In-vitro Cultivation of Trypanasoma sp. of eel, Monopterus albus

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Introduction

Trypanosomes are free living and some of them are parasitic to their host like amphibians, mammals, fish and bird. *Trypanosoma* tend to occur in tissue fluid or bloodstream of the host. Much research had been conducted to study about them including their morphology,life cycle and immunology. We had found that our Malaysian swamp eel (*Monopterus albus*) were infected by the unknown *Trypanosoma sp. In vitro* cultivation of the trypanosome is necessary in order to be able to harvest adequate parasites for further research into cell cycle stages or genomic study.

Materials and Methods

Eels (*Monopterus albus*) were obtained from fresh market and supplier around Kuala Terengganu. It was then maintained in the tank before further examination. Blood collection was done by injecting through the caudal vein using 23 - 24G needle and 1ml heparinised syringe. *Trypanosoma sp.* was examined using Hematocrit Centrifugation Technique (Woo, 1969). Culture medium was prepared based on the Jones and Woo, 1991 with a little bit modification. Blood agar diphasic medium was prepared using Bacto Starch Agar and Liquid medium contained of 50% dilution of BME (Eagle Basal Medium with Hank's Salt and L-glutamine; Gibco Laboratory) that supplemented with 2.5% (v/v) heat activated bovine serum, 25mM HEPES (pH 7.3) and Antibiotic-Antimycotic (GibcoBRL, Cat. No: 15240-062). The culture media was then inoculated with 0.5 ml of infected sterile blood. Cultured *Trypanosoma sp.*was observed every three hours for 24 hours and after that two days interval. PCR-RAPD was applied for genomic study of *Trypanosoma sp.* DNA extraction was done using phenol-chloroform method and then run through 0.8% agarose gel electrophoresis to determine the purity and quantity of DNA. Primer screening was then carried out using the GIBCO primer (A01-A20).

Results and Discussion

Experiment on culturing of *Trypanosoma sp.* in-vitro was done successfully in diphasic medium of Blood Bacto Agar and Eagle Basal Medium 50/2.5 as developed by S.R.M. Jones and P.T.K. Woo, 1991. At the time infected eel's blood was isolated, *Trypanosoma sp.* was in a very small number at matured stage and single form but different sizes. After 15hrs incubation, all individual assumed different shapes such as double divided form and tetra divided form or like a rosette form. Other forms that were detected in the culture medium were small stumpy form, bigger stumpy form and epimastigote form in a very small number. After 21hrs of incubation, small stumpy form, bigger stumpy form and epimastigote increased mostly double from before (at 15hrs), no more tetra divided form was seen. The tetra divided form rarely occurred in other cultivation of *Trypanosoma sp.* and may be this is an abnormal case. After 48hrs, the growths of the flagellates were still increasing but only double divided and epimastigote was seen. Small number of metacyclic trypomastigote form was seen after 72 hours and double divided and epimastigote form also increased. After 96 hrs more metacyclic trypomastigote was formed and high number of double divided and epimastigote forms were seen. Eagle Basal Medium gave better growth to the cultivation of the blood flagellate compared to other such as RPMI. It was widely used to culture other species of trypanosomes such as in growing *Trypanosoma catostomi* and *Trypanosoma phaleri.* Blood source also influence the growth and it act as the nutrient supply to the medium. L-glutamine in the Eagle Basal Medium was the co-factor of the trypanosomes growth. The growth profile was sigmoid shape, after reaching the maximum point the growth decreased. The factor which probably caused the decrease in number of growth could be substrate limitation, space limitation or accumulation of inhibitory product (P.F. Stanbury & A. Whitaker). Sigmoid growth profile pattern also occurred in culture of *Trypanosoma danilewskyi* (R. Wang & M. Belosevic, 1994), *T. catostomi* and *T. phaleri* (S.R.M. Jones & P.T.K. Woo, 1991) and *T.brucei, T. rhodensiense* and *T.gambiense* (Brun et al. 1981). PCR-RAPD was not successful in experiment because there was insufficient pure quantity and quality of DNA extracted. Hence the low yield affected banding patterns which were not clearly visible.

Conclusions

Trypanosoma sp. in-vitro was done successfully in diphasic medium of Blood Bacto Agar and Eagle Basal Medium 50/2.5

Benefits from the study

This study showed that Trypanosoma sp from the freshwater swamp eel could be cultivated in –vitro and hence further experiments can be carried out on the blood flagellates.

Patent(s), if applicable :
Nil

Stage of Commercialization, if applicable :
Nil

Project Publications in Refereed Journals:
Nil

Project Publications in Conference Proceedings
1. Jili, N., Mustafa B. and F.M. Shaharom-Harrison 1999. Morphology, morphometric and infection aspects of blood flagellate (*Trypanosoma sp*) in rice paddy eel (*Monopterus albus*, Zuiew) Malaysian Science & Technology Congress, 25-27 October, 1999. Ming Court Hotel, Kuala Lumpur, Malaysia.

2. Rustan E.Ismail, Faizah Shaharom Harison, A, M Samad (2000). In-vitro Cultivation of *Trypanasoma* sp. of eel, *Monopterus albus* using diphasic define medium. Malaysia Biotechnology Conference 2000. Monarch University Petaling Jaya, Selangor.

3. Rustan E. Ismail and F.M Shaharom-Harisson. Morphology of in vitro vultivated *Trypanasoma sp.* from blood of freshwater eel, *Monopterus albus.* Congress of Science and Technology Malaysia (COSTAM) 2001, MOSTE, Sutera Harbour, Kota Kinabalu, Sabah.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Mohd Rustan Effendie b. Ismail	In-vitro Cultivation of *Trypanasoma* sp. of eel, *Monopterus albus*	Biotechnology		
Anis Mazidah bt Abd Samad	A Study of Some Aspect On *Trypanosoma sp.* from Swamp Eel (*Monopterus Albus*)	Fish Health		

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