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ScienceDirect
European Journal of Protistology 47 (2011) 16–23
European Journal of PROTISTOLOGY
www.elsevier.de/ejop

Oramoeba fumarolia gen. nov., sp. nov., a new marine heterolobosean amoeboflagellate growing at 54 °C

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Received 31 March 2010; received in revised form 3 September 2010; accepted 6 September 2010

Abstract

An amoeba strain was isolated from marine sediment taken from the beach near a fumarole in Italy. The trophozoites of this new marine species transforms into flagellates with variable numbers of flagella, from 2 to 10. The strain forms round to oval cysts. This thermophilic amoeboflagellate grows at temperatures up to 54 °C. Molecular phylogenetic analysis of the small subunit ribosomal DNA (SSU rDNA) places the amoeboflagellate in the Heterolobosea. The closest relatives are *Stachyamoeba* sp. ATCC50324, a strain isolated from an ocean sample, and *Vrihiamoeba italica*, a recent isolate from a rice field. Like some other heterolobosean species, this new isolate has a group I intron in the SSU rDNA. Because of the unique place in the molecular phylogenetic tree, and because there is no species found in the literature with similar morphological and physiological characteristics, this isolate is considered to be a new genus and a new species, *Oramoeba fumarolia* gen. nov., sp. nov.

Keywords: Heterolobosea; ITS; New species; SSU rDNA; Thermophilic

Introduction

The class Heterolobosea was established for amoebae, which move with eruptive pseudopodia, have intranuclear orthomitosis (promitosis), have mitochondrial cristae which are flattened, often disc-like, and in which stacked Golgi bodies are absent (Page and Blanton 1985). At the time of establishing this class of protists, the Heterolobosea contained amoebae and amoeboflagellates. In the latter there is transformation from an amoeba stage into a temporary flagellate stage, and vice versa. Recently it was found that *Percolomonas* and *Pleurostomum*, which were originally placed within the heterotrophic flagellates, and *Stephanopogon*, originally placed in the Ciliophora, also belong to the Heterolobosea (Nikolaev et al. 2004; Park et al. 2007; Yubuki and Leander 2008; Cavalier-Smith and Nikolaev 2008). These three genera had remained incertae sedis for a long time, but molecular phylogenetic analysis places them within the Heterolobosea, a conclusion which is supported by ultrastructural data. However, in *Pleurostomum flabellatum*, one of the characteristics of Heterolobosea, mitochondrial cristae, were not observed (Park et al. 2007). In all the Heterolobosea a unique characteristic insertion in the secondary structure (helix 17-1) of the small subunit ribosomal

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doi:10.1016/j.ejop.2010.09.002

DNA (SSU rDNA) is found (Nikolaev et al. 2004), except in '*Macropharyngomonas halophila*', which has a basal position in the heterolobosean phylogenetic tree.

While the majority of heterolobosean species had been found in fresh water habitats and soil (Page 1988), some were isolated from marine (Page 1983) and even hypersaline habitats (Park et al. 2007). We report the isolation of a thermophilic marine amoeboflagellate from a beach near a fumarole. The strain was studied by light microscopy and the SSU rRNA gene sequence analysis shows that it belongs to a new genus in the Heterolobosea.

Materials and Methods

The strain under study, SAN2, was isolated from a sediment sample taken in October 2000 in Italy. The sediment, varying in temperature from 55 °C to 70 °C, originated from the beach near a fumarole at San Angelo on the island Ischia (40°43′N, 13°54′E). Ten ml of these sediment samples were incubated at 45, 50 and 55 °C in synthetic seawater BisTris buffered medium (SME-Medium) with 0.1% (v/v) glycerol and 0.05 (w/v) yeast extract. *Pseudomonas* sp. or *Marinobacter* sp. (Baumgartner et al. 2002) was added as food source. For growth on agar plates, agar was added to a final concentration of 1.8% to SME-Medium and food bacteria were spread on the plates. The strain was also grown in Page Amoeba Saline (PAS) (Page 1988), supplemented with 3% NaCl, and *Escherichia coli* as food source.

The morphology of moving amoebae attached to glass surfaces was studied at 50 °C using phase contrast optics with a Zeis Axiovert inverse microscope (Baumgartner et al. 2003). Length and breadth dimensions of 100 actively moving trophozoites were determined. The size of 20 cysts was measured after growth on agar plates. The number of flagella was counted on 100 cells.

The optimal growth temperature of the isolate was determined in liquid SME-Medium. In addition, agar plates with lawns of *Marinobacter* sp. were incubated at different temperatures. The diameter of the grazed area was measured at regular intervals (Baumgartner et al. 2003).

DNA extraction, amplification of the SSU rRNA gene by PCR, and sequencing were carried out as described previously (Baumgartner et al. 2002). The internal transcribed spacers (ITS), including the 5.8S rDNA, sequences were obtained as described by De Jonckheere and Brown (2005). The SSU rRNA sequence of strain SAN2, omitting 1047 bp of an intron, was placed into an alignment of selected Heterolobosea SSU rRNA sequences obtained from the on-line comprehensive ribosomal RNA database Silva (http://www.arb-silva.de/; Pruesse et al. 2007). The SSU RNA sequence from strain SAN2 was manually aligned to this set. A total of 741 of unambiguously aligned sites common to all sequences was retained for phylogenetic analysis. This alignment is available upon request. Phylogenetic trees were inferred by distance matrix neighbor-joining as implemented in ClustalX version 1.8 (Thompson et al. 1997), maximum likelihood (Felsenstein 1981) as implemented by the program PhyML version 2.4.5 (Guindon and Gascuel 2003), and by Bayesian analysis using MrBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001). The general time reversible (GTR) model of evolution (Tavaré 1986) was selected as the best model from 28 using the ModelFind program (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). The optimal parameters for the GTR model were estimated using the PhyML program. This model extended with gamma-shaped rate variation ($\alpha = 0.89$) with four rate categories and a proportion of 0.19 of invariable sites was used for both maximum likelihood (100 bootstrap samplings in PhyML) and Baysian analysis (MrBayes). To estimate Bayesian posterior probabilities, Markov Chain Monte Carlo (MCMC) chains were run for 105,000 generations until convergence and sampled every 100 generations (burn-in: 250 generations).

The final dataset for tree construction comprised 29 other Heterlobosea taxa (accession numbers): *Tetramitus aberdonicus* (AJ224888), *T. jugosus* (M9805), *T. entericus* (AJ224889), *T. rostratus* (M98051), *T. lobospinosus* (M98052), *T. thermacidophilus* (AJ621575), *Vahlkampfia avara* (AJ 22488), *V. inornata* (AJ22488), *Naegleria fowleri* (U80059), *N. andersoni* (U80057), *Willaertia magna* (X93221, X93223 and AY266315), *P. flabellatum* (DQ979962), *Tulamoeba peronaphora* (FJ222603), *Acrasis rosea* (AF011458), *Neovahlkampfia damariscottae* (AJ224891), *Paravahlkampfia ustiana* (AJ224890), *Heteramoeba clara* (AF011460), '*Plaesiobystra hypersalinica*' (AF011459), *Psalteriomonas lanterna* (X94430), *Sawyeria marylandensis* (AF439351), *Monopylocystis visvesvarai* (AF011463), *Stachyamoeba* sp. ATCC 50324 (AF011461), *Percolomonas cosmopolitus* (AF519443 and AF011464), *Stephanopogon minuta* (AB365646), '*M. halophila*' (AF011465), *Marinamoeba thermophila* (FM244741), *Vrihiamoeba italica* (AB513360) and the uncultured heterolobosean clone WIM43 (AM114803). The sequences of the Euglenozoa *Euglena gracilis* (AY029409) and *Trypanoplasma borreli* (AY028454) were used as outgroups.

Results

Most observations were performed on amoebae growing on *Marinobacte*r sp. although the isolate also grows well on *Pseudomonas* sp. and *E. coli*. Where indicated, observations were made while organisms were growing on *Pseudomonas* sp. or *E. coli*. In liquid medium the isolate grows optimally at a temperature of 50 °C, with an upper limit of 54 °C. The growth rate on agar plates was measured by the distance covered by the migrating ring of amoebae. On agar plates, the isolate grows at temperatures between 25 °C and 52 °C, but optimally at 50 °C. The amoebae move with eruptive pseudopodia and appear in two forms, triangular shaped (Fig. 1a