**Thermal adaptation in a marine-derived tropical strain of *Fusarium equiseti* and polar strains of *Pseudogymnoascu*s spp. under different nutrient sources**

**Running title:**

Thermal adaptation in marine-derived fungi

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**Abstract**

We documented relative growth rates and activities of extracellular hydrolytic enzymes (EHEs) of one marine-derived tropical strain of *Fusarium equiseti* originally isolated from Malaysia, and two polar strains of *Pseudogymnoascus* spp. from Antarctica and the Arctic, under varying temperatures and different nutrient conditions. Relative growth rates (R) and activities (RA) of protease, amylase and cellulase were screened in seawater nutrient assay plates augmented with either skim milk, soluble starch, or carboxylmethylcellulose with trypan blue, respectively, across culture temperatures between 5 and 40°C. Measures of R were fitted into third-degree polynomial and Brière-2 temperature-dependent models to estimate optimum temperatures for growth (*Topt*) and maximum growth rates (*Rmax*), and used to calculate temperature coefficients (*Q10*) and activation energies (*Ea*) for growth. All studied strains showed highest R and RA when grown under skim milk nutrient assay. *Topt* for growth in *F. equiseti* was 25°C, and 20°C in *Pseudogymnoascus* spp. Only *F. equiseti* showed cellulase activity. The data suggest a preference for protein-based over plant-derived substrates for metabolism in these fungal strains. The tropical *F. equiseti* could utilise higher levels of thermal energy for growth than the polar strains of *Pseudogymnoascus* spp., implying adaptation of these fungi to different biogeographical regions.

**Keywords**: Ascomycota; extracellular enzymes; growth rate; seawater; temperature.

**Introduction**

Fungi are present on various substrates in the marine environment, from coastal to deep-sea ecosystems. Some fungi are of terrestrial and/or freshwater origin, particularly those occurring in seawater and sediment in coastal areas, and therefore these fungi are generally called ‘marine-derived’ fungi (Pang et al. 2016). They play an important role as decomposers in marine environments, transforming dissolved or particulate organic matter (DOM and POM) into simpler chemical compounds that can be utilised by themselves or other consumers in the marine food web (Bianchi 2011). Eighty-percent of the organic matter, largely comprised of proteins, followed by carbohydrates, lipids, and nucleic acids, present in marine ecosystems is preserved in deltaic and coastal deposits (Hedges 1992, Fabiano and Danovaro 1998, Kujawinski 2011). Marine fungi mainly occur in coastal environments, which are typified by high organic matter content and productivity (Pang and Jones 2017).

*Fusarium* Link and *Pseudogymnoascus* Raillo are fungal genera that are common in environmental samples originating from lower (tropical) and higher (temperate and polar) latitudes, respectively. Representatives of the two genera are clearly halotolerant (Kochkina et al. 2007, Summerell et al. 2010). Some species are pathogenic to other living organisms. A majority of the well-characterised members of *Fusarium* (e.g. *F. oxysporum* Schltdl., *F. graminearum* Schwabe, *F. solani* (Mart.) Saccardo, *F. equiseti* (Corda) Saccardo) are cosmopolitan plant pathogens colonising aerial and root structures in plants, and sediments/soils in which their hosts occur (Summerell et al. 2010). Several *Fusarium* species have been reported to grow differentially in response to specific environmental triggers such as temperature and nutrient sources, and some are dimorphic, growing as yeasts at temperatures higher than 30°C (Szécsi and Magyar 2011). In comparison to *Fusarium*, there are currently fewer described *Pseudogymnoascus* species. However, *Pseudogymnoascus* spp. are often the most common fungal group in both temperate and polar soils (Kochkina et al. 2007). *Pseudogymnoascus* includes important species such as *P.* *pannorum* (Link) Minnis *et* D.L. Lindner which is a plant pathogen, and *P. destructans* (Blehert *et* Gargas) Minnis *et* D.L. Lindner, the causative agent of white-nose syndrome (WNS) in bats in Europe and North America (Hayes 2012, Verant et al. 2012).

Environmental factors such as temperature, nutrients, and salinity affect fungal growth rates and act as triggers in developmental pathways (Deacon 2006). Temperature is one of several important drivers of physiological, ecological, and evolutionary processes in living systems, including in fungi. Reaction rates and properties of compounds fundamental to cellular functions change with variability in temperature. Lower thermal limits for microbial growth are set by the slowing down of biological processes. Upper thermal limits for microbial growth, on the other hand, are set by the first cellular component or process that breaks down (Robinson 2001, Deacon 2006). To survive temperature fluctuations, fungi adjust phenotypic features to accommodate their physiological processes to specific thermal needs. Some species are known to alter their growth forms under different conditions through several mechanisms, including growing in pellets when grown in liquid culture, producing dormant cells called resting spores in unfavourable environmental conditions, and exhibiting dimorphism as seen in the model fungus *Histoplasma capsulatum* Darling, which can grow as a yeast from mould (thus becoming pathogenic) at 37°C in human bodies, and *vice versa* in the yeast *Candida albicans* (C.P. Robin) Berkhout (reverse morphogenesis) (Ignatov and Keath 2002, Nemecek et al. 2006, Liu et al. 2008). Fungi have been subjected to thermal treatments in a wide range of medically- and industrially-driven research, usually testing hypotheses on the relationship between fungal pathogenicity or function and temperature (Paterson and Lima 2010, Fisher et al. 2012). The influence of temperature on fungal adaptation to their ecological niche as decomposers, however, has received limited research attention, with most existing studies examining growth responses of terrestrial or freshwater fungi to different temperatures (Newsham et al. 2016).

With this background, we studied relative growth rates (R) and relative activities (RA) of three EHEs in a tropical marine strain of *Fusarium equiseti* and two polar strains of *Pseudogymnoascus* spp. across an experimental culture temperature range from 5 to 40°C. We hypothesised that fungal strains adapted to specific biogeographical regions would exhibit differences in growth rates and enzyme activities when provided in culture with different nutrient sources and subjected to temperature variation. The objectives of this study were (a) to determine the relationship between relative growth rates and temperature in the three strains when provided with different nutrient sources at culture temperatures between 5 and 40°C, (b) to determine the activation energy for growth in the three strains across the experimental temperature range, and (c) to profile relative activity of three EHEs (protease, amylase, cellulase) produced by the three strains across the experimental temperature range.

**Materials and methods**

**Marine-derived fungal strains**

One representative fungal strain from each of the tropical and polar (Arctic and Antarctic) latitudes was selected from the Institute of Ocean & Earth Sciences (IOES) and National Antarctic Research Centre (NARC) culture collections. These were *Fusarium equiseti*, originally isolated from a tropical beach vegetated with *Vitex rotundifolia* in Peninsular Malaysia, and two strains of *Pseudogymnoascus* (HND16 R2-1 sp. 2 and AK07KGI503 R2-1 sp. 3) respectivelyisolated from soil in maritime Antarctica and coastal sediment in the High Arctic (Krishnan et al. 2011, Ali et al. 2014). These strains were selected based on the following features: (a) ascomycetes isolated from the marine environment; (b) strains growing in the asexual state at ambient local temperature (26 – 29°C); and (c) strains that showed highest radial growth in stock plates in comparison to other strains originating from similar biogeographical regions.

**Maintenance of stock culture plates**

The three fungal strains were revived by sub-culturing onto seawater Potato Dextrose Agar medium (PDA; potato infusion 4.0 g/L, D(+)glucose 20.0 g/L, and agar-agar 15.0 g/L; Merck, Germany). After four weeks, each strain was sub-cultured again onto seawater PDA plates (30 psu Crystal Sea Marinemix; USA, 3.75% w/v salt mix dissolved in distilled water, stirred overnight, and filtered through a Whatman No 1 filter paper supplemented with 0.01% v/v chloramphenicol). The tropical strain was maintained at local ambient temperature (26 – 29˚C), while the polar strains were maintained at 15˚C.

**Preparation of fungal inoculants under different nutrient assays**

Growth and extracellular hydrolytic enzyme activity of the three strains were observed on a seawater Reasoner’s 2A-based (R2A; yeast extract 0.5 g/L proteose peptone 0.5 g/L, casein hydrolysate 0.5 g/L, glucose 0.5 g/L, soluble starch 0.5 g/L, sodium pyruvate 0.3 g/L, dipotassium hydrogenphosphate 0.3 g/L, magnesium sulphate anhydrous 0.024 g/L, agar-agar 15.0 g/L; Merck, Germany) medium supplemented with skim milk (0.4% w/v skim milk), soluble starch (0.4% w/v soluble starch), or cellulose (0.4% w/v carboxylmethylcellulose with 0.01% w/v trypan blue) (Merck, Germany) (modified from Margesin et al. 2003). All media were supplemented with 0.01% v/v chloramphenicol, and adjusted to a final salinity of 30 psu using sea salt. Trial 10 d growth experiments were performed at 25°C to standardise screening time-points across all subsequent experiments, taking into account the different growth rates between the selected strains.

One mycelial plug (7 mm diameter) from each fungal strain was inoculated onto each individual assay plate using a sterile cork borer and a pair of forceps. Inoculated assay plates were prepared in triplicates to study growth of the fungi in skim milk, starch, and cellulose nutrient assays and screening of protease, amylase, and cellulase was then carried out. Screening for growth and EHE activities took place at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40˚C during the mid-log phase of growth (*D3* of growth in *Fusarium equiseti*, and *D5* of growth in *Pseudogymnoascus* spp., data not shown).

**Relationship between temperature and fungal growth rates under different nutrient sources**

Temperature levels vary dramatically both spatially and temporally in both tropical and polar habitats because of the interaction of various abiotic factors governed by regional climates. Mean surface temperatures in the polar regions can be as low as -20°C, but increase to short-term maxima of 25°C or more, while shaded surface temperatures in the tropics narrowly range between 28 and 32°C (Nagelkerken 2009, Convey 2012, Peck 2015). Therefore, our experimental thermal regime was designed to encompass recorded land and sea surface temperatures typical of both tropical and polar regions, and the three fungal strains were grown at 5, 10, 15, 20, 25, 30, 35 and 40°C.

Relative growth rates (R d-1) of the three strains across the experimental temperature range were calculated using Eq. 1. Relative growth rates at different temperatures were calculated assuming exponential growth (Eq. 1). Calculated relative growth rates were then fitted into polynomial (Eq. 2) and Brière-2 (Eq. 3) non-linear models to obtain best-fitting thermal growth curves of these strains on each of the three nutrient sources, and at temperatures from 5 to 40°C (Lamb et al. 1984, Brière et al. 1999).

(Eq. 1)

In Eq. 1, *D0* and *Df* are the colony diameters measured before and after the experimental period, respectively, and *Δt* is the time span (d) of the incubation periods.

(Eq. 2)

(Eq. 3)

In Eq. 2, *a*, *b*, *c*, and *d* are equation constants. In Eq. 3, *a* and *m* are equation constants, and *Tmin* and *Tmax* the critical minimum and maximum temperatures, respectively. Curves fitted under Eq. 3 were calculated through unconstrained iterative non-linear regression based on the Levenberg-Marquadt algorithm. Information from the raw data was used to constrain the parameters *Tmin* (critical minimum temperature) and *Tmax* (critical maximum temperature) of Eq. 3 (e.g. *F. equiseti* did not grow at 5 and 35°C during the 3 d incubation period). Curves fitted under Eq. 3 were calculated through constrained iterative non-linear regression based on the quadratic sequential programming algorithm.

Curve estimates under the two nonlinear models were compared against three parameters: coefficient of determination (*R2*), residual sum of squares (*RSS*), and corrected Akaike information criterion (*AICc*) (van Boekel and Zwietering 2007). *R2* indicates goodness-of-fit of the models against the datasets. Estimates with lower or negative *AICc* values are preferred to represent the datasets*.* The *AIC* was calculated using Eq. 4.

(Eq. 4)

where *n* is the number of treatments, *RSS* is the residual sum of squares, and *p* is the number of parameters in the model.

Optimum temperatures (*Topt*) for growth for each strain under the different nutrient assays were calculated using Eq. 5 (Van Der Heide et al. 2006).

(Eq. 5)

Eq. 6 was used to calculate the growth rate at *Topt* (*Rmax* d-1) by substitution of *T* = *Topt* as shown in Eq. 3, where *a* and *m* are equation constants following the values estimated for Eq. 3. Eq. 6 was also used to manually counter-check parameter *a* values of Eq. 3.

(Eq. 6)

All non-linear regression (NLR) procedures were run in SPPS Statistics v.21 (IBM, USA).

**Relationship between temperature and thermal energy requirement for fungal growth under different nutrient assays**

To compare growth rates across the temperature series from 5 to 40°C, the temperature coefficients (*Q10*) were calculated across the different temperature intervals (Eq. 7). *R2* is relative growth rate at the higher temperature, *R1* is relative growth rate at the lower temperature, *T2* is the higher temperature, and *T1* is the lower temperature.

(Eq. 7)

An Arrhenius plot was used to plot the natural log of *k* (ln *k*) versus 1/*T* (*k* is estimated growth rate and *T* is absolute temperature in Kelvin) and to calculate the activation energy (*Ea,* J mol-1) required by the three fungal strains to initiate growth at each temperature between 5 and 40°C under the different nutrient sources. *Ea* values could be determined from the slope (-*Ea*/*R*) between temperature points on the resultant plots, and by solving Eq. 8.

(Eq. 8)

(Eq. 9)

Eq. 8 is the Arrhenius equation, in which *A* is the pre-exponential factor, *e* is Euler’s number (2.718), *R* is the gas energy constant (8.314 J mol-K), and *T* is absolute temperature in Kelvin. Eq. 9 is derived from Eq. 8.

**Screening of extracellular hydrolytic enzyme (EHE) activity and radial growth under three different nutrient sources between 5 and 40°C**

Extracellular hydrolytic enzyme (EHE) activity was assessed by the presence of a clear zone around the fungal colonies (colorimetric assessment). Protease and amylase activities were confirmed by flooding the nutrient assay plates with Coomasie Blue staining reagent and Lugol’s solution, respectively, while cellulase activity was indicated by decolouration of trypan blue around fungal colonies. Both diameters of fungal colonies and clear zones were measured and recorded to calculate relative enzyme activity (RA) indices. Values ≥ 1.0 indicate significant relative enzyme activity following Bradner et al. (1999). *RA* was calculated as follows:

(Eq. 10)

where *Dcz* is diameter of the clear zone around the fungal colony, and *Df* is the diameter of fungal colony after the 3 d incubation period for the tropical strain of *F. equiseti* and 5 d for the polar strains of *Pseudogymnoascus* spp.

**Statistical analyses of relative growth rate (R) and enzyme activity (RA)**

Mean and standard deviation of Rand RA from all datasets were calculated. The effects of temperature on R and RAvalues in the three different nutrient assays were analysed using multivariate analyses of variance (*m*ANOVA). When significant differences were detected, a *post hoc* Bonferroni test (*P* < 0.05) was applied to examine pairwise differences. Paired t-tests were applied to further examine these significant differences. All analyses were performed using SPSS Statistics v.21 (IBM, USA).

**Results**

**Thermal growth optima and relative growth rates**

Relative growth rates were calculated for each strain under different nutrient assay plates incubated between 5 and 40°C. These were then fitted into third-degree polynomial and Brière-2 non-linear temperature-dependent models. The third-degree polynomial model, while parsimonious to Brière-2, had no biologically-meaningful parameters. This model also had *R2* values consistently higher than those estimated by the Brière-2 model, but the resulting curves were not suitable to describe biological growth. This was further supported by AICc values estimated by the Brière-2 model, with majority of these being consistently lower than those estimated by the third-degree polynomial model. All the parameters estimated by these two models, and temperature-dependent curves estimated by the third-degree polynomial model are presented in Supplementary information Table S1.

Figure 1A-C show the relative growth rates of the three studied strains grown under the three nutrient sources (skimmed milk, soluble starch, carboxylmethylcellulose) between 5 and 40°C. The growth curves estimated for *Pseudogymnoascus* spp. were similar and consistent under all three nutrient sources, with *Topt* of the Arctic strain being 20°C ± 0.2 and *Rmax* = 0.49 d-1, and *Topt* for the Antarctic strain being 20°C ± 0.3 and *Rmax* = 0.50 d-1 ± 0.2. These two strains had *Tmax* = 30°C. Growth curves estimated for *F. equiseti* were similar except when grown under carboxylmethylcellulose. The *Rmax* value at *Topt* in *F. equiseti* was three times higher than that of *Pseudogymnoascus* spp. grown under skim milk or soluble starch, and twice that obtained under carboxylmethylcellulose. *Topt* of *F. equiseti* as estimated by the Brière-2 model was 24.9°C, and *Rmax* was the highest when grown in skim milk (*Rmax* = 1.49 d-1) (Figure 1A). *Rmax* values were progressively lower when grown under the other two substrates, as shown in Figures 1B and 1C (*Rmax* = 1.44 d-1 and 1.05 d-1, respectively). *Tmax*of *F. equiseti* was 35°C.

**Temperature coefficient (*Q10*) and activation energy (*Ea*) for growth**

The temperature coefficient (*Q10*) and activation energy (*Ea*) values were calculated using estimates by the Brière-2 nonlinear temperature-dependent model to determine the ratios of relative growth rates at a higher temperature to a lower one across the experimental temperature range. *Q10* values could only be calculated and compared where growth occurred in the three fungal strains across the temperature series. Therefore, we present here the data obtained from datasets on relative growth rates under skim milk and carboxylmethylcellulose sources, since there were marked differences in EHE activities and radial growth between polar *Pseudogymnoascus* spp. and the tropical *F. equiseti* as noted above. Calculated values of *Q10* are presented in Table 1.

The calculated *Q10* coefficients decreased with increasing temperature for all three strains when grown under the skim milk and carboxylmethylcellulose nutrient assays.  *Q10* values in *F. equiseti* were generally higher than those of *Pseudogymnoascus* spp..

Using the Arrhenius plot, the activation energy values of radial growth in the three strains were estimated (Figures 2A and 2B). Figure 2A shows energy requirements in the form of the activation energies (*Ea*) for these strains to grow across the experimental temperature range. Activation energy for growth in these strains (*Ea*) decreased with increasing thermal energy, obeying the Arrhenius behaviour of exothermic reactions (Sims 2013). *Pseudogymnoascus* spp. and *F. equiseti* generally required a lower *Ea* when grown in plates augmented with skim milk than those augmented with carboxylmethylcellulose. *Ea* also declined with increasing temperature; a negative *Ea* was calculated when growth in these strains occurred.

**Trade-off between growth and enzyme activity**

The polar strains of *Pseudogymnoascus* spp. showed only protease and amylase activities across the experimental temperature range after the 5 d incubation period (Figures 3A and 3B). Cellulase activity was recorded only in *F. equiseti*, from 5 to 30°C (Figure 3C). Relative enzyme activity (RA) indices were the highest at temperatures at which relative growth rates in these fungal strains were the lowest i.e. at 5°C and between 30 and 40°C. The same pattern of enzyme activity was observed in the tropical strain of *Fusarium equiseti* incubated across the full experimental temperature range for 3 d, and which had the lowest relative growth rate at 5°C. Colony diameter measurements could not be carried out after *F. equiseti* was incubated at 35 and 40°C because no filamentous growth was observed. Calculated RA indices for protease, amylase, and cellulase activities were low in *F. equiseti*.

Figure 3 also highlights important pairwise comparisons identified in the *m*ANOVA analyses. There were significant differences in RA values of EHEs across the three fungal strains, *F*56,286 = 29.21, *P* < 0.001; Wilk's Λ = 0.022, partial *η2* = 0.85. Protease and amylase activities were higher at the maximum temperature for growth (*Tmax* = 30°C) in *Pseudogymnoascus* spp. than at the optimum temperature (*Topt* = 20°C) (*P* < 0.001) (Figures 3A and 3B). *Tmax* is a term equivalent to the upper critical limit (*CLu*) used in Verant et al. (2012). In Figure 3B, amylase activity at 5°C, at which the lowest growth rate occurred in both strains of *Pseudogymnoascus* spp., was also significantly higher than at their *Topt*for growth (*P* < 0.05, and *P* < 0.001, respectively). A similar result was obtained when comparing cellulase activity in *F. equiseti* at 5°C, and at the temperature with highest growth rate, 30°C (*P* < 0.001) (Figure 3C).

*Pseudogymnoascus* spp. grew on carboxylmethylcellulose assay plates without showing any observable cellulase activity. Calculated RA indices were low but consistent across the range of culture temperatures between 5 and 30°C in *F. equiseti*. As illustrated in Figures 4A and 4B, measures of fungal colony and clear zone diameters were comparable in *F. equiseti*. Radial growth under all nutrient assays was greatest at 30°C. Radial growth, however, was lower on carboxylmethylcellulose plates than on skim milk and soluble starch. Clear zone diameters indicative of cellulase activities were also larger than fungal colonies. This resulted in RA > 0 in *F. equiseti* from 5 to 30°C, with the highest RA recorded at 5°C.

**Discussion**

Determining thermal energy requirements for growth in *Fusarium equiseti* and *Pseudogymnoascus* spp. under different nutrient sources is key to understanding how these fungal strains are adapted to natural ambient temperatures, and respond to changes in their natural habitats. A large amount of carbon is stored as high molecular weight organic matter in the environment, and temperature is the primary factor driving both fungal growth and hydrolytic processes of organic matter (German et al. 2012). In the current study, in comparison to *F. equiseti*, *Pseudogymnoascus* spp. exhibited consistently low relative growth rates when grown under different nutrient sources across the experimental culture temperatures. This suggests that polar strains of *Pseudogymnoascus* spp. require lower thermal energy than the tropical *F. equiseti* to grow optimally, consistent with the generally low temperatures and nutrient availabilities (as proxies for thermal and chemical energy, respectively) that are characteristic of the polar regions.

Living fungal cells are in effect a system containing a mixture of biochemicals with pre-existing potential energy, and their reaction rates increase with higher input of thermal energy; this results in lower activation energy (*Ea*) required to catalyse growth-related reactions in these strains (Battley 2013, Peck 2015). *Pseudogymnoascus* spp. grew optimally at around 20°C, and exhibited relatively high *Q10* values for growth rates between 5 and 10°C, and lower values at higher temperatures. These data indicate that these polar strains of *Pseudogymnoascus* spp. are psychrophilic/psychrotolerant, while the possession of elevated *Q10* values at low environmental temperatures has also been proposed as a mechanism of stress tolerance adaptation in terrestrial biota of the polar regions, allowing advantage to be taken of short-term increases in microclimate temperatures (Convey 1996). Several studies have reported optimal temperatures for growth in *Pseudogymnoascus* spp. to range between 15 and 20°C (Zucconi et al. 1996, Krishnan et al. 2011, Hayes 2012). The mesophilic strain of *F. equiseti* studied here, on the other hand, is adapted to the greater availability of thermal energy at progressively lower latitudes. Thus, it had a higher thermal optimum of approximately 25°C, and could tolerate a temperature as high as 30°C for growth. Other members of *Fusarium* have also been reported to grow and reproduce optimally between 25 and 30°C (Marin et al. 1995, Doohan et al. 2003). In nature, competitive interactions can restrict growth of a fungus to a much narrower temperature range (Deacon 2006). Furthermore other factors can influence the thermal response characteristics of fungi, such as symbiotic interactions between fungi and other host organisms which can increase host tolerance to temperature stress (Redman et al. 2002).

Fungal EHEs are better understood in pathogenesis than in other ecological processes, including nutrient cycling. Proteases, in particular, are common virulence factors in fungal strains preying upon nematodes and insects, and are primary biocatalysts of mycoses in animals including humans (Huang et al. 2004, Yang et al. 2007). To take advantage of various substrates available in marine environments, fungi such as those studied here may secrete a suite of EHEs that are active under the pH levels and high salinity that are characteristic of such environments. Halotolerance in EHEs obtained from marine microbes has largely been identified and characterised in bacterial groups and in the marine water column, and knowledge of the expression and roles of fungal EHEs in coastal environments is very limited at present (Arnosti et al. 2014). Our data demonstrate that the *F. equiseti* and *Pseudogymnoascus* spp. studied can produce a range of functional EHEs that would allow utilisation of major environmental substrates. The ability of *F. equiseti* to secrete cellulases illustrates the versatility of this group of opportunistic microorganisms. Members of *Fusarium* are most commonly associated with plant substrates, including living plant hosts where they can be rampant pathogens. Therefore, they occur where vegetation is abundant, most often in lower latitudes including in coastal environments (Summerell et al. 2010). St. Leger et al. (1997) argued that versatility in nutrient exploitation may not result from specific adaptation, and rather reflects ability to exploit a wide range of substrates when they become temporarily present. However, our data indicate an absence of cellulolytic activity in marine *Pseudogymnoascus* spp.

Our data do not permit the inference that *F. equiseti* showed no EHE activity based on the formula proposed by Bradner et al. (1999) alone. Furthermore, *Pseudogymnoascus* spp. and *F. equiseti* did not share similar colony morphologies; *Pseudogymnoascus* spp. had thick colonies with slower radial growth rates, while *F. equiseti* were widespread and grew more rapidly in culture plates. Contradicting results between growth and cellulase activity as seen in *Pseudogymnoascus* spp. might be explained in two ways: (a) the strains were degrading minute amounts of starch that was present in R2A due to it being a simpler organic substrate than carboxylmethylcellulose, and/or (b) the strains may possess cell-wall associated cellulases (also known as endoglucanases); such enzymes would not be released into the media, and thus not detected in the assays used here (Adams 2004). Previous enzyme screening studies have drawn mixed conclusions on cellulase activity in *Pseudogymnoascus*. Antarctic strains of *P. pannorum* isolated from various sites showed moderate to no cellulase activity at 25°C in non-soluble enzyme assays (Fenice et al. 1997, Duncan et al. 2008, Krishnan et al. 2011). However, *Pseudogymnoascus* strains associated with deep-sea sponges in the Irish Sea were reported to show cellulase activity (Batista-García et al. 2017). Nutrient profiles across Arctic, Antarctic and tropical coastal waters are similar, with proteins, followed by carbohydrates and lipids, being the largest components of characterised organic matter present in the marine water column. Our data might suggest that polar strains of *Pseudogymnoascus* spp. have evolved in an environment where complex plant-based substrates such as celluloses are scarce. Therefore, they have adapted to use proteins and other simpler carbohydrates for extracellular digestion over celluloses.

It is possible that members of *Pseudogymnoascus* prevalent at higher latitudes are under selective pressure to survive in habitats with a lower diversity of nutrient sources than is typical at lower latitudes. Schneider et al. (2012) provided evidence of local temporal EHE adaptation in Austrian temperate forests, where litter nutrient content and stoichiometry of C:N:P affect the decomposer community and activity. Fungi were found to be the main producers of EHEs, and ascomycetous EHEs were succeeded by those of basidiomycetes with the seasonal transition from spring to summer as the forest floor was increasingly littered with recalcitrant lignin-containing substrates. Spatial adaptation in EHEs has also been demonstrated (German et al. 2012), with cold-active cellulose-degrading β-glucosidase from higher latitudes being more temperature-sensitive than that from lower latitudes. Similar observations, however, are still lacking for microbial communities in coastal/marine sediments (Hyde and Lee 2015).

The enzyme screening data obtained in the current study clearly demonstrate that the proteases and amylases produced by both tropical *F. equiseti* and polar *Pseudogymnoascus* spp. were both cold-active (i.e. capable of functioning at low temperature) as well as thermotolerant, exhibiting activity across the experimental temperature range to as high as 40°C. Our data suggest a trade-off between radial growth and EHE activity across the experimental temperature range from 5 to 40°C in the three strains studied. However, higher RA values exhibited by these strains at temperatures at which growth was lower may also be a consequence of enzyme kinetics and functional efficiency rather than increased enzyme production rate by fungal cells. Our data indicate a clear energy trade-off when a specific type of EHE was produced and secreted extracellularly, as seen with *F. equiseti* showing lower growth rates while exhibiting higher relative celluloytic activity in the R2A-based carboxylmethylcellulose assay than in the skim milk and starch nutrient assays (the latter screening for protease and amylase, respectively). This suggests that the expression of EHEs degrading more chemically complex environmental substrates such as celluloses may require higher metabolic energy in fungi, diverting cellular energy resources away from growth processes. Complete enzymatic degradation of cellulose utilises at least three enzymes - an endoglucanase, an exocellobiohydrolase and a β-glucosidase (Mansfield et al.1999, Karlsson et al. 2002).

**Conclusions**

The marine-derived strain of *F. equiseti* studied here was mesophilic and had a higher thermal optimum for growth, indicating adaptation to the higher level of thermal energy available in the tropics than the polar regions. The psychrophilic/psychrotolerant strains of *Pseudogymnoascus* spp. are representatives of a genus prevalent at higher latitudes. Our data indicate that proteins may be a more accessible source of nutrients for *F. equiseti* and *Pseudogymnoascus* spp. than complex carbohydrates, and gave some evidence consistent with the presence of an energy trade-off between fungal growth and EHE activity.

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**List of figures**

**Figure 1:** Temperature-dependent growth curves of the Arctic marine strain of *Pseudogymnoascus* sp. (yellow), the tropical strain of *Fusarium equiseti* (red), and the Antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated onto R2A-based (A) skim milk, (B) soluble starch, and (C) carboxylmethylcellulose nutrient assay plates. Coloured points with error bars indicate observed mean relative growth rates with standard deviations. *Topt* is optimum temperature for growth, and *Rmax* is maximum relative growth rate.

**Figure 2:** (A) Arrhenius plots (left) of measured growth rates and (B) Activation energy

(*Ea*) of growth (right) in the Arctic strain of *Pseudogymnoacus* sp. (yellow), tropical strain of *Fusarium equiseti* (red), and Antarctic strain of *Pseudogymnoascus* sp. (blue) inoculated onto R2A-based (i) skim milk, and (ii) carboxylmethylcellulose assay plates across the experimental temperature series between 10 and 30°C.

**Figure 3:** Mean relative activity of (A) protease, (B) amylase, and (C) cellulase in the Arctic marine strain of *Pseudogymnoascus* sp. (yellow), tropical strain of *Fusarium equiseti* (red), and Antarctic strain of *Pseudogymnoascus* sp. (blue) at temperatures between 5 and 40°C. Error bars indicate standard deviation. Horizontal brackets highlight pairwise comparison of relative enzyme activity between 5 (lowest growth rate, moderate activity) and 20°C (lowest activity, highest growth rate), and between 20 and 30°C (highest activity, no growth) among the Arctic and Antarctic strains in Figure 1A and 1B, and between 5 (lowest growth rate, moderate activity) and 30°C (highest growth rate, lowest activity) in the tropical strain in Figure 1C. Significance: \* - *P* < 0.05, \*\*\* - *P* < 0.001. Only positive relative activity indices are shown.

**Figure 4:** Mean colony and clear zone diameter measures in the tropical strain of *Fusarium equiseti* inoculated onto R2A-based (A) skim milk, (B) soluble starch, and (C) carboxylmethylcellulose nutrient plates. Red dashed lines indicate initial colony diameter (7 mm). Error bars indicate standard deviation. Only temperatures at which filamentous growth occurred during the 3 d incubation period are shown.

**Supporting information**

**Table S1:** Descriptive statistics and parameter values for best-fit functions of each fungal strain grown under different nutrient assays.