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ScienceDirect

European Journal of Protistology 50 (2014) 33–39

European Journal of PROTISTOLOGY

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A *Colpoda aspera* isolate from animal faeces: In vitro cultivation and identification

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Received 22 February 2012; received in revised form 1 August 2013; accepted 1 August 2013
Available online 18 October 2013

Abstract

A colpodean ciliate was found in the faeces of experimental rabbits. It was initially cultivated in medium mixed with 2% (w/v) rabbit faeces. Subsequently, two chemically defined media, designated CA-1 and CA-2, were found to be suitable for axenical cultivation of the ciliate. The maximum abundance of the ciliate isolate in the CA media was $1–2 \times 10^5$ cells/ml. The ciliate isolate was further identified with silver impregnation and molecular analysis. Features of the left oral polykinetid, somatic dikinetids, and sliverline pattern were similar to those of *Colpoda aspera* as described by Foissner (1993). The 18S small subunit ribosomal RNA gene of the ciliate isolate shared 99% sequence identity with that of *C. aspera*, with 100% coverage, and formed a sister clade in the phylogenetic tree with the reference *C. aspera* isolate. In addition, the trophozoite of *C. aspera* could proliferate over a temperature range from 25–37 °C. When resting cysts were cultivated in CA-1 medium at 30–35 °C, 98.2% of the trophozoites were detached from the cyst wall after 7 h.

Keywords: Colpoda *aspera*; Chemically defined medium; 18S ribosomal RNA gene; Excystment

Introduction

Colpodean ciliates are extremely common, especially in terrestrial habitats such as mosses, soils, and leaf litter (Lynn 2008). More than 170 species of Colpodea and more than 20 species of *Colpoda* have been monographed by Foissner (1993) based on morphological characters. However, compared with other protists, especially parasites such as *Eimeria*, *Toxoplasma* and *Plasmodium*, *Colpoda* has been poorly studied, as colpodeans have little influence on humans or domestic animals (Shen 1999). However, researchers have recently shown a growing interest in colpodeans studying the life cycle of colpodeans including a resting cyst stage. Other main research foci were on their potential as bioindicators of environmental quality and their possible development as eukaryotic vectors to express vaccine antigens or therapeutic proteins (Aguilar-Díaz et al. 2011; Brunk 1999; Forge et al. 1993). In this study, we report a reniform-like ciliate that is occasionally found in the faeces of experimental rabbits and chickens. The ciliate was identified as an isolate of *Colpoda aspera* Kahl, 1926 based on morphological and molecular characteristics. In addition, chemically defined media (designated CA media) were designed and successfully used to clone the *C. aspera* isolate. Additionally, growth condition and proliferation pattern in the CA media were investigated.

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http://dx.doi.org/10.1016/j.ejop.2013.08.001

Material and Methods

Isolation and cultivation

Colpoda aspera was first detected in the faeces of rabbits and also observed in the faeces of chickens. These rabbits and chickens were reared in different isolators in the animal house of our institute. Colpodeans were isolated from rabbit faeces and cultivated in sterilised medium mixed with 2% (w/v) rabbit faeces and phosphate-buffered saline (PBS, pH 7.4) at 30–35 °C, in a statically placed centrifuge tube. A few ciliates in a volume of 10 μl of the culture supernatant were transferred to a new tube containing the mixed medium, which was prepared as follows: fresh rabbit droppings were ground to a powder after baking at 80 °C for 24 h. The faecal powder was mixed with sterilised PBS at a proportion of 2% (w/v). The ciliates were subcultured in the mixed medium every 4 d.

Chemically defined media designed to axenically cultivate the ciliate

In order to obtain a clonal strain of *C. aspera*, several chemical recipes were designed and tried, and two media supplemented with 500 U/ml penicillin and 0.5 mg/ml streptomycin, named CA-1 and CA-2, respectively, were found to be suitable for axenic cultivation of this *Colpoda* species. A few ciliates in 10 μl of culture supernatant from the mixed medium were inoculated into CA medium, cultivated in centrifuge tubes at 30–35 °C, and subcultured every 4 d. To clone the *Colpoda* species, a 96-well cell culture plate was inoculated with only one ciliate cell per well in 100 μl CA medium. Four days later, the ciliate culture from axenic wells was transferred as described above. After three passages in a 96-well cell culture plate, the ciliate culture from axenic wells was transferred into screw-capped bottles with CA media and cultivated at 30–35 °C. CA-1 medium was composed of 2.5 g tryptone, 2.5 g proteose peptone, 1 g sodium acetate trihydrate, 1 g glucose, 0.1 g yeast extract, 0.1 g magnesium sulphate, 0.002 g vitamin B1, 1 g dipotassium hydrogen phosphate, 0.15 g sodium silicate, 1.2 g sodium chloride, 0.06 g sodium sulphate, 0.065 g calcium chloride, 0.035 g magnesium chloride, and 0.05 g ferric chloride in 1 l of distilled water. CA-2 medium was composed of 20 g proteose peptone, 1 g yeast extract, 2 g glucose, 0.03 g Sequestrene (Na-Fe-EDTA, supplied by Sinopharm Chemical Reagent Co., Ltd., China), 0.15 g sodium silicate, 1.2 g sodium chloride, 0.06 g sodium sulphate, 0.065 g calcium chloride, 0.035 g magnesium chloride, and 0.05 g ferric chloride in 1 l of distilled water.

Microscopic observation

The morphology of live and silver-impregnated specimens was investigated under a light microscope at 1000 × magnification. The abundance in CA media was estimated under a light microscope at 100 × magnification. Different containers, temperatures, and nutritional components were investigated for ciliate cultivation. Excystment in chemically defined medium was investigated for the resting cysts of the ciliate.

Silver impregnation

Silver impregnation was carried out according to the 'wet' sliver nitrate method and the silver carbonate method of Foissner (1993). Ciliate cells from CA culture were purified by centrifugation before the process of silver impregnation.

Molecular characterisation

Ciliate cells from one clone in CA culture were purified by centrifugation. The purified cells were directly used as the polymerase chain reaction (PCR) template. The 18 S small subunit (SSU) ribosomal RNA gene of the ciliate was amplified by PCR, in which 2.5 μl of the purified cells (about 10 ciliates) were used as the template with a pair of universal primers: Euk A (5′-AACCTGGTTGATCCTGCCAGT-3′) and Euk B (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) (Medlin et al. 1988). The PCR programme was as follows: predenaturation at 94 °C for 130 s, followed by 35 cycles with denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 130 s, and a final extension at 72 °C for 10 min. The products were separated by gel electrophoresis on a 1.0% agarose gel stained with Golden View (supplied by Beijing Solarbio Science & Technology Co., Ltd., China). The target DNA band was purified using a gel extraction kit (supplied by Omega Bio-Tek, USA). The purified DNA fragment was cloned into the pEASY-T1 Simple cloning vector. DNA sequencing was performed using universal M13 primers. The sequences were uploaded into the Basic Local Alignment Search Tool programme to search for the most similar reference sequences. The phylogenetic analysis was performed using the MegAlign programme (supplied by DNAstar Inc., USA). The tree was constructed using the Clustal W method of the MegAlign programme. The reference sequences of other ciliates are listed in Table 1.

Results

Isolation and cultivation in chemically defined media

When the ciliate was cultivated in the mixed medium with 2% (w/v) faecal powder at 30–35 °C, the maximum abundance was about 5×10^4 cells/ml on the third day after inoculation. Abundance decreased markedly on the fifth day and resting cysts emerged in the subsequent period. Six months after inoculation, a small number of vegetative cells