RESEARCH ARTICLE

A new Thraustochytrid, strain *Fng1*, isolated from the surface mucus of the hermatypic coral *Fungia granulosa*

Moshe Harel[1,2], Eitan Ben-Dov[1,3], Diana Rasoulouniriana[1], Nachshon Siboni[1], Esti Kramarsky-Winter[4], Yossi Loya[4], Ze'ev Barak[2], Zeev Wiesman[1] & Ariel Kushmaro[1]

[1]Department of Biotechnology Engineering and National Institute for Biotechnology, Ben-Gurion University of the Negev, Be'er-Sheva, Israel; [2]Department of Life Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel; [3]Achva Academic College, M.P. Shikmim, Israel; and [4]Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Correspondence: Ariel Kushmaro, Department of Biotechnology Engineering and National Institute for Biotechnology, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel. Tel.: +972 8 647 9024; fax: +972 8 647 2983; e-mail: arielkus@bgu.ac.il

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Abstract

Recent evidence suggests that there is a dynamic microbial biota living on the surface and in the mucus layer of many hermatypic coral species that plays an essential role in coral well-being. Most of the studies published to date emphasize the importance of prokaryotic communities associated with the coral mucus in coral health and disease. In this study, we report the presence of a protist (*Fng1*) in the mucus of the hermatypic coral *Fungia granulosa* from the Gulf of Eilat. This protist was identified morphologically and molecularly as belonging to the family *Thraustochytridae* (phylum *Stramenopile*, order *Labyrinthulida*), a group of heterotrophs widely distributed in the marine environment. Morphological examination of this strain revealed a nonmotile organism *c.* 35 μm in diameter, which is able to thrive on carbon-deprived media, and whose growth and morphology are inoculum dependent. Its fatty acid production profile revealed an array of polyunsaturated fatty acids. A similar protist was also isolated from the mucus of the coral *Favia* sp. In light of these findings, its possible contribution to the coral holobiont is discussed.

Introduction

A number of studies have shown that there is a dynamic microbial biota living on the surface and in the tissue of many coral species, although the role of the biota in the reef ecosystem remains, for the large part, a mystery (Kushmaro *et al.*, 1996; Rohwer *et al.*, 2001, 2002; Ritchie & Smith, 2004; Koren & biota Rosenberg, 2006). These microorganisms include commensal, endosymbiotic and parasitic forms. Together with the scleractinian host, these microorganisms comprise what is termed a holobiont (Kushmaro *et al.*, 1996; Santavy & Peters, 1997; Harvell *et al.*, 1999; Rohwer & Kelly, 2004; Johnston & Rohwer, 2007). One possible function of microorganisms found on coral surfaces is conceivably to provide them with protection from pathogens (Rohwer *et al.*, 2002; Reshef *et al.*, 2006; Ritchie, 2006; Rosenberg *et al.*, 2007). Another role is to supply the coral host with nitrogen and phosphorus not provided by their symbiotic zooxanthellae (Sorokin, 1973, 1978; Anthony, 1999, 2000; Rosenfeld *et al.*, 1999; Anthony & Fabricius, 2000). The transfer of nutrients from the surroundings occurs via the highly productive, mucus-rich microlayer, which extends a few millimetres above the surface tissue of the coral (Paul *et al.*, 1986). This mucus layer also acts as a growth medium for microorganisms and assists the coral in entangling prey or particulate food (Schlichter & Brendelberger, 1998; Goldberg, 2002).

Specialized mucus cells present in the coral epidermis secrete mucus (Goldberg, 2002). Intact mucus contains polymers that form a highly hydrated viscoelastic polymeric gel, consisting of fucose, arabinose, mannose, galactose and glucose residues as well as a number of fatty acids and proteins (Ducklow & Mitchell, 1979; Krupp, 1981; Meikle *et al.*, 1988). The differences in the ratio of protein to carbohydrate, glucose content and amino acid composition of mucus from different corals demonstrate that there is no universal chemical composition to coral mucus (Meikle *et al.*, 1988). Therefore, this niche is specific to the coral

and is different from the surrounding seawater environment.

In an attempt to identify the microorganisms that inhabit the mucus layer enveloping hermatypic corals in the Gulf of Eilat (see Kushmaro & Kramarsky-Winter, 2004; Kramarsky-Winter *et al.*, 2006), we have recently succeeded in isolating an axenic culture (based on microscopic observation and 18S rRNA gene analysis) of fungus-like protists belonging to the *Thraustochytridae*. Thraustochytrids are widely distributed marine heterotrophs (saprophytic or occasionally parasitic) that are believed to have major impacts on the marine environment (Raghukumar *et al.*, 1994, 2000; Honda *et al.*, 1998; Kimura *et al.*, 2001; Fan *et al.*, 2002; Anderson *et al.*, 2003; Kvingedal *et al.*, 2006). Some species were found to be potential sources for valuable bioactive compounds such as polyunsaturated fatty acids (PUFA) or carotenoids (Lewis *et al.*, 1999; Fan *et al.*, 2001; Carmona *et al.*, 2003).

Thraustochytrids are a family in the order *Labyrinthulida* (Raghukumar, 2002; Leander *et al.*, 2004) that are considered by Patterson (1999) to be part of the *Stramenopiles*. Morphologically, they are characterized by a cell wall arranged in a lamellar form (Darley *et al.*, 1973). Almost all species develop ectoplasmic extensions from one or more points on the cell. This branched network of plasma membrane extensions is generated by a unique organelle termed the bothrosome (or sagenogenetosome), which is located at the periphery of the cell. Many of the species exhibit a gliding mobility associated with these ectoplasmic networks (Perkins, 1973; Leander & Porter, 2001; Raghukumar, 2002; Leander *et al.*, 2004). They possess multi-staged life cycles. The vegetative stages of most of these organisms consist of single, granular cells, which are globose to subglobose, measuring 4–20 μm in diameter (Raghukumar, 2002). Most Thraustochytrids reproduce by means of zoospores, which possess a long anterior, tinsel flagellum along with a short, posterior, whiplash flagellum (Porter, 1990; Raghukumar, 2002). The phylogeny of this group is based on the similarity between their 18S rRNA gene sequences, cell morphology and reproductive mode (Darley *et al.*, 1973; Cavalier-Smith, 1978, 1993; Honda *et al.*, 1999; Leander & Porter, 2001). To date, six genera and 38 species have been identified molecularly under the *Thraustochytridae* (Honda *et al.*, 1999, http://www.ncbi.nlm.nih.gov/Taxonomy).

In the present paper, we describe a Thraustochytrid isolated from the mucus layer collected from the surface of the scleractinian coral *Fungia granulosa*. This protist (strain *Fng1)* was identified phylogenetically (by 18S rRNA gene and 16S mitochondrial rRNA gene sequences), morphologically (by light and electron microscopy), as well as by its fatty acid profile and growth characterizations. The possible role of this PUFA-producing microorganism in the coral holobiont is discussed.

Materials and methods

Sampling methods

Using SCUBA, an individual hermatypic coral *F. granulosa* (*Fungiidae*) and fragments from four colonies of the *Favia* sp. corals were collected at a depth of 15 m from the Gulf of Eilat (29.8°N, 34.55°E), Israel, in January 2004 and November 2006, respectively. The corals were collected in individual plastic bags, brought to the surface and rinsed in filtered seawater. The surface mucus was then immediately sampled using a sterile pipette or a funnel. Approximately 500 μL of this mucus was immediately inoculated to B1 agar plates (Bremer, 2000) for isolation. This medium consists of 0.1% peptone (Sigma), 0.1% yeast extract (Sigma) and 1.5% bacteriological agar (Fluka) (Jensen & Fenical, 1994) in 0.22 μm filtered seawater with 500 mg L^{-1} streptomycin sulphate (Sigma) and 300 mg L^{-1} penicillin G (Sigma). The antibiotics were prefiltered in a 0.22 μm filter and added to the mixture after it was autoclaved and its temperature was brought down to room-temperature levels. Optimization of culture growth was carried out in B1 liquid and solid media with a sea water base (Bremer, 2000). Growth was monitored in cultures incubated at 20–30 °C and at a pH of 7.5. Samples from *Favia* sp. corals were first grown on B1 liquid media before being transferred to solid B1 agar plates and treated as above.

Biochemical characterization of *Fng1*

We chose to characterize the isolate from *F. granulosa*. In order to characterize the cultured organisms, carbon sources, which are essential for an organism to develop, were used. The essential carbon sources provide the criteria in the identification of a new taxon (Staley & Krieg, 1989). We used mineral medium (SM medium) containing 3.6% NaCl, 0.1% NH_4NO_3, 0.02% $MgSO_4 \cdot 7H_2O$, 0.1% K_2HPO_4, 0.1% $CaCl_2 \cdot 2H_2O$, 0.015% KCl, 0.005% yeast extract (as a vitamins source), 0.1% $FeSO_4 \cdot 6H_2O$, 0.1% $ZnSO_4 \cdot 7H_2O$, 0.1% $MnSO_4$ or 0.1% $MnCl_2$ and 1.5% bacteriological agar. To this medium we added a different carbon source each time, as shown in Table 1 (Insam, 1997; Choi & Dobbs, 1999) for single carbon source utilization analysis. The percent carbon source added was 0.5%. SM medium with 0.5% peptone and a treatment with 0.005% yeast extract (as a vitamin source) without additional carbon sources were used as controls. An axenic culture of *Fng1* (based on microscopic observations of colonies from subsequent plating on B1 agar and 18S rRNA gene sequence analyses), which had grown in a shaker for 2 days in Marine broth 2216 (HiMedia Laboratories, Mumbai, India), was centrifuged at the maximum speed for one minute; the supernatant was removed. The remaining pellet was washed twice with sterile inert salt medium containing 4% NaCl, 0.68% KH_2PO_4 and

Table 1. List of PCR primers

Primer	Length (bp)	Sequence (5′ → 3′)	Target
8F*	26	GGATCCAGACTTTGATYMTGGCTCAG	Bacterial 16S rRNA gene
1512R†	30	GTGAAGCTTACGGYTAGCTTGTTACGACTT	Bacterial 16S rRNA gene
NSF4‡	18	CTGGTTGATYCTGCCAGT	Eukaryote 18S rRNA gene
NSF370‡	18	AGGGYTCGAYYCCGGAGA	Eukaryote 18S rRNA gene
NSF573‡	19	CGCGGTAATTCCAGCTCCA	Eukaryote 18S rRNA gene
NSF963‡	18	TTRATCAAGAACGAAAGT	Eukaryote 18S rRNA gene
NSR1787‡	18	CYGCAGGTTCACCTACRG	Eukaryote 18S rRNA gene
Thr404f§	26	CTGAGAGACGGCTACCACATCCAAGG	*Thraustochytridae* 18S rRNA gene
Thr1017r§	27	GACTACGATGGTATCTAATCATCTTCG	*Thraustochytridae* 18S rRNA gene

*Based on Felske *et al*. (1997) and modified by shortening three bases from its 5′ end.
†Based on Felske *et al*. (1997).
‡From the European Ribosomal RNA Database (http://bioinformatics.psb.ugent.be/webtools/rRNA/index.html).
§Primers were designed using PRIMER3 (http://frodo.wi.mit.edu).

0.115% NH_4HCO_3 in distilled water and resuspended in the same medium. Aliquots of 50 μL of *c*. 10^{10} cells mL^{-1} were applied to duplicates of agar plates with different carbon sources; the dishes were incubated at 22 °C and were examined under a stereoscope twice: 1 week and 1 month after the inoculation. In order to ascertain whether there is development of ectoplasmic extensions from the colonies a 72-h continuous microscopic observation was carried out on samples in B1 liquid medium (Bremer, 2000).

Fatty acid analysis

The isolated strain was cultured in 1 L of B1 liquid medium with a sea water base at 26 °C and incubated on a horizontal shaker at 150 r.p.m. for 72 h, under a natural light/dark regime to produce a high biomass. The cells were collected by centrifugation and washed from the remaining media in phosphate-buffered saline (PBS) × 3. Fatty acids were isolated in two stages of fractionation. Initially, the samples were fractionated in 2-mL vials with chloroform and water. The chloroform fraction was collected and evaporated with N_2. The dried samples were dissolved with hexane and fractionated with water. The hexane fraction was used for methylation according to the procedure reported by Dionisi *et al*. (1999). Dried samples (100 mg) were *trans*-esterified using 1 mL of methanolic HCl (1.5 M) and 1 mL methanol at 80 °C for 10 min. Two milliliters of water was added and, after mixing and low-speed centrifugation, the upper phase was collected for GC-MS. The operating GC (Agilent Technologies, 6890N Network GC system, Germany; with an HP-5.ms column and Flame Ionization Detector) conditions were as follows: the column temperature was increased from 120 to 190 °C at a rate of 4 °C min^{-1}, the injector temperature was set to 250 °C and the detector temperature was set to 300 °C. The linear velocity of the nitrogen carrier gas was 30 cm s^{-1}; hydrogen was 20–30 cm s^{-1} and air was 300 cm s^{-1}. Fatty acids were identified both by their retention time and their mass spectrum (Agilent Technologies, 5973 Network Mass Selective Detector, model G 2589A, Germany) according to the GC-MS library (Nist02, Mass Spectral Library). The percentage of each acid was calculated according to the formula: % X = (Area X × 100)/ Total Area.

Transmission electron microscopy (TEM)

Colonies from an agar plate were fixed with 3% glutaraldehyde in 0.22 μm filtered sea water for 1 h, then washed and gently mixed with 3% bacteriological agar, treated with osmium, dehydrated, embedded in Araldite epoxy resin and sectioned at 70–80 nm (Hoppert & Holzenburg, 1998). The resulting sections were stained with uranyl acetate and lead citrate and examined using a JEM-1230 transmitted electron microscope at an 80-kV excitation voltage.

DNA extraction and PCR amplification

Genomic DNA from newly formed axenic thraustochytrid colonies was extracted using the NucleoSpin-Food kit (Macherey-Nagel, Düren, Germany). Amplification of rRNA gene was performed simultaneously with universal primer pairs for the 16S rRNA gene (8F and 1512R) and the 18S rRNA gene (NSF4 and NSR1787), as described in Table 1. All the PCR primer pair amplifications were carried out using a Mastercycler gradient thermocycler (Eppendorf, Westbury, NY) as follows: initial denaturation (4 min at 95 °C), followed by 30 cycles of denaturation (30 s at 94 °C), primer annealing (40 s at 50 °C), a primer extension (90 s at 72 °C) and a final extension step (20 min at 72 °C). Sequencing was performed on an ABI PRISM 377 DNA Sequencer (Applied Biosystems) for 16S and 18S rRNA genes with a set of universal and specific primers (see Table 1).

Phylogenetic analysis

The 18S rRNA gene sequences belonging to the *Thraustochytridae* family were obtained from the NCBI GenBank (Benson *et al.*, 2000; Wheeler *et al.*, 2000) with the basic local alignment search tool BLAST network service (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). A phylogenetic tree (Fig. 3) was constructed by the neighbour-joining method (Saito & Nei, 1987) with the MEGA package (Kumar *et al.*, 2004), using bootstrap resampling analysis (Felsenstein, 1985) for 100 replicates to estimate the confidence of tree topologies.

Nucleotide sequence accession number

The rRNA gene sequences were deposited in the NCBI GenBank and given accession numbers AY870336 and EU264109–EU264112 (18S rRNA gene) and AY870337 (16S rRNA gene).

Results

Isolation and culture of *Fng1*

Strain *Fng1* was isolated from the mucus of the coral *F. granulosa*. In addition, four morphologically similar strains were isolated from four different *Favia* sp. corals. The colonies isolated from *F. granulosa* in all the agar plates showed identical morphology and colour and were cultured to achieve axenic cultures. Once an axenic culture was achieved, it was thereafter reinoculated on marine media and thus a continuous culture was developed.

The colonies tended to form aggregates in liquid media. In solid media (marine agar plates), the time that was required by the cells to form a colony depended on the cell density in the inoculum. Spread inoculum formed colonies within 2–3 weeks while the same concentration of cells in the form of a drop took 2 days to form colonies on the agar plates. The colonies formed were *c.* 0.5 mm in diameter (Fig. 1a) and appeared yellowish on B1 and marine agar plates. Growth was achieved at a range of temperatures (20–30 °C) with an optimal growth at 22 °C. *Fng1* was found to grow on media with a salinity range of 0.75–10% NaCl with optimal growth at 3.5% NaCl.

Biochemical characterizations of *Fng1*

By comparing the amount, size and distribution of the *Fng1* colonies on a variety of growth media, we assessed their biochemical character. We compared *Fng1* colonies grown on agar plates with peptone with those grown on a variety of plates with single carbon sources and a basic mineral medium–SM agar (with limited viable carbon source) (Table 2). The evaluation was indexed into three levels representing the rate of growth on a certain carbon source. Optimal growth was observed on peptone-, glycerol- and sucrose-enriched media, and no growth was observed on agar plates with L-Arginine and TWEEN® 20 (Table 2).

The lipid profile of *Fng1* demonstrated a diverse spectrum, with about 75% of long-chain unsaturated fatty acids of the total fatty acids present (Table 3). The strain constituted 50% of oleic acid (including two ω7 and ω10 isomers). In addition, *Fng1* constituted PUFAs as 10% of α-linolenic acid (ALA, 18:3), 1.7 % of docosahexaenoic acid

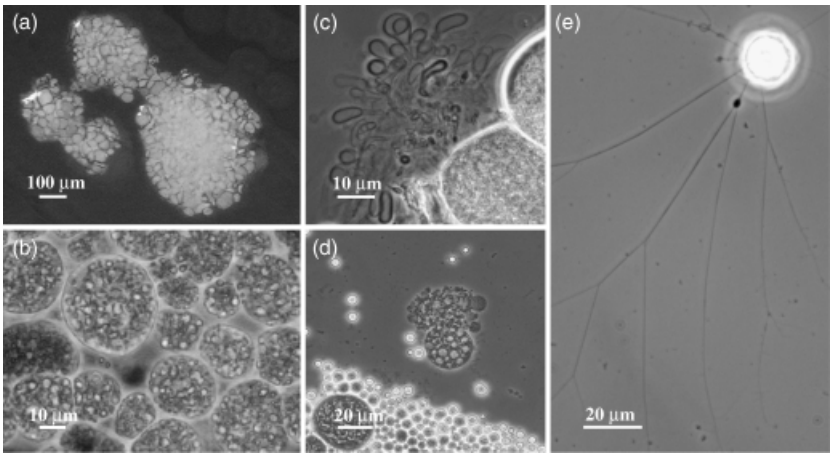

Fig. 1. Colonies of strain *Fng1*. (a) Colonies were c. 0.5 mm in diameter and appeared yellowish on B1 and Marine agar plates. Notice the individual vegetative cells that form the colony and were seen easily through the stereoscope. (b) Microscopic view of individuals from the same colony. The cells were spherical and granular, 35 ± 15 μm in diameter. (c) Bulbous extensions arising from one or more points on the cell surface were seen only in cells that were inoculated on poor media. Note that the length and thickness of the extensions were variable. (d) Colonies that grew on Marine agar; an 'unwrapping' was visible and the inner content spilled out. (e) Individual *Fng1* after 3 days of incubation in B1 liquid medium in a closed 200 μL container.

Table 2. Physiological characteristics of strain *Fng1*

Utilization of carbon source	Growth reaction
TWEEN® 20	−
D-Glucose	+
Glycerol	+++
Sucrose	+++
Ethanol	+
D-Mannitol	+
D-Mannose	++
D-Galactose	++
Maltose	+
D-Glucosamine hydrochloride	+
D-(−)-Arabinose	+
α-Lactose monohydrate	+
L-Arginine	−
L-Asparagine	++
Peptone	+++
Mineral medium (SM)	+

+ represents levels of growth of colonies; each + is an estimation of a logarithmic change. −, not detected.

Table 3. Mean values for fatty acids in thraustochytrid *Fng1* strain measured as a percent of fatty acid profile

Relative concentration (%)	Number of isomers	Molecular formula	Fatty acid
0.2	1	14:0	Methyl tetradecanoate
0.3	1	15:0	Pentadecanoic
14.4	1	16:0 iso	Palmitic
0.2	1	17:0	Heptadecanoic
3.5	1	18:0	Stearic
0.3	1	20:0	Eicosanoic
1.5	1	16:1ω7	Palmitoleic
50.0	2	18:1ω7 and ω10	Oleic*
11.0	1	18:2ω7	Linoleic
10.0	6	18:3ω3	α-Linolenic
0.7	1	20:1ω9	Eicosenoic
0.3	1	20:5ω3	Eicosapentaenoic
1.7	1	22:6ω3	Docosahexaenoic
5.9	–	–	Others†

*Oleic acid comprises two ω7 and ω10 isomers.
†Other fatty acids included cyclopropaneoctanoic acid and unidentified long fatty acids.

(DHA, 22:6) and 0.3% of eicosapentaenoic acid (EPA, 20:5), which belongs to ω3-polyunsaturates.

Morphological characterizations of *Fng1*

The vegetative cells were spherical and granular, 35 ± 15 μm in diameter (Fig. 1b). When inoculated on nutrient-poor media such as B1 agar or on 10% B1, the cells produce bulbous extensions from one or more points on the cell surface. The length and thickness of the extensions changed within each cell (Fig. 1c). No morphological differences were observed between cultures that were inoculated on B1 agar and those inoculated on marine agar media.

In some cases, light microscopy revealed an opening in the cell wall and a release of the cell's inner contents (Fig. 1d). Three- to four-hour incubations in B1 liquid medium in a closed container revealed the development of ectoplasmatic networks. Some of the ectoplasmatic networks grew to 100–200 μm length following a 3-day incubation period (Fig. 1e). TEM of samples from the axenic cultures of *Fng1* revealed a variety of cells between 6 and 50 μm. The larger cells were enveloped by a cell wall made up of a lamellar structure *c.* 100 nm in thickness (Fig. 2). From the inner side of the cell wall, a 20–60 nm gap filled with unidentified vesicle-like structures was noted (Fig. 2c). Inside the cell (Fig. 2a and b), nuclei with nucleoli, numerous mitochondria, golgi and endoplasmic reticulum were observed.

Phylogeny

Comparison of the 18S rRNA gene sequence of *Fng1* (1634 bases; AY870336) and *Favia* sp. isolates *Rs-Fav-1* to *Rs-Fav-4* (EU264109–EU264112) with the database of GenBank (http://www.ncbi.nlm.nih.gov) positioned these organisms as members of the *Thraustochytridae* (phylum – *Stramenopiles*, order – *Labyrinthulida*). The similarity of the 18S rRNA gene sequence of *Fng1* was between 97% and 99% to sequences *Rs-Fav-1* to *Rs-Fav-4* isolated from the Faviid corals (Fig. 3). Moreover, there was an 86% similarity of the 18S rRNA gene from *Fng1* to *Thraustochytrium aureum* (AB022110) and *Ulkenia profunda* (AB022114 and L34054), the closest characterized species (Fig. 3). As shown in Fig. 3, there was also high sequence similarity (97%) to three unidentified Thraustochytrids: MBIC11060, MBIC11063 and MBIC11077 (AB183653, AB183654 and AB183659, respectively). In addition, there was a 73% similarity between the mitochondrial 16S rRNA gene of *Fng1* (1320 bases; AY870337) to that of *T. aureum* (AF288091). The sequencing of the mitochondrial 16S rRNA gene as well as the 18S rRNA gene of *Fng1* was carried out at least three times, with a set of universal and specific primers (see Table 1), from different cultures on each occasion. In all the events, the results were identical.

Discussion

Thraustochytrids are cosmopolitan organisms that have been isolated from estuarine and marine habitats throughout the world. Although originally positioned under the fungi, the Thraustochytrids have been reclassified under the order *Labyrinthulida* based on cell wall composition (Darley *et al.*, 1973), the fact that their ectoplasmic net extensions seem to be generated by the unique organelle the bothrosome (Perkins, 1973) and by molecular analysis using 18S rRNA gene sequences (Cavalier-Smith *et al.*, 1994; Honda *et al.*, 1999).

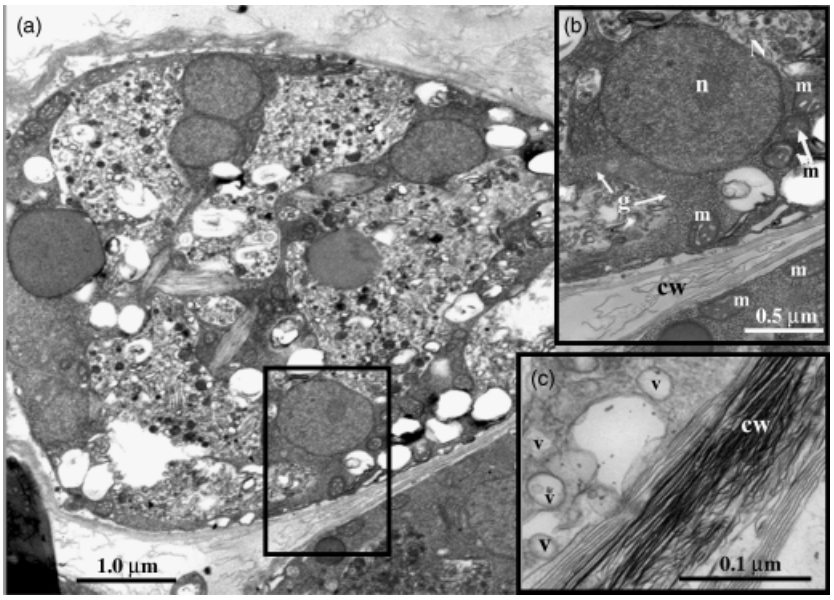

Fig. 2. Transmission electron micrographs of *Fng1*. (a) Parent cell with a lamellar cell wall. (b) Higher magnification nucleus 'N', nucleolus 'n', mitochondrion 'm', golgi apparatus 'g' and lamellar cell wall 'cw', were visible. (c) Photomicrograph of the structure of the lamellar cell wall; note the vesicle-like structures ('v') that are juxtaposed to the cell wall ('cw').

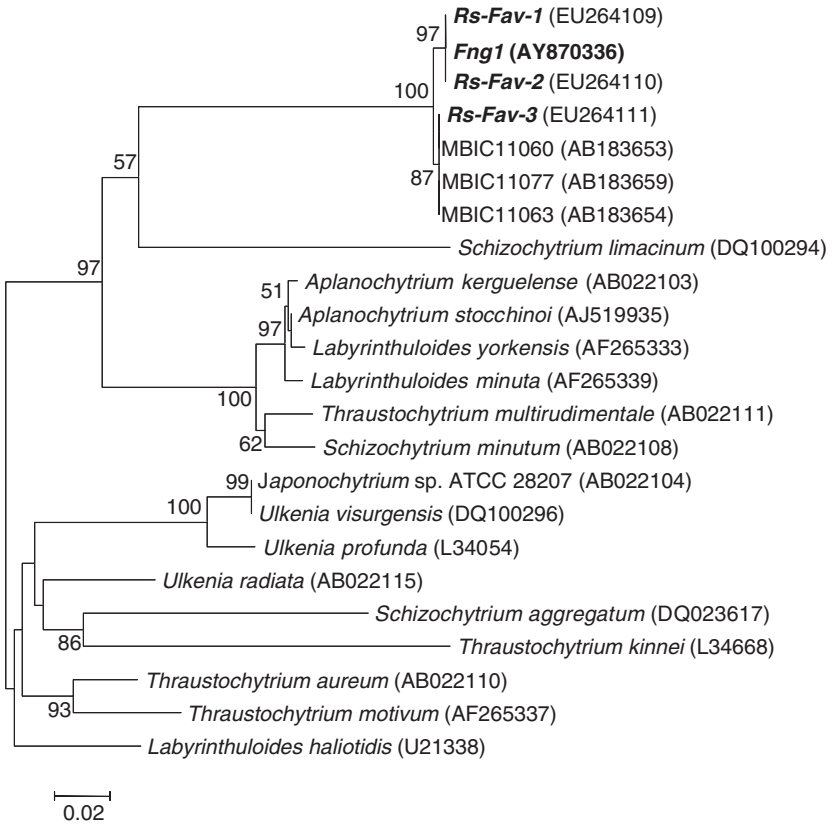

Fig. 3. Neighbour-joining 18S rRNA gene phylogenetic tree showing the relationship between *Fng1* and representative species of the *Thraustochytridae*. Note that *Fng1*, *Rs-Fav-1*, *Rs-Fav-2* and *Rs-Fav-3* clearly form a distinct lineage within the family. The scale bar represents two substitutions per 100 nucleotide positions. Bootstrap probabilities are indicated at branch nodes.

In the present study, we succeeded in isolating and culturing a Thraustochytrid species strain *Fng1* from the mucus of the fungiid coral *F. granulosa*. In addition, four similar Thraustochytrid species (*Rs-Fav-1* to *Rs-Fav-4*; EU264109–EU264112) were isolated from *Favia* sp. corals with similarities between 97% and 99% to *Fng1*. This indicates that latter Thraustochytrid or closely related species may be found in association with Faviid corals in the northern gulf of Eilat. Phylogenetically, *Fng1* clearly forms a distinct lineage in the *Thraustochytridae* cluster. As shown in Fig. 3, the 18S rRNA gene of *Fng1* (AY870336) displayed a high sequence similarity (97%) to three other *Thraustochytridae* species MBIC11060, MBIC11063 and MBIC11077 (AB183653, AB183654 and AB183659, respectively) that have been deposited in GenBank as unidentified species. The similarity between the mitochondrial 16S rRNA gene of *Fng1* (AY870337) to that of *T. aureum* (AF288091) further strengthens its position in the *Thraustochytridea*.

Comparison of *Fng1* morphology with that of the described Thraustochytrid species (Watson & Ordal, 1957; Darley *et al.*, 1973; Harrison & Jones, 1973; Raghukumar, 1988, 2002; Porter, 1990; Honda *et al.*, 1998, 1999; Rinkevich, 1999; Bremer, 2000; Fan *et al.*, 2002; Anderson *et al.*, 2003; Leander *et al.*, 2004; Kvingedal *et al.*, 2006) revealed that it is larger ($35 \pm 15\,\mu m$ in diameter) than any documented Thraustochytrid. Microscopic observations showed that *Fng1* produced bulbous extensions from one or more points on the cell surface only under oligotrophic conditions (Fig. 1e and c), an observation that is in agreement with observations from previous studies on other species of Thraustochytrids (Raghukumar, 2002). In many cases, the bulbous extensions, described previously as ectoplasmic networks, originated from an organelle identified as a bothrosome (Perkins, 1973; Cavalier-Smith *et al.*, 1994; Leander *et al.*, 2004). Raghukumar (2002) suggested that these ectoplasmic extensions may aid in increasing the surface area of the cell and may contain hydrolytic enzymes that are surface-bound or are secreted into the surrounding medium, helping in the digestion of organic material. He further suggests that they may also anchor the cells to surfaces. In *Fng1,* ectoplasmic network extensions were observed between 3 and 72 h after inoculation in an undisturbed liquid sample (Fig. 1e); this may indicate how these organisms anchor into the mucus on the coral surface.

The optimal growth observed for this organism was in a medium with 3.5% NaCl and at 22 °C. This demonstrates that it is well adapted to its marine environment. The formation of clusters in broth media and the different growth patterns observed when inoculated as a drop vs. spread on the plate indicate that the individuals in *Fng1* prefer to grow as aggregations. This type of growth may be advantageous due to the critical mass of extracellular enzymes secreted by the community that may facilitate the use of carbon sources. Our study showed that *Fng1* can grow using multiple carbon sources (e.g. peptone) as well as on select carbon sources (e.g. glycerol and sucrose). This characteristic may allow the organisms to exploit the changing environment of the coral mucus (Ducklow & Mitchell, 1979; Meikle *et al.*, 1988). Kramarsky-Winter *et al.* (2006) previously suggested that there is a mutualistic relationship between *Stramenopile* protists (which include Thaustochytrids) and hermatypic corals such as Fungiids and Favaids. It was further speculated that the Stramenopiles may provide part of the nutritional requirements of these corals (Kramarsky-Winter *et al.*, 2006). Reef-building corals are known to be rich in fatty acids (Latyshev *et al.*, 1991), which are believed to play an important role in coral wellbeing (Bachok *et al.*, 2006). Indeed, it was demonstrated that during bleaching events there is a substantial decrease in lipids and polyunsaturated fatty acids in affected *Pavona frondifera* colonies (Bachok *et al.*, 2006). The difference in the fatty acid content between bleached and non-bleached coral colonies has been attributed to differences in the presence of symbiotic algae in the coral tissue (Bachok *et al.*, 2006). Interestingly, in general, massive corals were shown to lose less fatty acid during bleaching events than branching species (Yamashiro *et al.*, 2005). Moreover, these massive growth forms and solitary fungiid corals are known to survive bleaching events better than branching species (Hoeksema, 1991; Loya *et al.*, 2001). It is therefore possible that thraustochytrids and other *Stramenopiles* found on the surface and in the mucus of these corals may provide nutritional sources including polyunsaturated fatty acids, allowing these coral hosts to survive such events. Indeed, the fatty acid analysis of *Fng1* thraustochytrid revealed above 75% unsaturated fatty acids, with ω3-PUFA as a significant component (12% of the total fatty acids present; Table 3). ω7-palmitoleic acid, ω7-linoleic acid, ω9-eicosenoic acid and ω7 and ω10 isomers of oleic acid were also present.

Marine bacteria are known to both produce and utilize PUFAs (Watanabe *et al.*, 1994; Russell & Nichols, 1999). It is possible that the maintenance and distribution of these bacteria on the coral surface depend in part on the utilization of the fatty acids produced by the coral's eukaryote symbionts, indicating the importance of the eukaryote protists for the holobiont. This strengthens the Coral 'Probiotic Hypothesis', according to which the coral lives in a symbiotic relationship with a diverse metabolically active microbial population (Reshef *et al.*, 2006). When environmental conditions are altered, the accompanying microbial biota undergo changes as well, a shift that may aid the coral holobiont in adapting to the new conditions. It is therefore possible that in some cases corals can fend off diseases or develop resistance to certain microbial-driven diseases by 'active' or passive shifting of their associated microbial communities

(Reshef *et al.*, 2006) including thraustochytrids. Ongoing research is being carried out in our laboratory in order to further our understanding of *Fng1* as well as other Thraustochytrids in the coral holobiont and the reef ecosystem.

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