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Komunikasi Pendek/*Short Communication*

Survivability of *Acanthamoeba* Strains Isolated from Clinical and Environmental Specimens During Axenization

(Kemandirian Strain *Acanthamoeba* Pencilan Klinikal dan Persekitaran Semasa Proses Aksenisasi)

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ABSTRACT

Acanthamoeba is a free living protozoa that can cause keratitis and granulomatous amoebic encephalitis. Physiological characteristics of this amoeba are found to have a medical importance in which it can be related to the pathogenicity potential of the organism. This study was carried out to investigate the physiological characteristics of survivability during axenization. Six Acanthamoeba strains from three clinical isolates (HSB 1, HKL 48 and HKL 95) and three environmental isolates (PHS 2, PHS 11 and PHS 15) were used in this study. Axenization test was done by treating cysts with hydrochloric acid (3%) and Page saline containing Gentamicin (100 µg/ml). Cysts were then cultured into PYG enrich media, incubated at 30°C and the presence and proliferation of trophozoites of Acanthamoeba were observed. This study showed that PHS 15, HSB 1, HKL 48 and HKL 95 could be axenized but they have poor proliferation rate in PYG enrich media. The result showed that the difference between both clinical and environmental isolates was observed in two strains; PHS 2 and PHS 11. This indicates that there is a possibility that the physiological traits of strains from both isolates are the same and strains from the environment are able to show the pathogenic potential and capable of causing infection to human.

Keywords: Axenization, Survivability, Acanthamoeba, Clinical and environmental strains

ABSTRAK

Acanthamoeba adalah protozoa hidup bebas yang mampu menyebabkan penyakit keratitis Acanthamoeba dan ensefalitis amebik bergranuloma. Ciri fisiologikal ameba ini didapati mempunyai kepentingan perubatan yang boleh dikaitkan dengan potensi patogenisiti. Kajian ini dilakukan untuk mengkaji ciri fisiologikal Acanthamoeba dari aspek kemandirian semasa aksenisasi. Enam pencilan Acanthamoeba iaitu tiga pencilan klinikal (HSB 1, HKL 48 dan HKL 95) dan tiga pencilan persekitaran (PHS 2, PHS 11 dan PHS 15) telah digunakan dalam kajian ini. Ujian aksenisasi dilakukan dengan merawat sista menggunakan asid hidroklorik (3%) dan salin Page yang mengandungi larutan Gentamicin (100 µg/ml). Sista ini kemudiannya dikultur di dalam media pengkayaan PYG, dieramkan pada suhu 30°C dan pemerhatian dilakukan untuk melihat kehadiran trofozoit dan pembiakannya. Bagi keputusan kemandirian aksenisasi, PHS 15, HSB 1, HKL 48 dan HKL 95 boleh diaksenisasi namun tidak dapat membiak dengan baik dalam media pengkayaan PYG. Hasil kajian mendapati perbezaan antara pencilan klinikal dan persekitaran hanya berlaku pada dua pencilan; PHS 2 dan PHS 11 iaitu semasa proses aksenisasi sahaja. Ini menunjukkan terdapat kemungkinan ciri fisiologikal pencilan dari kedua-dua persekitaran ini adalah sama dan pencilan dari persekitaran mampu menunjukkan potensi patogenisitinya sekaligus boleh mengakibatkan jangkitan kepada manusia.

Kata kunci: Aksenisasi, Kemandirian, Acanthamoeba, Strain klinikal dan persekitaran

INTRODUCTION

Acanthamoeba is a free living amoeba that is widely distributed in nature (Khan et al. 2002; Khan 2003). It is also known as amphizoic amoeba because it is capable of existing both as free living organism in nature and in parasitic form in host (Szenasi et al. 1998). Human are easily expose to this amoeba because of its wide distribution in environment (Schuster & Visvesvara 2004). *Acanthamoeba* is capable causing two main diseases, keratitis *Acanthamoeba* and granulomatous amoebic encephalitis (Visvesvara et al. 2007). However, *Acanthamoeba* keratitis is a relatively rare disease compared on the other forms of infectious keratitis such as bacterial and fungal keratitis (Ibrahim 2007). In a country which has high prevalence of people wearing contact lens, 85-88% of *Acanthamoeba* keratitis cases reported occurred in this population. Meanwhile, *Acanthamoeba* is rarely diagnosed as the cause of keratitis to those who are not wearing contact lens, which results in the late diagnosis of the case (Dart et al. 2009; Radford et al. 2002).

Acanthamoeba can be grown in the absence of external live food organism. This is typically referred to as axenic culture which indicate that no other living organism is

present (Khan 2006). Meanwhile, axenization is the process of excystation of cyst to trophozoite in liquid nutrient. Axenic also refers to a state acquired by laboratory means; in other words artificial (Houk 1964). Axenic culture is used for research work requiring a culture system free from bacterial contaminants (Garcia 2007) since presence of contaminant bacteria could inhibit molecular and biochemical research (Khan 2008). This study was conducted to observe survivability of *Acanthamoeba* Malaysian strainsin axenic culture and improve laboratory method in research involving axenic technique.

Six *Acanthamoeba* strains from three clinical isolates (HSB 1, HKL 48 and HKL 95) and three environmental isolates (PHS 2, PHS 11 and PHS 15) were used in this study. Plate of *Acanthamoeba* cyst was incubated with 3% hydrochloric acid for two days. The cysts were then harvested into microcentrifuge tube and the plate was washed with Page saline for a few times to ensure there was no cyst left. The cysts suspension was then centrifuged at 1500 rpm, and the suspension was then immersed in Page saline containing 100 µg/ml Gentamicin for four hours. Later, the cyst was washed two times with Page saline containing Gentamicin. After the last wash, the cysts suspension was then cultured in 1ml PYG enriched media (4% peptone, 0.75% yeast, 1.5% glucose, 5% serum bovine albumin, 1% vitamin and 100 µg/ml Gentamicin). The plate was incubated at 30°C overnight and the presence of trophozoites and proliferation of *Acanthamoeba* was observed using inverted microscope.

TABLE 1. Ability of Strains of *Acanthamoeba* spp. to be Axenized

Strains	Axenization
HSB 1	+
HKL 48	+
HKL 95	+
PHS 2	–
PHS 11	–
PHS 15	+

Indicator:

\+ Presence of trophozite
– Presence of cyst

TABLE 2. *Acanthamoeba* Survivability in Axenic Media

Strains	Axenized
HSB 1	–
HKL 48	–
HKL 95	+
PHS 2	–
PHS 11	–
PHS 15	+

Indicator:

– Failed to proliferate

In axenization process, four strains (PHS 15, HSB 1, HKL 48 and HKL 95) can be axenized, but cannot proliferate well in axenic culture thus we predicted that they cannot survive in axenic medium fora long duration. To continue to exist, organism need to propagate to ensure their existence in future. From environmental strains, only PHS 15 can be axenized. Eventhough clinical strains have been isolated from similar environment (hot spring), maybe as different trait in each strain thus producing different results. Meanwhile, all clinical strains can be axenized, showing that clinical strains could adapt in this condition.

Walochnik et al. (2000) stated that few isolates could not live in axenic culture. Some *Acanthamoeba* species are naturally fastidious and needs the bacteria as a source of food, so they are not easily axenised (Maghsood et al. 2005). Besides that, genetic differentiation is also another cause why trophozoite failed to survive in axenic medium.

Acanthamoeba trophozoites readily adapt to the perfect environment. When they are confronted with nutrition surplus, it may lead to a down regulation of genes that are no longer required. Shutting off mechanisms that become unessential under changed environmental conditions has been reported for several single-cell organisms (Kohsler 2008). After adapting for a certain period of time in phagocytosis condition in the laboratory, it may be hard for *Acanthamoeba* to change the mechanism to pinocytosis only. Oxygen level is also an important factor during axenization. Weekers and Vogels (1994) stated that oxygen level should be kept above 90% saturation, thus preventing oxygen limitation and cyst formation.

Peptone yeast glucose media have been used widely in various *Acanthamoeba* study but, different concentrations were used. Some strains and possibly new species, however, required a richer medium consisting of PYG base supplemented with serum and a vitamin mixture (Schuster & Visvesvara 1998). In this study, concentration of 4% peptone, 0.75% yeast and 1.5% glucose, added with 5% fetal bovine serum, 1% vitamin and Gentamicin have been used to produced good axenization result compared to other concentration.

From the result, one environmental strains (PHS 15) can be axenized eventhough it cannot proliferate in axenic media. This showed similarity with the other clinical isolates used in this study. In conclusion there is a possibility that environmental strains can exhibit characteristic like clinical strains and may become potential cause of infections.

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