Arrhenian growth thermodynamics in a marine-derived tropical *Fusarium equiseti* and polar *Pseudogymnoascus* spp. in a liquid culture system

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**ABSTRACT**

We hypothesised that the activation energy ($E_a$) of growth in a marine-derived tropical strain of *Fusarium equiseti* and polar strains of *Pseudogymnoascus* spp. grown for 10 days in a liquid culture system comprised of seawater Mueller-Hinton Broth would differ across the same experimental culture temperature range. The specific growth rates (SGRs) obtained from these experiments were fitted into third-degree polynomial and Brière-2 temperature-dependent models to estimate optimum temperatures for growth ($T_{opt}$) and maximum SGR ($SGR_{max}$) of the selected strains. Estimates of SGR values from the Brière-2 model were used to calculate the temperature coefficient ($Q_{10}$) and $E_a$ for growth in all three fungal strains across the experimental culture temperature range. Our findings indicated that *F. equiseti* is better adapted to utilising higher levels of thermal energy for growth than either *Pseudogymnoascus* strain, consistent with general definitions that classify the former as a mesophile and the latter as psychrophiles. A progressive increase in pH was recorded in the liquid culture system during the growth of *F. equiseti* and *Pseudogymnoascus* spp., suggesting that these strains could tolerate more alkaline conditions for growth until nutrient resources were exhausted, as has been noted in some other fungal studies.

**1. Introduction**

Fungi adapt to the availability of thermal energy in the environment. A majority of the studied species can be given a thermal classification based on their thermal optima and range for growth, which often reflects their distribution across the globe. The four conventional thermal classes of microorganisms in relation to their growth temperature optima are: (i) psychrophily ($\leq 15^\circ C$), (ii) mesophily (25–40°C), (iii) thermophily (45–80°C), and (iv) hyperthermophily (> 80°C). Microorganisms that exhibit optima between 20 and 40°C but are able to grow (although very slowly) at 0 °C are psychrotolerant, while those that exhibit growth optima ≥ 40°C but have maximum (lethal) temperatures ≤ 50°C are thermotolerant (Madigan et al., 2014). As a kingdom, fungi are ubiquitous, occurring across marine and terrestrial habitats, and species of terrestrial origin also occur in the marine environment. Fungi that occupy terrestrial habitats but are also found in/on marine substrates are termed as ‘marine-derived fungi’ (Pang and Cowen, 2013).

Fungal adaptation to temperature has long been studied in the context of understanding their responses to thermal stress, which affects proliferation processes in natural and artificial environments. Some of these responses have been quantified through molecular approaches, including measuring the expression of heat shock proteins (Hsp; most commonly Hsp90) and antifreeze proteins (AFPs) (Robinson, 2001). Physiological responses have also been measured by measuring fluidity of the plasma membrane and the production of principal fungal metabolites, such as polyols (glycerol, mannitol, etc.), the sugar alcohol ergosterol, and trehalose (Niemenmaa et al., 2008; Xiao et al., 2010; Cowen, 2013).

Growth rates, being a physical property of growth, can also be used as an indicator of response towards varying environmental conditions and, hence, be instrumental in understanding microbial adaptation to temperature. For bioenergeticists, this concept is used to investigate the flow and conversion of thermal energy into and out of cells (von Stockar et al., 2006). The limited available literature on microbial growth thermodynamics have focussed on yeast, bacterial, and microalgal populations grown under controlled experimental conditions (Sandler and Orbey, 1991), and information on growth thermodynamics in filamentous fungi remains lacking. Filamentous fungi are multicellular organisms growing in a network of hyphae known as mycelia, forming...
pellets as they grow in liquid cultures (Casas Lopez et al., 2005), which makes their growth challenging to measure using standard spectrophotometric approaches. The relationship between growth rates and thermal adaptation can be linked based on the following premises: (i) temperature is a scale of thermal energy; (ii) temperature is a factor of growth; and (iii) growth rates are a physical vector of growth over time. Therefore, a viable approach to describing and understanding thermal adaptation in different fungal species found in specific bioclimatic regions is through relating their growth rates to thermal energy requirements.

There are three principle laws of thermodynamics (four when the zeroth law is considered) that conceptualise the transformation of energy across different systems and scales – from a system as large as the Earth, to the microscopic scale of living cells: (i) the first law of thermodynamics states that when energy passes into or out of a system, the system’s energy changes in accordance to the law of conservation of energy (e.g., thermal energy is converted into kinetic energy measured through biomass production within a closed culture system); (ii) the second law of thermodynamics states that the entropy (disorder) of a system increases with energy input (e.g. a microbial cell reproduces more daughter cells with increasing temperature until nutrient sources are exhausted); and (iii) the third law of thermodynamics states that the entropy of a system approaches a constant value as the temperature approaches absolute zero (0 K or −273.15 °C). If one system has the same temperature as another that it is in contact with (e.g. the environment and a living microbial cell that it surrounds), both are in thermal equilibrium with each other. If they are initially at different temperatures, they will eventually achieve thermal equilibrium (this is also known as the zeroth law of thermodynamics). According to the law of conservation of energy, energy can neither be created nor destroyed; it can only be transformed from one form to another, e.g. potential to kinetic energy. Microbial growth reactions are considered spontaneous and irreversible. Therefore, they are coupled with a production of entropy in which microbial cells are treated as an open system (Sandler, 1991; Sandler and Orvey, 1991; Batley, 1998; von Stockar and Liu, 1999; von Stockar et al., 2006; Batley, 2013). Hence, we hypothesise that fungal strains adapted to different thermal ranges will utilise different levels of thermal energy to carry out growth processes, with cold-adapted strains requiring less thermal energy for growth than mesophiles.

A principal thermodynamic theory that is very pertinent to the investigation of thermal adaptation in biological systems from a physical perspective is the Arrhenius law. The law states that rate of reactions depends on the energy state of molecules involved in the reaction, where only those molecules above a certain energy state can complete the reaction. This energy threshold is called the activation energy ($E_a$); at higher temperatures, a larger proportion of molecules present are above the $E_a$, making reaction rates faster (Logan, 1982). The limitation of the Arrhenius law is that it assumes that only temperature changes in the system, not allowing interactions of many factors such as multiple reactions running concurrently in a cell. However, a very large body of data published over the past 100 years have demonstrated that biological rates predominantly follow the Arrhenius relationship over the normal biological temperature range (Peck, 2015). Therefore, in the current study, we used growth rates as a physical indicator of thermal adaptation in fungi, also deriving $E_a$ for growth in our studied fungal strains to test our underlying hypothesis that the $E_a$ of growth in a marine-derived tropical strain of Fusarium equiseti Corda (Sacc.) and polar strains of Pseudogymnoascus spp. Rillou would differ across the same experimental culture temperature range. With this background, in a previous study (Tajuddin et al., 2017) we subjected three marine-derived fungal strains, obtained from tropical and polar regions and growing under three different solid-state nutrient assays, to an experimental thermal treatment over a temperature range from 5 to 40 °C. This experimental temperature range was set to reflect the range of land and sea surface temperatures (LST and SST) of tropical, temperate, and polar regions (https://neo.sci.gsfc.nasa.gov/). We determined the growth energetics of the mesophilic F. equiseti and two cold-adapted Pseudogymnoascus strains based on relative growth rates (RGR). The study found that Pseudogymnoascus spp. utilised less thermal energy for growth than did F. equiseti. The growth reactions in these strains obeyed the Arrhenius behaviour of exothermic reactions, where $E_a$ for growth decreased with increasing temperature (Sims, 2013). The present study aims to develop the concept introduced by Tajuddin et al. (2017) by incorporating information based on specific growth rates (SGR), which can be obtained through a liquid-state culturing system. The objectives of this study were: (a) to determine the relationship between SGRs and temperature in the three strains at culture temperatures between 5 and 40 °C using a proteinaceous nutrient source, (b) to determine the $E_a$ for growth in the three strains across the experimental temperature range, and (c) to compare the growth energetics of these strains when cultured under liquid-state culture systems.

2. Materials and methods

2.1. Experimental regime

The three fungal strains, a tropical strain of Fusarium equiseti (strain code FEQ006) and the Arctic and Antarctic strains of Pseudogymnoascus spp. (HND16 R2-1 sp. 2 and AK07KG503 R2-1 sp. 3, respectively), used in the current study were selected as described by Tajuddin et al. (2017). Each growth experiment was run for 10 days, at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C. The culture medium used in the experiment contained Mueller Hinton Broth [(MHB; meat infusion 2.0 g l\(^{-1}\), casein hydrolysate 17.5 g l\(^{-1}\); starch 1.5 g l\(^{-1}\); average initial pH 6.8) (Merck, Darmstadt, Germany)] medium dissolved in artificial seawater with a salinity adjusted to 30 [(Crystal Sea Marine Mix; Marine Enterprises International) (Baltimore, MD, USA), 3.75% w/v salt mix dissolved in distilled water, stirred overnight and filtered through a Whatman no. 1 filter paper (Merck; Darmstadt, Germany)]. Each conical flask was inoculated with 25 ml of culture medium. Forty conical flasks were prepared for each temperature experiment for each fungal strain. Of these, the planned sampling regime required 33 culture flasks to be sampled by the end of each experiment, allowing some redundancy (Krejcie and Morgan, 1970). Three culture flasks were sampled every 24 h, including for $D_w$ weight measurements (total of 11 sampling days). Each culture flask was inoculated with two mycelial pieces (each 4 mm in diameter) from stock culture plates. Each set of culture flasks were positioned randomly in an orbital shaking incubator set at 120 rpm. All culture flasks were assigned with random numbers generated using Research Randomizer v.4.4 (Urbania and Plous, 2013). These numbers were randomised again, and sampling was carried out sequentially based on these randomised numbers.

Temperatures in the incubator were monitored manually and recorded daily throughout the experiments. Positions of the culture flasks were randomised again on the fifth day of every experiment to further minimise the potential for influence of any systematic variation in conditions within the incubator. Before every sampling of biomass, triplicates of 15 mL tubes containing one piece of Whatman no. 1 filter paper each were labelled, and weighed on an analytical balance to obtain their initial dry mass. Every 24 h over the 10-day incubation, wet biomass from three randomly selected culture flasks from each set of experiments was sampled by filtering through the prepared Whatman no. 1 filter papers using a vacuum pump. The samples of wet biomass were collected into the respectively labelled tubes, and stored at −80 °C before being freeze-dried for 24 h after the experiments were completed. After freeze-drying, they were weighed using an analytical balance to obtain the final dry biomass. Filtered culture medium was sampled for pH measurement before disposal.
2.2. Temperature-dependent growth

Growth data for each fungal strain throughout the 10-day experiment were collected from \( D_0 \) until \( D_{10} \). Total biomass values obtained from triplicates of samples, including that of the agar from the initial mycelial plugs, were averaged. The average biomass reading from \( D_0 \) was then used to subtract the weight of dried agar from subsequent average biomass readings collected from \( D_1 \) to \( D_{10} \). In this way, data for dry mycelial biomass for each day \( t \) were obtained, using the following formula:

\[
M_{tn} = B_{tn} - B_0
\]

Where

\[
M_{tn} = \text{mycelial biomass at } t = n, n \leq 10
\]

\[
B_{tn} = \text{mean biomass at } t = n
\]

\[
B_0 = \text{mean biomass at } t = 0
\]

Mean mycelial biomass values from each dataset were used to generate raw growth profiles and visualise growth trends. The means of mycelial biomass were transformed into natural logarithm values to evaluate exponential growth in each fungal strain at the various culture temperatures. The start of exponential growth was determined from the values given by natural-log transformations. Also, the natural log values were fitted to a straight line through a linear function \( (y = mx + c; y = \text{value of transformed dry biomass}; m = \text{slope of the straight line plot}; x = \text{the point in time expressed in unit } d; c = \text{point of } y\text{-intercept}) \) to obtain specific growth rate \( (k, \text{referred to as SGR henceforth}) \) values for each dataset.

Specific growth rate \( (\text{SGR}, \text{d}^{-1}) \) values obtained from previous analysis of exponential growth in each strain at the various culture temperatures served as constants in two sigmoidal growth models, the Gompertz (Eq. (2)) and Richards’ logistic (Eq. (3)) growth models, to obtain best-fit growth curves (Gompertz, 1825; Richards, 1959; Peleg and Corradini, 2011).

\[
Y_t = Ae^{-Be^{-(ct)}}
\]

(2)

In Eq. (2), \( Y_t \) is biomass, at time \( t \), \( A \) is upper asymptote, \( B \) is displacement along the x-axis, \( k \) is specific growth rate, and \( e \) is Euler’s number \((2.7183)\).

\[
Y_t = A + \frac{K - A}{(C + Qe^{-dt})^{1/n}}
\]

(3)

In Eq. (3), \( Y_t \) is biomass, \( A \) is lower asymptote, \( K \) is upper asymptote, \( k \) is specific growth rate, \( n \) is the variable that fixes the inflection point, \( Q \) is the variable that controls inflection degree, and \( C \) is 1.0. Curve estimates under the two nonlinear models were compared against three parameters: coefficient of determination \( (R^2) \), residual sum of squares \( (\text{RSS}) \), and corrected Akaike information criterion \( (\text{cAIC}) \) (van Boekel and Zwietering, 2007). \( R^2 \) indicates goodness-of-fit of the models against the datasets. Estimates with lower or negative \( \text{cAIC} \) are preferred to represent the datasets. All statistical analyses were performed in SPSS Statistics v.21 (IBM, New York, USA).

2.3. Requirement of thermal energy across the experimental temperature range in a liquid-state culture system

All specific growth rate estimates obtained from the previous fitting exercise using the Richards’ logistic regression model were then fitted into the temperature-dependent Brière-2 nonlinear model as shown in Eq. (4) (Brière et al., 1999).

\[
\text{SGR} = a(T - T_{\text{min}})(T_{\text{max}} - T)^{1/m}
\]

(4)

Projected SGR values calculated using Eq. (4) were then applied to Eqs. (5)–(7) to determine activation energy \( (E_a) \) for growth in Fusarium equiseti and Pseudogymnoascus spp. grown in liquid cultures. To compare growth rates across the temperature series from 5 °C to 40 °C, the temperature coefficients \( \left( Q_{10}\right) \) were calculated across the different temperature intervals (Eq. (7)). \( \text{SGR}_1 \) is the SGR at higher temperature, \( \text{SGR}_2 \) is the SGR at lower temperature, \( T_2 \) is the higher temperature, and \( T_1 \) is lower temperature.

\[
Q_{10} = \frac{\text{SGR}_2}{\text{SGR}_1}^{10/(T_2 - T_1)}
\]

(5)

An Arrhenius plot was used to plot the natural log of \( k \) versus \( 1/T \) (is estimated growth rate and \( T \) is absolute temperature in Kelvin) and to calculate the activation energy \( (E_a, \text{J mol}^{-1}) \) required by the three fungal strains to initiate growth at each temperature between 5 °C and 40 °C. \( E_a \) values can be determined from the slope \( -(E_a/R) \) between temperature points on the resulting plots, and by solving Eq. (6).

\[
k = Ae^{-E_a/RT}
\]

(6)

\[
E_a = -(\text{slope}) (R)
\]

(7)

Eq. (6) 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 \((8.314 \text{ J mol}^{-1} \text{K}^{-1})\), and \( T \) is absolute temperature in Kelvin. Eq. (7) is derived from Eq. (6).

2.4. Statistical analyses of dry biomass

Means, standard deviations, and standard errors of the mean of dry biomass values were calculated for each set of three flasks sampled. The effects of varying temperatures on fungal growth as assessed by dry biomass data were analysed using repeated measures analysis of variance (rANOVA) in SPSS Statistics v.21 (IBM, New York, USA). The null hypothesis \( (H_0) \) of the analysis was that there was no significant difference in the mean dry biomass sampled from the 10-day growth experiments at temperatures between 5 and 40 °C. The significance level set for the analysis was \( a = 0.05 \).

3. Results

3.1. Temperature-dependent growth across the experimental culture temperature range

The values of parameters and statistics to obtain best-fitting growth curves under the two sigmoidal growth models, Gompertz and Richards’ logistic growth models, are given in Supplementary Information. All available datasets obtained from dry biomass measures of \( F. \) equiseti and \( P. \) pseudojymnoascus spp. were better fitted under the Richards’ logistic growth model than under Gompertz’ (Fig. 1A–C) although both models gave similar results. Values of \( R^2 \) and \( \text{RSS} \) calculated from subtracting the mean dry mycelial mass values obtained every 24 h with the values obtained from the Richards’ logistic growth equation were consistently lower than those obtained with the Gompertz’ equation. Therefore, growth curves calculated under the Richards’ logistic model were chosen to profile the growth of \( F. \) equiseti and \( P. \) pseudojymnoascus spp. across all completed experimental culture temperatures.

Fig. 1 shows the growth profiles of \( F. \) equiseti (Fig. 1B) and \( P. \) pseudojymnoascus spp. (Fig. 1A and C) at six experimental temperatures. The tropical strain of \( F. \) equiseti exhibited the highest specific growth rate at 30 °C \((k = 2.94 \text{ d}^{-1})\). Growth of \( F. \) equiseti at this temperature was accelerated but quickly arrested by \( D_2 \). Highest dry mycelial biomass in \( F. \) equiseti was observed at 25 °C by \( D_{10} \) of growth \((k = 1.80 \text{ d}^{-1}, 140.0 \text{ mg})\). Coupled with the result obtained from the analysis of exponential growth in this tropical strain at 25 °C, the strain was yet to reach the stationary phase by the end of the 10-day experiment. Lowest specific growth rate in the strain was observed at 5 °C \((k = 0.41 \text{ d}^{-1})\). \( F. \) equiseti was still in exponential growth phase by \( D_{10} \) at this temperature.
Growth rate patterns in the Arctic and Antarctic strains of *Pseudogymnoascus* sp. were similar. However, the log phase of growth in Arctic *Pseudogymnoascus* sp. started at around \( D_3 \) of growth, and growth rates were generally lower than those of the Antarctic strain. The highest growth rate was recorded at 25 °C and the lowest at 5 °C (\( k = 0.85 \) and \( k = 0.21 \) \( d^{-1} \), respectively). The Antarctic strain of *Pseudogymnoascus* sp. generally entered the log phase of growth at around \( D_2 \). The highest dry mycelial biomass in the former was produced at 25 °C by \( D_9 \) before their growth declined, suggesting cell death (autolytic phase) beyond this time-point. Highest growth rate in this strain was also recorded at 25 °C (\( k = 1.45 \) \( d^{-1} \), 130.0 mg).

*Pseudogymnoascus* sp. showed the lowest growth rate at 5 °C (\( k = 0.36 \) \( d^{-1} \)), and was still in the exponential phase of growth until the end of the experiment at 5 °C.

Fig. 1. Sigmoidal time-dependent growth curves of (a) the Arctic strain of *Pseudogymnoascus* sp., (b) tropical strain of *Fusarium equiseti*, and (c) Antarctic strain of *Pseudogymnoascus* sp., calculated under the Richards’ logistic model at temperatures between 5 and 30 °C. Temperature levels are indicated by colours as shown in the legend.

Fig. 2. Temperature-dependent growth curves of the Arctic strain of *Pseudogymnoascus* sp. (yellow), the tropical strain of *Fusarium equiseti* (green), and the Antarctic train of *Pseudogymnoascus* sp. (blue) inoculated into seawater-based Mueller Hinton Broth. Coloured circles with error bars indicate mean specific growth rates. Values in brackets are \( T_{opt} = \) optimum temperature for growth, and \( SGR_{max} = \) maximum specific growth rate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Activation energy (\( E_a \)) of growth

Table 1 shows a consistent decline in \( Q_{10} \) coefficients with increasing temperature for all three strains. \( Q_{10} \) values in *Fusarium equiseti* were generally higher than in *Pseudogymnoascus* sp. in each temperature series A through E. Fig. 3 shows the Arrhenius plot and activation energy (\( E_a \)) for growth in *Fusarium equiseti* and *Pseudogymnoascus* sp. based on SGR values. In comparison with our previous analyses based on RGR, we could estimate \( E_a \) at lower temperature levels, i.e. between 5 and 25 °C, in this study. These analyses confirm that *Fusarium equiseti* required the greatest energy for growth, followed sequentially by the Antarctic and Arctic *Pseudogymnoascus* sp. (\( E_a = 1.9 \) and \( 16.5 \) kJ mol\(^{-1}\), respectively).
respectively). Negative $E_a$ in the Arctic and Antarctic *Pseudogymnoascus* spp. at 25 °C indicated that the fungal cells were starting to absorb less energy for growth than at 20 °C ($E_a = -176.2$ and $-46.4$ kJ mol$^{-1}$, respectively).

### 3.3. Variability in biomass production

Repeated measures analysis of variance (rANOVA) of all obtained dry biomass datasets were performed. Mauchly's Test of Sphericity performed separately on the dry biomass data of *F. equiseti*, and the Arctic and Antarctic strains of *Pseudogymnoascus* sp., respectively, across the six temperatures tested indicated in each case that the assumption of sphericity had been violated, meaning that variances of the differences between all combinations of related, levels or groups were not equal (respectively, $\chi^2 = 63.17$, $P < 0.001$; $\chi^2 = 55.69$, $P < 0.001$; $\chi^2 = 46.32$, $P < 0.001$). Therefore, degrees of freedom were corrected using the Huynh-Feldt estimate of sphericity ($\varepsilon = 0.73$; $\varepsilon = 0.86$; $\varepsilon = 1.00$, respectively) (Mauchly, 1940; Greenhouse and Geisser, 1959; Huynh and Feldt, 1976). Results of rANOVA with corrected sphericity showed that the effect of different culture

### Table 1

Temperature coefficient ($Q_{10}$) of specific growth rates (SGR) in *Fusarium equiseti* and *Pseudogymnoascus* spp. across five temperature series in a seawater-based liquid-state culture system.

<table>
<thead>
<tr>
<th>Temperature series</th>
<th>ArcP</th>
<th>TropF</th>
<th>AntP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = 5–10 °C</td>
<td>11.11</td>
<td>14.58</td>
<td>12.52</td>
</tr>
<tr>
<td>B = 10–15 °C</td>
<td>3.48</td>
<td>4.90</td>
<td>3.66</td>
</tr>
<tr>
<td>C = 15–20 °C</td>
<td>1.84</td>
<td>2.89</td>
<td>2.02</td>
</tr>
<tr>
<td>D = 20–25 °C</td>
<td>1.03</td>
<td>2.14</td>
<td>1.25</td>
</tr>
<tr>
<td>E = 25–30 °C</td>
<td>0.10</td>
<td>1.51</td>
<td>0.54</td>
</tr>
</tbody>
</table>

(ArcP) Arctic strain of *Pseudogymnoascus* sp.  
(TropF) Tropical strain of *Fusarium equiseti*.  
(AntP) Antarctic strain of *Pseudogymnoascus* sp.
temperatures on dry biomass obtained from liquid cultures of the three strains were significant, each with large effect sizes ($F(3.66,80.54) = 25.55, P < 0.001$, $\eta^2 = 0.77$; $F(4.28,94.31) = 29.55, P < 0.001$, $\eta^2 = 0.57$; $F(5.00,110.9) = 96.72, P < 0.001$, $\eta^2 = 0.82$, respectively). Pairwise comparison between dry biomass data across the six temperatures tested for *F. equiseti* showed that the means obtained were significantly different from each other, except for those obtained at 15 and 30 °C ($P = 0.72$), and 20 and 25 °C ($P = 1.0$). Across the five temperatures tested for the Arctic strain of *Pseudogymnoascus* sp. the means obtained were significantly different from each other except between 10 and 25 °C ($P = 0.197$), and 15 and 20 °C ($P = 0.056$), 15 and 25 °C ($P = 0.404$), and 20 and 25 °C ($P = 0.064$). Across the five temperatures tested for the Antarctic *Pseudogymnoascus* sp. strain the means obtained were significantly different from each other except between 5 and 30 °C ($P = 0.668$), and 15 and 20 °C ($P = 1.0$).

### 3.4. Change in pH levels during growth

The pH levels of the seawater-based Mueller Hinton Broth culture medium were recorded throughout the entire growth experiment. Our preparation resulted in an initially slightly acidic medium (pH 6.5–7.0). However, pH levels increased with time when *F. equiseti* and *Pseudogymnoascus* spp. were grown in this medium across all culture temperatures at which growth occurred from pH 6.6 to 9.1 in all three strains by the end of the growth experiment. The relationship between fungal biomass and pH increment was intermediate in *F. equiseti* ($R^2 = 0.66$), while not significant in the Arctic and Antarctic *Pseudogymnoascus* spp. ($R^2 = 0.11$ and 0.32, respectively) (Fig. 4).

A further rANOVA was conducted on all culture medium pH datasets to determine the effect of temperature on pH levels in the culture medium over time, and to measure variability in differences in the pH measures. Mauchly's Test of Sphericity on the pH data from cultures of *F. equiseti*, and the Arctic and Antarctic strains of *Pseudogymnoascus* sp. between 5 and 30 °C tested, again indicated that the assumption of sphericity had been violated in each case ($\chi^2 = 54.83, P < 0.001$; $\chi^2 = 101.49, P < 0.001$; $\chi^2 = 91.11, P < 0.001$, respectively). Therefore, degrees of freedom were corrected using a Huynh-Feldt estimate of sphericity ($\epsilon = 0.99$; $\epsilon = 0.99$; $\epsilon = 0.58$, respectively). Results of rANOVA with corrected sphericity showed that the effect of different culture temperatures on pH levels of culture medium inoculated with the three strains were significant, each with large effect sizes (respectively, $F(4.95,108.9) = 1064.91, P < 0.001$, $\eta^2 = 0.98$; $F(4.96,109.0) = 177.78, P < 0.001$, $\eta^2 = 0.89$; $F(2.88,63.4) = 175.64, P < 0.001$, $\eta^2 = 0.89$). Pairwise comparison between pH data across the six temperatures tested for *F. equiseti* showed that the means obtained were significantly different from each other, except for those obtained at 20 and 25 °C ($P = 1.0$). Also, the pairwise comparison of pH data across the six temperatures tested for the Arctic strain of *Pseudogymnoascus* sp. showed that the means obtained were significantly different from each other, except for those obtained at 10 and 15 °C ($P = 0.564$). Means of pH values from culture medium of the Antarctic *Pseudogymnoascus* sp. were not significantly different for those obtained at 10 and 30 °C, 15 and 25 °C, and 15 and 30 °C ($P = 1.00$, respectively).

### 4. Discussion

The survival of a species in a natural habitat is ultimately dependent on its ability to reproduce at a rate sufficient to balance death. Microorganisms are remarkably versatile in terms of physiological features. Different environmental factors governing processes in a habitat influence the physiology of organisms. Clearly, the performance of a species in the inevitably restricted conditions of a culture medium does not necessarily reflect that in the natural environment. However, pragmatically, to gain insight into the behaviour and capabilities of a species in its natural habitat, the use of laboratory culture approaches where environmental factors are known and controlled is a valuable tool (Brock, 1971).

*Fusarium* and *Pseudogymnoascus* can be used as model genera in relating thermal adaptation to global fungal distribution through reductionism. Mesophily and psychrophily have been observed in many fungal taxa which are predominantly abundant in tropical and polar regions, respectively. Psychrophilic and psychrotolerant fungi are predominant in polar substrates. However, polar environments also experience considerable environmental variability due to short-term climatic as well as seasonal or longer-term variations (Peck, 2015). Microbial microhabitat temperature can reach as high as at least 25 °C in soils during boreal and austral summers in polar regions of the Northern and Southern Hemispheres, as well as being strongly influenced by short-term climatic events (Convey, 1996; Krishnan et al., 2011).

Fungal members of various economically-important and widespread genera such as *Aspergillus* and *Penicillium* have been classified as mesophiles, with highest growth rates observed between 25 °C and 30 °C. Representatives of *Aspergillus* and *Penicillium* tend to occur at lower latitudes in tropical and temperate regions, and have also been isolated from marine substrates (Meletiadis et al., 2001; Larsson, 2009). Commonly isolated cold-adapted fungal strains from higher latitudes (cool temperate and polar regions) include members of the genera *Thelebolus* Tode and *Hebeloma* (Fr.) P. Kumm (Geml et al., 2012; Henríquez et al., 2014). As observed from our $Q_{10}$ data from the Arctic and Antarctic *Pseudogymnoascus*, the possession of elevated $Q_{10}$ values and lower $E_a$ at low environmental temperatures has also been proposed as a

![Fig. 4. The relationship between recorded biomass and change in pH in the three fungal strains during the 10-day growth experiment (indicated by the coefficient of determination, $R^2$).](image-url)
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).

Fungal growth rates can be calculated using measurement of hyphal length of single-spore inoculates, colony diameter in solid-state media, and wet or dry biomass under liquid-state culture conditions. Technical criteria set for these techniques are usually arbitrary, but sizes of mycelial plugs are standardised or fixed within an experimental setup (Liu et al., 2008; Fuhr et al., 2011). The first two techniques are limited by the measures obtained being two-dimensional. Thus, colony depth or thickness are not considered when measuring growth. However, measures obtained using these two techniques are still relevant, as, for instance, fungal growth in natural environments is assessed by colony spread to evaluate the severity of fungal infection. Biomass measures obtained from liquid-state culture approaches, in contrast, integrate three-dimensional growth, as well as allowing measures of changes in other parameters such as pH and salinity.

Considering the results of the current study based on liquid-state media and our previous study using solid-state media (Tajuddin et al., 2017), both studies showed that T_{opt} values obtained in the different culture media were consistent, and the profiles of E_{d} for growth in *Fusarium* and *Pseudogymnoascus* spp. were very similar. These strains also showed better growth performance under liquid culture conditions, perhaps due to the continuous aeration of the cultures throughout the entire experiment (Casas Lopez et al., 2005; Liu et al., 2008; Gomaa and El Bialy, 2009). Critical maximum temperature (RGR_{max} versus SGR_{max}) values in each strain were similar even though grown in liquid than solid media culture, with SGR_{max} in *F. equiseti* from our present study being 31.7 °C, and in Arctic and Antarctic *Pseudogymnoascus* spp. 30.7 and 33.1 °C, respectively. All strains exhibited growth beyond the maximum growth rate values that were previously estimated using RGR values, with the values being 1.05 d^{-1} in *F. equiseti*, and 0.49 and 0.51 d^{-1}, in the Arctic and Antarctic *Pseudogymnoascus*, respectively (Tajuddin et al., 2017). As a result, T_{opt} values estimated using SGR were greater than those estimated using RGR. *Fusarium equiseti* grew optimally at 30 °C while *Pseudogymnoascus* spp. grew optimally at 25 °C under liquid conditions. Although these were derived from the Brière-2 projections of the SGRs, the calculated E_{d} for growth in *F. equiseti* was least positive at 25 °C; suggesting that very little E_{d} was required for the catalysis of optimal growth reactions in this strain at 25 °C, and the cells were starting to experience stress at temperatures beyond 25 °C. Similar observations were obtained from the two polar strains of *Pseudogymnoascus* spp., where E_{d} was least positive at 20 °C before turning negative at 25 °C.

The growth of both *F. equiseti* and *Pseudogymnoascus* spp. in liquid culture led to progressive increase in the pH of the medium. Some fungi are known to alter pH according to substrate availability within a culture system. St. Leger et al. (1999) demonstrated that the fungus *Metarhizium anisopliae* (Metschn.) Sorokin actively excreted ammonium ions (NH_{4}^{+}) to increase pH levels of the culture medium as they continued to grow under a closed culture system, thereby optimising protease activity under alkaline conditions. The data obtained in the current study are consistent with the strains tested expressing a similar ability as seen in *M. anisopliae*. Fungal amylases showed optimum activity between pH 4.0 and 7.0 in some studies (Yamanaka, 2003; Sunita et al., 2012; Ominyi et al., 2013). Proteaceous substrates are the most abundant particulate organic matter in the marine environment, followed by carbohydrates, and lipids (Hedges, 1992; Fabiano and Danovaro, 1998; Kujawinski, 2011). Correspondingly, proteases are found to be more highly expressed in environmental samples than other subclasses of hydrolyses including glycolhydrolyses (e.g. amylases, cellulases, and ligninases) and lipases (Dong et al., 2014). Proteases generally function optimally under alkaline pH levels between 8 and 10. Some proteases can be alkalophilic, functioning best at more extreme pH levels between 11 and 14. Substrates in the marine environment, which are continuously exposed to seawater temporarily and/or spatially, have an average pH level of approximately 7.5–8.0 (Marion et al., 2011).

5. Conclusions

The data obtained in this study demonstrated that fungal strains originating from different bioclimatic regions are adapted to specific ranges of ambient temperature. The marine-derived tropical strain of *F. equiseti* is mesophilic, with optimal E_{d} for growth in liquid-state cultures being 31.2 KJ mol^{-1} at 25 °C. In contrast, Arctic and Antarctic *Pseudogymnoascus* spp. grow optimally at 20 °C, with optimal E_{d} for growth of these two strains being 1.9 and 16.5 KJ mol^{-1}, respectively. Activation energies (E_{d}) for growth calculated based on SGRs of the polar strains were negative beyond 20 °C for *Pseudogymnoascus* spp. Therefore, *Pseudogymnoascus* spp. are psychrotolerant. *Fusarium equiseti* requires a greater E_{d} for growth than do the polar strains of *Pseudogymnoascus* spp., indicating that these strains are characterised by adaptation to ambient temperatures typical of their original habitats. A progressive increase in pH was observed during the growth of all three strains in the liquid culture system, suggesting that the fungal strains tested could tolerate varying pH in culture medium for growth.

Conflicts of interest

None.

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Appendix A. Supplementary data

 Supplementary data to this article can be found online at https://doi.org/10.1016/j.polar.2018.10.005.

References


