to a drug compared to another population from the same species. A study on *Plasmodium falciparum* in 4 Thailand-Myanmar border areas using RFLP

Restriction Fragment Length Polymorphism) and nucleotide sequencing technique have detected genetic variation (mutation) due to resistance to chloroquine.⁹ Similar study also showed genetic resistance of *P. Falciparum* to chloroquine and pyrimethamine in Indochina and the Western Pacific.¹⁰

Molecular approaches also useful in studying the extent of genetic diversity, infection dynamics or population structure.¹¹ Random Amplified Polymorphic DNA (RAPD) study has been used in differentiating 3 populations of human filarial parasite *Wuchereria bancrofti* in South India.¹² While microsatellite marker was used in generating genetic diversity and population structure of *Schistosoma mansoni* within human infrapopulations in Mwea, central Kenya.¹³ **etics?**

Techniques to choose

Genetic data of parasite could be obtained from many sources. Genomics uses data arising from karyotypic analysis, genetic and physical mapping of traits and anonymous markers, DNA sequencing and bioinformatic prediction of function-structure relationships.⁷ Since karyotyping analysis already left behind compared to other techniques, the writer exclude this technique in the discussion.

RFLP is a technique to differentiate DNA using specific restriction enzymes resulting in DNA cutting pattern. Differences in the cutting pattern indicate differences in the sequencing of samples DNA we analyze, these differences affecting the recognition site of the restriction enzymes. RFLP study of Serine-Rich *Entamoeba histolytica* Protein (SREHP) generated 13 different profiles from 23 *E. histolytica* isolates from Cameroon, Zimbabwe and South Africa, and 20 profiles were generated from 38 *E. histolytica* PCR positive stool samples from South Africa. The results might indicate that African isolates of *E. histolytica* may possess extremely complex genetic structures independent of geographic location.¹⁴

In the study of population differentiation on specific parasite, mutation is not the only factors that cause DNA variation. Genetic drift and gene flow might also take part. RAPD study on *Wuchereria bancrofti* population in South India showed higher genetic

variation in an urban area compared to several rural areas surrounding it. The study suggested that urban parasite populations appeared to be a pool of parasite population originating from surrounding rural areas.¹⁵

Another technique to choose for analyzing genetic of parasite is sequencing. This technique offer higher resolution in DNA differentiation. There are three categories of sequence data: complete or nearly complete genomic sequences; genome-survey sequence (GSS) tags; and expressed sequence tags (ESTs).⁸ Fong et al¹⁶ have successfully compared three clinical *Plasmodium knowlesi* isolates from Klang Valley, peninsular Malaysia to the older peninsular Malaysia isolates, and those obtained from Sarawak and Thailand.

Molecular study also found to be useful in detecting gene flow between parasites host. High gene flow was found between humans and dogs on a microsatellite assay of *Schistosoma japonicum* populations in Philippine. This founding gives a consideration that current chemotherapy-based control programs may be inefficient if humans are rapidly re-infected by animal host reservoirs.¹⁷

Regardless of its positive features in parasites control, molecular genetics also have some weakness. Its rapid process requires the presence of some highly-tech equipments, thus require higher cost on the investment. Furthermore, these equipments could not be used on the field where electricity is limited. Molecular studies also require additional knowledge to help us to build an understanding. Some molecular processes require knowledge in mathematical modeling at a population level, particularly in genetics and immunology.¹⁸

Summary

Recently there are a lot of molecular genetics techniques ranging from low resolution to higher resolution, simple to the most complex. These techniques help us on studies related with the control of parasites. Despite of their limitation molecular genetics techniques will become popular choices in parasites control. techniques help us on studies related with the control of parasites. Despite

Table 1. Summary of some researches in molecular genetics application on parasite control

Species	Technique	Result	Reference No.
<i>Brugia malayi</i>	Conventional PCR and Real Time- PCR	Both C-PCR and RT- PCR detected 2 out of 18 samples as positive from non-concentrated genomic DNA preparations. After the DNA samples were pooled and concentrated, both C-PCR and RT-PCR detected <i>B. malayi</i> DNA amplifications in 7 out of 18 samples. However one sample which showed faint band in C-PCR was detected as highly positive in RT-PCR.	6
<i>Wuchereria bancrofti</i>	RAPD	The Nei's gene diversity between the individual populations in the 2 areas (one urban and another rural) was comparatively greater (0.3372 ± 0.1462 & 0.2830 ± 0.1764) than that of populations in another village (0.0490 ± 0.1373).	12
<i>Schistosoma mansoni</i>	Microsatellite	The mean number of alleles per locus was 8.22–10.22, expected heterozygosity in Hardy–Weinberg equilibrium was 0.68–0.70, and pairwise F_{ST} values ranged from 0.16% to 3.98% for the 12 infrapopulations. Low levels of genetic structure were also found, suggesting high levels of gene flow among infrapopulations.	13
<i>Entamoeba histolytica</i>	PCR-RFLP	Thirteen different profiles were generated from 23 <i>E. histolytica</i> isolates from Cameroon, Zimbabwe and South Africa while 20 others were generated from 38 <i>E. histolytica</i> PCR positive stool samples from South Africa.	14
<i>Plasmodium knowlesi</i>	Sequence analysis on the mitochondrial COXI gene	Multiple alignment showed that the three clinical <i>Plasmodium knowlesi</i> isolates from Klang Valley, peninsular Malaysia were more similar to the older peninsular Malaysia isolates than to those from Sarawak and Thailand.	16

of their limitation, molecular genetics techniques will become popular choices in parasites control.

This approach will give a bright future in controlling parasites in the aspect of drug development such as vaccine design^{19,20}. Furthermore, this approach is useful in phylogenomic study, helping parasite taxonomist to identify parasite and grouping them into correct taxonomic classes. The increasing of available sequence information from online database such as GenBank (www.ncbi.nlm.nih.gov/genbank/) would also help us in designing specific primer to use for disease detection using PCR amplification. Nevertheless, their usage might become a routine in parasite laboratory.

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