Isolation and culture of Thraustochytrids from Scottish marine environments: why do these protists synthesise high amounts of unsaturated lipids?

Thraustochytrids are an enigmatic group of marine protists, increasingly exploited by the nutraceutical industry as a source of lipid rich in omega 3 fatty acids, both for human consumption and animal feeds. Although these protists are ubiquitous in the marine environment, our knowledge of their ecology is limited. Thraustochytrids have been identified in all major oceans and marine biomes (sea-ice, open-ocean environments during post-bloom conditions and in the deep sea) and are particularly abundant in coastal habitats. These protists are thought to fulfill a similar role to bacteria, being major decomposers of organic material. Surprisingly their biomass in the marine environment can often exceed that of bacteria, suggesting a major, yet largely unstudied role in nutrient regeneration and decomposition of organic material. In addition, specific strains of thraustochytrids are often intimately associated with a wide range of invertebrate taxa including sponges, hydroids, bivalves and zooplankton. An extraordinary feature of thraustochytrids is their propensity to accumulate high quantities of lipid, which at times can exceed 70% of the dry mass of culture biomass. The composition of this lipid is also unusual in containing unprecedented amounts of omega 3 fatty acids. However, the functional significance of these lipids in the life cycle of thraustochytrids is unknown.

Fig 1. Thraustochytrid sampling locations in vicinity of SAMS.

We isolated strains of thraustochytrids from marine habitats local to Oban with the aim of culturing them under different growth conditions. These protists have a multi-stage life-cycle, developing through sporangium, vegetative and free swimming zoospores. Thraustochytrids are particularly prevalent in organic rich sediments, often associated with invertebrates and macroalgae, such as kelp and zostera. All these potential sources of thraustochytrids are readily accessible from the SAMS laboratory by research vessel and foreshore access (Fig 1).

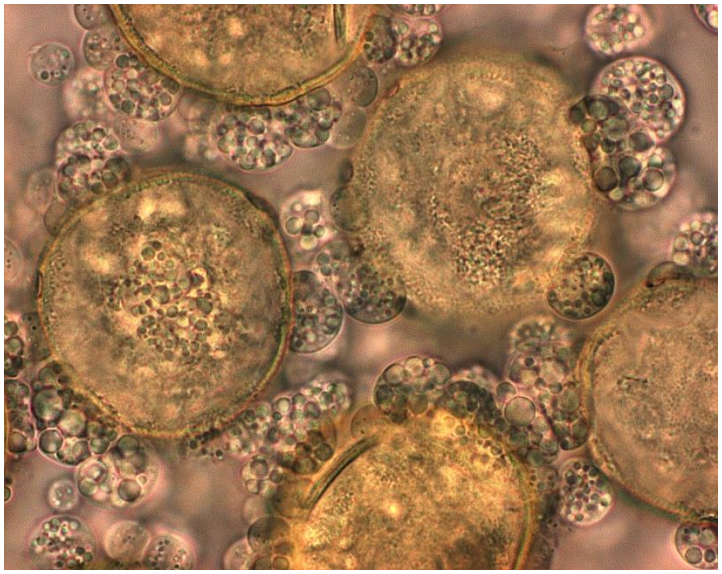

Fig 2. Thraustochytrids were isolated using pollen grains as 'bait'. Thraustochytrid colonies are clearly visible attached to pollen grains. The small dark spherical objects are lipid droplets.

Table 1. Isolated colonies were regularly check for diagnistic features linked to taxonomic affiliation and used in conjunction with 18S rRNA sequencing.

Sample	Colonies isolated	Morphological features in pollen grains/ brine shrimps	Growth in liquid media	Molecular characterization
1	Elevated, cream, small cells, compact (slow growth)	***Schizochytrium sp.*** Small cells. Refractive cells with inclusions. (flower-like). Spores cleavage like ***U. amoeboidea***, but zoospores were rare. Net poor developed.	Pellets (Cryopreservation)	Low PCR amplification
3	Confluent, cream-yellow, big cells	***Thraustochytrium aggregatum*** (photos and cells sizes)	Small aggregates (Cryopreservation)	*Oblongichytrium* sp. **X**
4	Plane, pink, vitraux like with very big and irregular cells	Globose cells, refractives, with inclusions and broad cell walls. Extensive network. Some colonizing pollen grains, other free. ***Ulkenia radiata* (or *visurgensis?*)**	Homogeneous culture (Cryopreservation)	*Ulkenia visurgensis*
7	Confluent, cream, small cells	No zoospores in pollen sea water cultures, but zoospores on *Artemia*. Big cells with lipid droplets and small refractive cells with binary fission. Zoospore formation as *Ulkenia amoeboidea* (**Schizo net - *U. amoeboidea***)	Big and irregular aggregates	*Oblongichytrium* sp.
9B	Confluent, cream, small cells	Neither zoospores nor pollen colonized. Big Schizo-like cells with lipid droplets, attached among them and to the bottom by a prominent net, small refractive cells (that could be the amoebospores) (**Schizo net - *U. amoeboidea***)	Big and irregular aggregates (Cryopreservation)	*Oblongichytrium* sp.
10C	Confluent, cream, big cells, cerebroid aspect	***Thraustochytrium aureum***. Dark globose cells with broad cell walls	Small aggregates (Cryopreservation)	*Thraustochytrium kinnei*, *Thraustochytrium* sp., *Thraustochytrium aureum*
11A	Confluent, cream-yellow, big cells, lax	***Thraustochytrium aggregatum*** (several photos)	Small aggregates	*Thraustochytrium kinnei*, *Thraustochytrium* sp.
AB	Confluent plus elevations, yellow, very big cells	**Chytrid!!!**		*Oblongichytrium* sp.
AC	Confluent, cream, small cells	**Schizo net - *U. amoeboidea.*** Net very developed. No zoospores observed	Big and irregular aggregates	*Oblongichytrium* sp.
BB, BC	Confluent, cream, small	**Schizo net - *U. amoeboidea.*** Full of zoospores	Big and irregular aggregates	BB: *Oblongichytrium* sp. BC: low PCR amplification

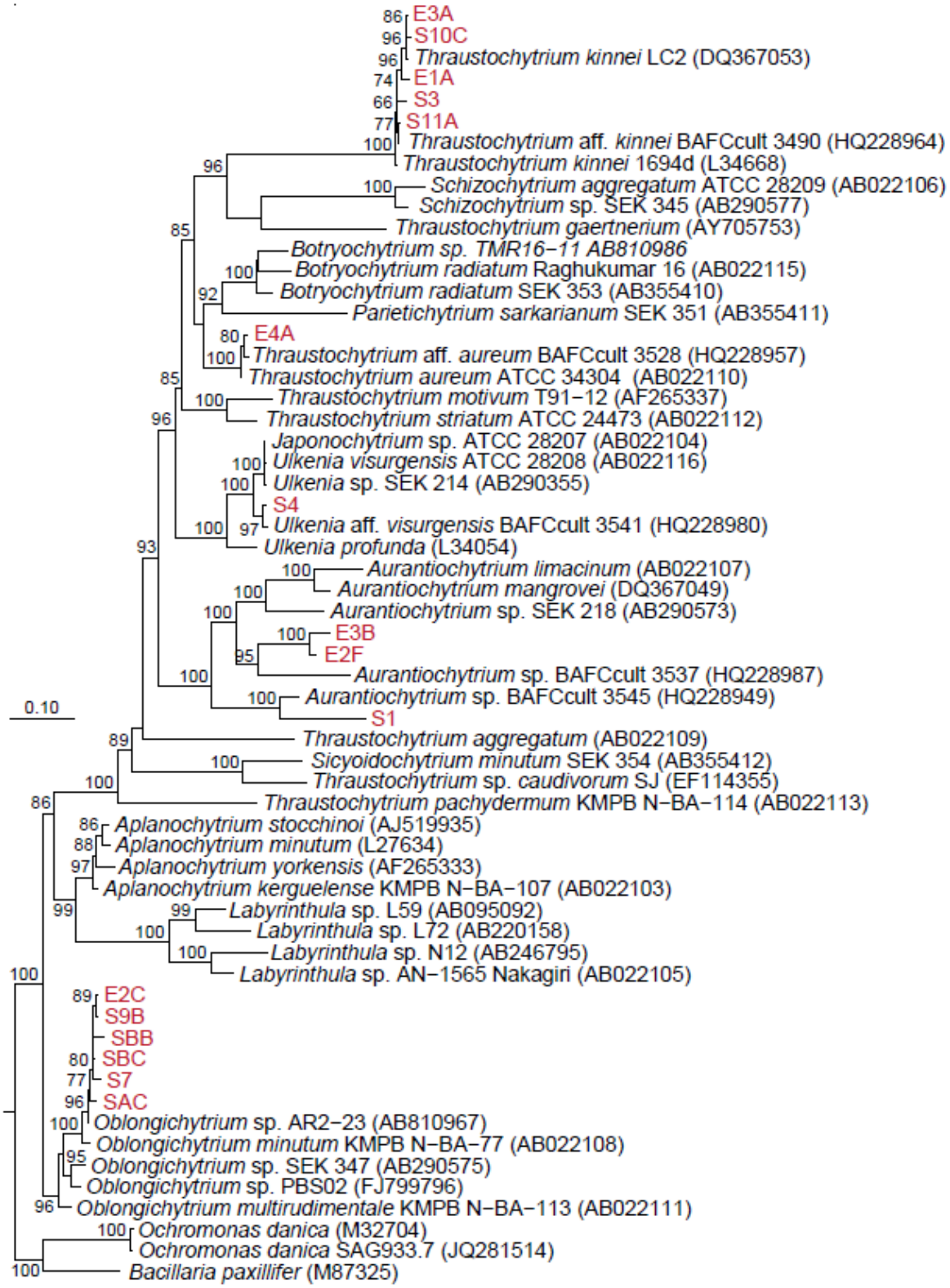

Fig. 3 Phylogenetic tree (SSU rRNA) of strains isolated from the environment immediately adjacent to SAMS (S strains) and deep water locations (140 m) in Loch Etive (E strains).

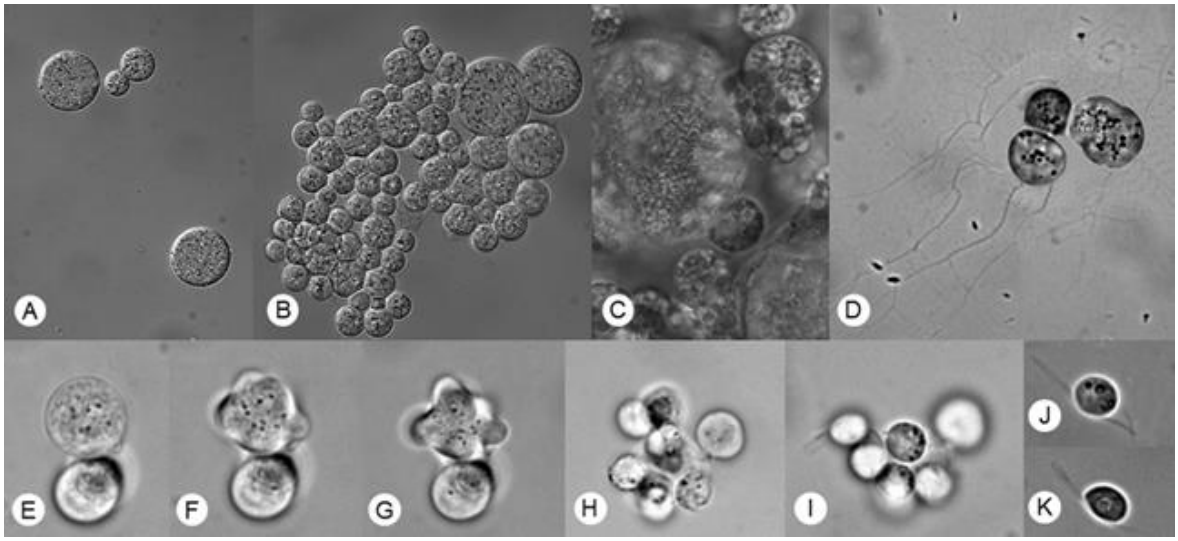

Fig. 4 Morphological features of *Ulkenia visurgensis* isolate S4. A and B: Vegetative cells growing in MC-BHB medium before (A) and after (B) cryopreservation. C to K: Diagnostic features in pollen seawater cultures. C: Vegetative cells attached to *Junglans nigra* pollen grains. D: Prominent network developed by a clump of vegetative cells. E to G: Amoeba merging from sporangium and suffering an irregular cleavage. H and J: Group of non-motile zoospores, joining together prior acquiring motility. J and K: Heterokont zoospores before (J) and after (K) cryopreservation.

Cryopreservation - Long term maintenance of the thraustochytrid strains is desirable if this biological resource is to be preserved for future studies. All strains isolated during the MASTS fellowship were successfully cryopreserved and subsequently revived. Growth characteristics post cryopreservation for strain S4 indicate a lag in growth, although after 7 days cell densities and PUFA compositions were identical for both treatments (Fig 5, Table 2).

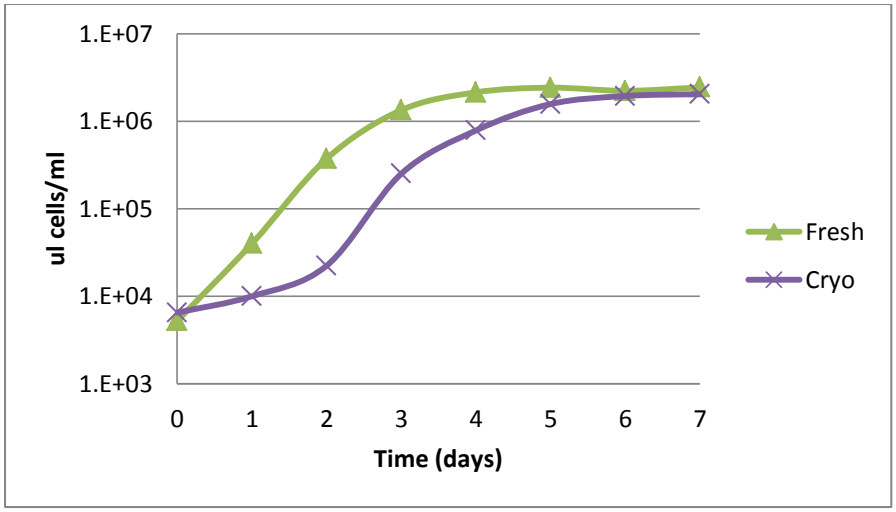

Figure 5: Growth of *U. visurgensis* isolate S4 before and after cryopreservation for 7 days.

Table 2. Polyunsaturated fatty acid composition of S4 before and after cryopreservation. Cuktures grown for 7 days.

PUFA (%)	**Fresh cells**	**Cryopreserved cells**
20:4(n-6)	12.35 ± 0.74	12.18 ± 0.95
20:5(n-3)	15.76 ± 0.72	14.77 ± 0.19
22:4(n-6)	6.88 ± 1.35	7.58 ± 1.06
22:5(n-6)	19.74 ± 0.88	18.51 ± 1.13
22:5(n-3)	3.08 ± 0.02	3.21 ± 0.05
22:6(n-3)	42.18 ± 2.44	43.75 ± 3.01

Summary- Thraustochytrids are clearly abundant in the marine habitats adjacent to SAMS and constitute an important biotechnological resource, especially given their propensity to produce high levels of omega 3 rich PUFA. Ongoing research at SAMS is exploring the potential of thraustochytrids for generating high quality feed stocks for the aquaculture industry.