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Short Communication

Algal polysaccharide utilisation by saprotrophic planktonic marine fungi

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ABSTRACT

The functional roles that marine mycoplankton fulfil are poorly understood, resulting in a lack of knowledge of their ecology. Here we show, using DNA Stable Isotope Probing with ¹³C-labelled diatom polysaccharide microgels, that mycoplankton assimilate algal-derived particulate organic carbon (POC), identifying two genera, *Malassezia* and *Cladosporium*, which are active saprotrophs in coastal waters. We subsequently isolated polysaccharide-utilising *Cladosporium* strains from the same ecosystem and that are well-represented in marine mycoplankton assemblages. At the study site, *Cladosporium* occurs across multiple years and is associated with diatoms. During growth with the polysaccharide laminarin, *Cladosporium* spp. secrete the extracellular carbohydrate-active enzyme glucan 1,3-β-glucosidase. These results show that some marine mycoplankton have a saprotrophic functional role in processing algal polysaccharides. Mycoplankton may, therefore, be involved in the trophic transfer of phytoplankton produced POC in marine food webs, and because bacterioplankton occupy the same niche, potential interactions maybe taking place that are yet to be characterised.

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1. Introduction

A base-line understanding of the diversity of marine mycoplankton has been established (Richards et al., 2015; Taylor and Cunliffe, 2016; Tisthammer et al., 2016), however the functional roles that fungi fulfil within marine ecosystems remain largely speculative. Marine mycoplankton could be saprotrophic, decomposing particulate organic carbon (POC) via extracellular enzymes and feeding on dissolved organic carbon (DOC) decomposition products osmotrophically (Richards et al., 2012). In support of this theory, by correlating marine mycoplankton abundance and extracellular enzyme activity, a study off Chile indicated that mycoplankton have roles in processing high-molecular-weight (HMW) algal-derived biopolymers (Gutiérrez et al., 2011).

Polysaccharide microgels, including transparent exopolymer particles (TEP), are principally formed from the abiotic coagulation

of dissolved biogenic precursor molecules and can constitute up to 40% of marine POC (Passow, 2002; Engel et al., 2004). Phytoplankton, in particular diatoms, are a major source of microgel precursor molecules, which are excreted during regular metabolic processes (Passow, 2002).

Currently there is a lack of direct evidence of marine mycoplankton saprotrophy, including the identity of taxa involved in processing algal-derived POC and the biological mechanisms used (e.g. enzymes). Such information is needed to establish an understanding of the roles that mycoplankton are fulfilling in the marine carbon cycle (Worden et al., 2015). This study used a combination of culture-independent (DNA-SIP and metabarcoding) and culture-dependent (cultivation and proteomics) approaches to identify and characterise marine mycoplankton that actively utilise algal-derived polysaccharides.

2. Materials and methods

¹²C and ¹³C-labelled polysaccharide microgels were produced from axenic cultures of the marine diatom *Phaeodactylum tri-cornutum* using established methods (Taylor and Cunliffe, 2017) (Supplementary Methods; Supplementary Table 1). Surface

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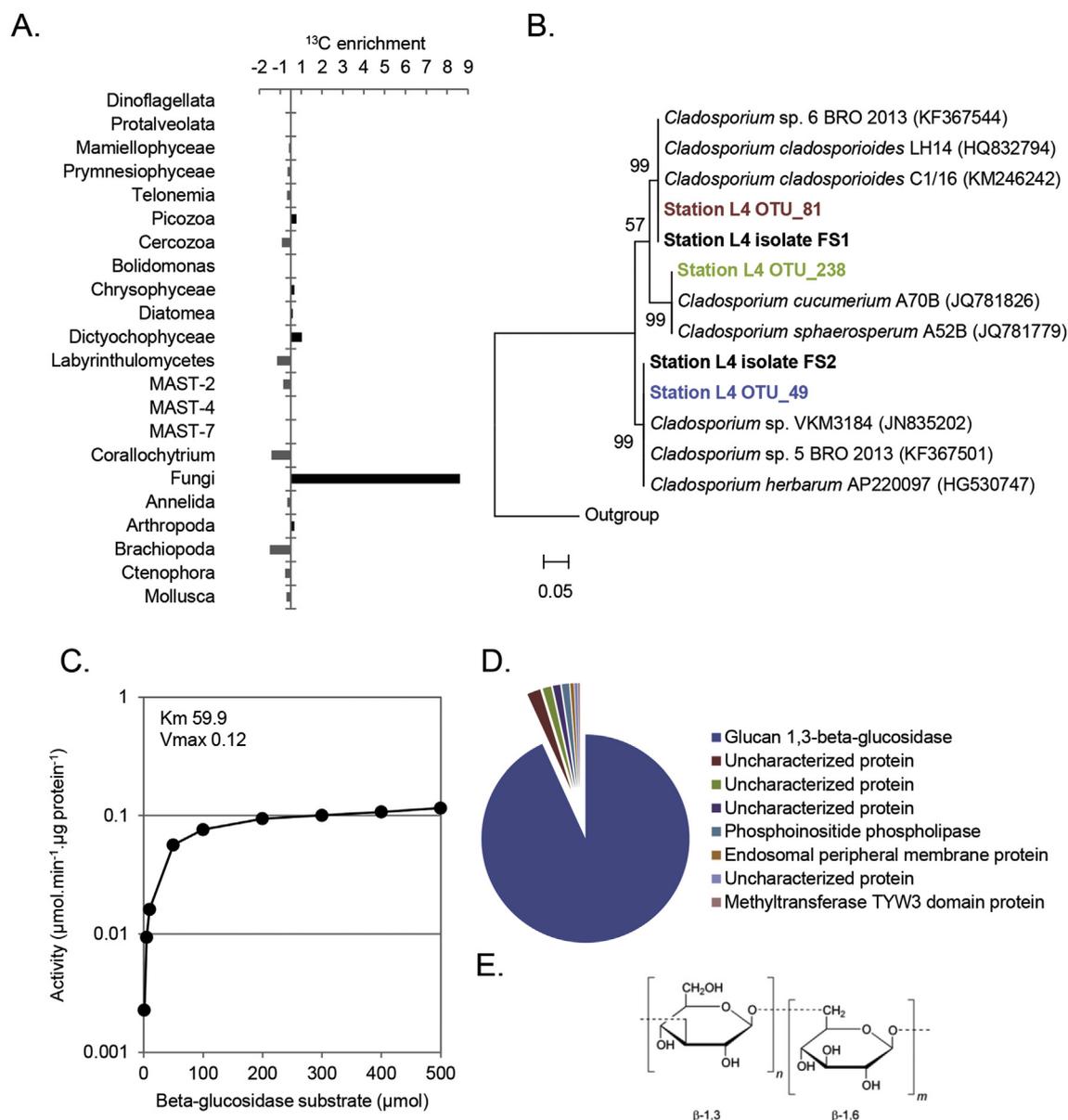


Fig. 1. (A) Comparison of the abundance of 18S rRNA gene sequences in libraries created from ^{13}C -labelled and ^{12}C control gradient fractions. Taxa >0 are those enriched in the ^{13}C libraries relative to the ^{12}C control libraries, indicating that they had assimilated ^{13}C -labelled algal polysaccharide. Taxa <0 are those not enriched in the ^{13}C libraries, indicating that they had not assimilated the ^{13}C -labelled algal polysaccharide. (B) Phylogenetic analysis of the algal polysaccharide-utilising *Cladosporium* strains FS1 and FS2 isolated from seawater collected from Station L4 and *Cladosporium* operational taxonomic units (OTUs) in 44 Fungi-specific ITS gene libraries generated by metabarcoding of plankton DNA samples collected between January 2008 and June 2013 from surface waters also at Station L4 (Taylor and Cunliffe, 2016). The tree is based on a maximum likelihood method using a Tamura-Nei model. The numbers on the nodes indicate bootstrap percentages and the scale bar represents sequence divergence. (C) *Cladosporium* FS2 extracellular β -glucosidase activity determined by measuring the hydrolysis of 4-methylumbelliferyl β -D-glucopyranoside (MUGlc) to the fluorescent 4-methylumbelliferone (MUF) across a range of concentrations in samples loaded in a 96-well plate using a fluorometer (CLARIOstar[®], BMG Labtech). Maximal velocity (V_{max}) and the Michaelis-Menten constant (K_m) were calculated via a Lineweaver-Burk plot of the Michaelis-Menten equation: $V = (V_{max} \times [S]) / (K_m + [S])$. (D) *Cladosporium* FS2 extracellular 'secretome' proteins produced during growth in liquid minimal media with the algal polysaccharide laminarin as a sole carbon and energy source. (E) Chrysolaminarin/laminarin a linear $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 6)$ glucan.

seawater was collected from Station L4 in the Western English Channel (Supplementary Fig. 1; Supplementary Table 2) and used to setup DNA-SIP incubations. After 18 h incubation, DNA was extracted from filtered seawater samples and DNA-SIP performed using established protocols (Neufeld et al., 2007), with fractions assessed by quantifying buoyant density and DGGE analysis of 18S rRNA genes. Specific 'light' and 'heavy' DNA fractions were analysed by sequencing the V9 region of the 18S rRNA gene on an Ion Torrent PGM (Life Technologies) and analysed as previously described (Taylor and Cunliffe, 2014).

P. tricorutum exudates are dominated by chrysolaminarin, a

glucan identical to laminarin (Ford and Percival, 1965). We therefore used laminarin to isolate saprotrophic mycoplankton because it is commercially available and chemically defined. Seawater was again collected from Station L4 and plated onto marine minimal agar plates containing laminarin (2% w/v) (Cunliffe, 2016). For assessment of extracellular β -glucosidase activity and proteomics, after growth in liquid media, biomass was removed by filtration. β -glucosidase activity was quantified via hydrolysis of 4-methylumbelliferyl β -D-glucopyranoside (Hoppe, 1983). Extracellular proteins were assessed using LC-MS (Orbitrap, ThermoFisher Scientific), with peptide spectra searched against the Universal Protein

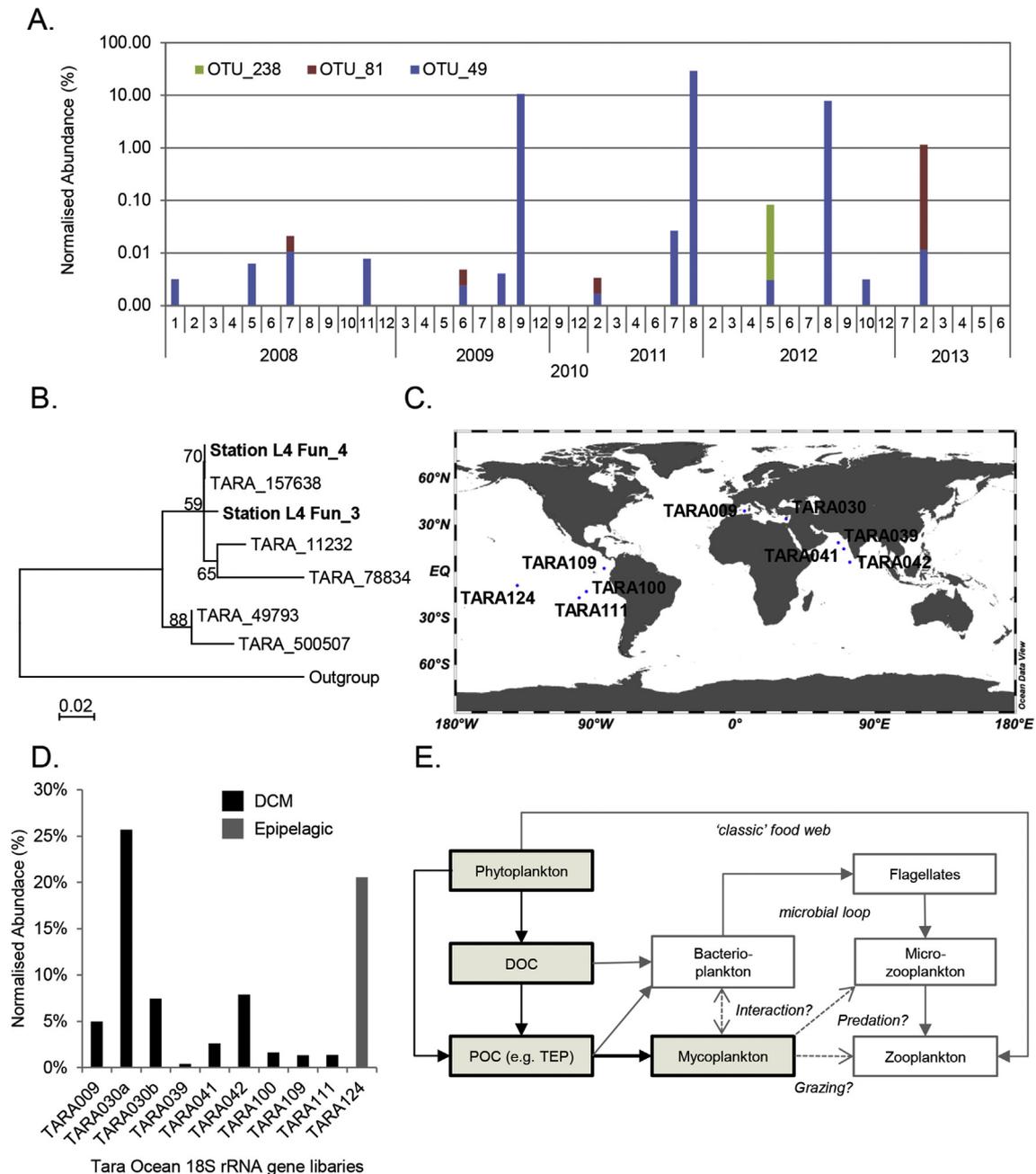


Fig. 2. (A) Change in the relative abundance of *Cladosporium* OTUs from Fig. 1B in the month-specific ITS gene libraries collected between January 2008 and June 2013 from surface waters at Station L4. (B) Phylogenetic analysis of the active saprotrophic *Cladosporium* OTUs Fun_3 and Fun_4 identified by ^{13}C -labelled diatom-produced POC DNA-SIP in this study and *Cladosporium* OTUs in the 18S rRNA gene libraries produced from the Tara Ocean Expedition (de Vargas et al., 2015). The tree is based on a maximum likelihood method using a Tamura-Nei model. The numbers on the nodes indicate bootstrap percentages and the scale bar represents sequence divergence. (C) Locations of the top ten Tara Ocean Expedition libraries from the epipelagic zone and deep chlorophyll maximum (DCM). (D) Relative abundance of *Cladosporium* OTUs in the mycoplankton communities of the top ten Tara Ocean Expedition libraries from the epipelagic zone and deep chlorophyll maximum (DCM). (E) Conceptual diagram of the ecological advance proposed in this study by providing empirical evidence of active marine mycoplankton saprotrophy and identifying specific taxa that utilise algal-derived polysaccharide-based POC with links to phytoplankton.

Resource fungal database.

3. Results

Comparison of the 18S rRNA gene libraries generated from the ^{13}C -labelled and ^{12}C -control fractions showed that fungi were enriched in the ^{13}C incubations relative to other plankton groups (Fig. 1A), showing that mycoplankton had assimilated the polysaccharide containing microgels. Enriched OTUs showed closest matches with the genera *Malassezia* (Basidiomycota) and

Cladosporium (Ascomycota) (Supplementary Table 3). Two polysaccharide-utilising cultures were subsequently isolated, FS1 and FS2, which were identified by sequencing the PCR amplified ITS region and showing that they both matched the genus *Cladosporium* (Fig. 1B).

We subsequently focused on *Cladosporium* FS2 because the strain is related to the most dominant *Cladosporium* at Station L4 (see below). The FS2 extracellular protein extract showed β -glucosidase activity (Fig. 1C) and was dominated by the CAZyme glucan 1,3- β -glucosidase (Fig. 1D, Supplementary Table 4), which

performs the exohydrolysis of β -D-glucose units from the non-reducing ends of (1 \rightarrow 3)- β -D-glucans, such as chrysolaminarin (Fig. 1E), and demonstrated that marine *Cladosporium* have the capacity to decompose diatom polysaccharides.

We examined the distribution of *Cladosporium*-assigned OTUs in 44 Fungi ITS gene libraries generated from DNA samples collected over 6 yr at Station L4 (Taylor and Cunliffe, 2016). Three OTUs were identified OTU_81, OTU_49 and OTU_238 (Fig. 1B), with OTU_81 and OTU_49 homologous to the gene sequences amplified from isolates FS1 and FS2 respectively. OTU_49 most frequently occurred at Station L4 and on three occasions was >1% of the ITS gene libraries (Fig. 2A). Changes in *Cladosporium* OTU abundances were compared to fluctuations in co-occurring phytoplankton blooms, including diatoms (Supplementary Fig. 3). Increased OTU_49 abundance was associated with the increased abundance of the diatom genus *Lep- tocyllidrus* (r 0.909; p < 0.01), and the increased abundance OTU_238 associated with the abundance of the diatom genus *Chaetoceros* (r 0.503; p < 0.01). Previous studies have shown that both diatom genera are important TEP producers (Passow, 2002).

To determine the distribution of *Cladosporium* in the open ocean, the 18S rRNA gene libraries produced from the Tara Ocean Expedition were also examined (de Vargas et al., 2015). *Cladosporium* OTUs identified in the Station L4 DNA-SIP experiments matched *Cladosporium* OTUs in the Tara libraries (Fig. 2B). The top ten Tara libraries in which *Cladosporium* OTUs were most abundant included sites in the Pacific and Indian Oceans (Fig. 2C). *Cladosporium* OTUs accounted for up to 26% of the mycoplankton communities, with nine of the libraries collected from the deep chlorophyll maximum (DCM) and coinciding with high phytoplankton biomass (Fig. 2D).

4. Discussion

Stringent analysis of 130 18S rRNA gene libraries from six coastal marine sites showed that *Malassezia* and *Cladosporium* are consistent components of other coastal mycoplankton assemblages (Richards et al., 2015), and as with *Cladosporium* shown here, we have previously shown that *Malassezia* are abundant specifically at Station L4 (Taylor and Cunliffe, 2016). Both genera have also been shown in other studies to be prevalent in other pelagic marine ecosystems, including the open ocean (Amend, 2014; Wang et al., 2014).

The functional roles that aquatic fungi undertake in food webs and associated biogeochemical processes are currently poorly understood (Grossart and Rojas-Jimenez, 2016). Here we provide empirical evidence of active marine mycoplankton saprotrophy, identifying specific taxa that utilise algal-derived polysaccharide microgels (Fig. 2E). Utilisation of algal-derived polysaccharides is a role already established in some marine bacterioplankton (Buchan et al., 2014; Taylor and Cunliffe, 2017), suggesting that possible ecological interactions (e.g. competition, syntrophy) between saprotrophic mycoplankton and bacterioplankton could be taking place that are yet to be understood. Marine fungi, including *Cladosporium*, are potential prey for some zooplankton (Hu et al., 2015), indicating that mycoplankton may have a carbon transfer role from algal-derived POC to higher trophic levels (Fig. 2E). Saprotrophic mycoplankton activity in the coastal carbon cycle must now be quantified and, with the discovery that fungi also dominate living biomass on marine snow (Bochdanský et al., 2017), the activity of mycoplankton in the open ocean should also be explored.

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Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2017.08.009>.

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