**Title: Effects of Temperature on Extracellular Hydrolase Enzymes from Soil Microfungi**

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**Abstract**

Soil microbes play important roles in global carbon and nutrient cycling. Soil microfungi are generally amongst the most important contributors. They produce various extracellular hydrolase enzymes that break down the complex organic molecules in the soil into simpler form. In this study, we investigated patterns of amylase and cellulase (which are responsible for breaking down starch and cellulose, respectively) relative activity (RA) on solid media at different culture temperatures in fungal strains from Arctic, Antarctic and tropical soils. Fungal isolates from all three regions were inoculated onto R2A media supplemented with starch for amylase and carboxymethylcellulose and trypan blue for cellulase screening. The isolates were then incubated at 4, 10, 15, 20, 25, 30, 35 and 40°C and examined for activity after 5 and 10 d, for tropical and polar isolates respectively. The data obtained indicate that the polar fungal strains exhibited similar patterns of amylase and cellulase RA. Both Arctic and Antarctic fungi showed highest RA for amylase and cellulase at 35°C, while colony growth was maximised at 15°C. Colony growth and RA of the polar isolates were negatively correlated suggesting that, as temperatures increase, the cells become stressed and have fewer resources available to invest in growth. Unlike polar isolates, tropical isolates did not exhibit any trend of colony growth with temperature, rather having idiosyncratic patterns in each isolate. The low enzyme production and RA levels in the tropical strains may suggest both a low ability to respond to temperature variation in their natural thermally stable tropical habitats, as well as a level of thermal stress limiting their enzyme production ability.

Key words: amylase, cellulase, soil microfungi, Arctic, Antarctic, tropical

**Introduction**

Soil microbes play a vital role in soil food webs and global biogeochemical cycles, including the cycling of nitrogen, carbon, phosphorus and sulphur (Addo-Bediako et al. 2000). Bacteria, fungi and archaea are important members of soil microbial communities, playing specific roles in the soil ecosystem. For instance, fungi and Gram-positive bacteria degrade complex compounds while Gram-negative bacteria act on less complex compounds (Tveit et al. 2015). However, fungi are the dominant decomposers of soil organic matter (de Graaff et al. 2010).

In polar environments, fungi play a vital role in cellulose decomposition (Kurek et al. 2007). Fungi from Arctic soils may even continue to catalyse biogeochemical processes under snow cover while general environmental conditions above the snow are too extreme for biological activity (Fahnestock et al. 1998; Florczak et al. 2013). Arctic permafrost soils currently store large amounts of carbon, approximately 98-Gt (Øvstedal and Smith 2001). If fungi were to have access to and decompose this stored organic carbon as permafrost recedes, substantial CO2 would be released to the atmosphere, thereby acting as a major positive feedback to global climate change (Robinson 2001; Sturm et al. 2005; Tortella et al. 2008). Polar fungi are also involved in symbiotic interactions with plants and algae, as are those in less extreme areas (Sturm et al. 2005). The symbiotic relationships of fungi in polar regions are epitomised in the success of lichens in these extreme environments (Nye 1961), as well as in mychorrhizal and dark septate fungal symbioses (Newsham et al. 2009).

Extracellular enzymes are a direct expression of microbial activity and function, potentially influenced by the cell’s surrounding environment, meaning that environmental impacts on this activity (for instance through rising temperature) in microfungi can be measured and analyzed (Sinsabaugh et al. 1993). These enzymes are secreted into the cells’ immediate environments. Extracellular hydrolase enzymes (EHEs) generally function to degrade soil organic matter (SOM), with the products subsequently being absorbed and used by the producing cells (Marx et al. 2001; Krishnan et al. 2016).

Soil microfungi have also been shown to contribute significantly in the bioremediation of hydrocarbon-contaminated soil in, for exmple, Antarctica (Aislabie et al. 2001; Ferrari et al. 2011), Kalimantan (tropical Borneo) (Chaillan et al. 2004) and Kuwait deserts (Radwan 2008). Fungal extracellular enzymes are potentially very important in biotechnology, because of the advantages they bring in areas such as cost and time effectiveness and consistency (Burhan et al. 2012) and, in the case of cold-adapted biological sources, energy savings (Feller and Gerday 2003). For instance, a majority of the amylases available commercially today are derived from fungal sources such as *Aspergillus*, *Penicillium* and *Rhizopus* (Manivannan and Kathiresan 2007). Cellulases also have many applications in biotechnology including, for instance, in cotton processing, paper recycling, stone washing and as food additives (Bilal et al. 2015).

In the present study, we focused on the effects of temperature on secreted amylase and cellulase relative activity (RA), as these enzymes have been reported widely in fungal studies globally but not from the polar regions (Tortella et al. 2008; Brindha et al. 2011). To our knowledge, this is the first report on EHE activity screening combined with temperature manipulation for soil microfungi sourced from different latitudes on solid media. This baseline comparative study across a global environmental gradient, including Arctic, Antarctic and tropical soil microfungi, may reveal important clues about microbial responses to climate change.

**Materials and Methods**

**Soil fungal isolates**

Soil fungal isolates from Antarctica, the Arctic and the tropics were obtained from the National Antarctic Research Centre fungal culture collection, University of Malaya, Kuala Lumpur. Antarctic isolates were originally obtained from King George Island Antarctica, collected during the austral summer 2007/08 (GPS locations (62º10’24.5”S 58º56’45.3”W, 62º12’34.7”S 58º55’33.0”W, 62º11'37"S 58º59'35"W and 62º12’00.7”S 58º57’35.6”W). Arctic isolates were obtained from Ny-Ålesund (78º54’60”N 11º55’60”E) and Hornsund (76°56'60"N 15°45'60"E) (Svalbard), collected in the boreal summers of 2006 and 2010, respectively. Tropical isolates were obtained from Rimba Ilmu, University Malaya, Kuala Lumpur (3º 07’ 56”N 101º 39’ 29”E) in October 2014 (Figure 1). Fungal isolates were sub-cultured onto PDA media in triplicate. Polar fungal cultures were maintained at 4°C or 25°C (following initial thermal classification as psychrophilic, psychrotolerant, mesophilic), and tropical isolates at 25°C. Psychrophiles have optimum growth at 15°C or lower and are unable to grow at 20°C and above, while psychrotolerant organisms have their maximum temperature for growth above 20°C (Morita 1975). Mesophiles have a minimum temperature for growth between 5 and 10°C and maximum temperature above 25°C (Robinson 2001).

The soil fungal isolates were identified using morphological characteristics and later confirmed by molecular identification. Taxonomic elements were not included in the present study, which focused on enzyme-related research. However, to confirm the strains’ taxonomical positions, we used available sequence data and a molecular approach using three genes (Internal Transcribed Spacer (ITS), Large Subunit (LSU), Small Subunit (SSU), which is sufficient for species confirmation. A summary of strains pre-screened for amylase and cellulase activities is given in Online Resource 1.

**Temperature treatment for assessment of relative enzyme activity of amylase and cellulase**

Six isolates showing the highest RA from the Antarctic, five from the Arctic and three from the tropics were selected from the pre-screening for amylase and cellulase activity (Online Resource 1). These isolates were incubated at temperatures of 4, 10, 15, 20, 25, 30, 35 and 40°C using the enzyme screening method as described below.

**Screening of amylase and cellulase relative activities**

RA for extracellular amylase and cellulase activity were assessed following Margesin et al. (2003). R2A agar (casein acid hydrolysate 0.5g L-1, yeast extract 0.5g L-1, proteose peptone 0.5g L-1, dextrose 0.5g L-1, soluble starch 0.5g L-1, dipotassium phosphate 0.3g L-1, magnesium sulphate 0.024g L-1, sodium pyruvate 0.3g L-1, agar 15g L-1) was supplemented with either soluble starch (Cat number: S9765 Sigma Aldrich) (0.4% w v-1) for amylase or carboxymethylcellulose (Cat number: 419338 Sigma Aldrich) and trypan blue (Cat number: 76146 Sigma Aldrich) (0.4% and 0.01% w v-1) for cellulase activity.

The agar plug assay method was applied to all tested fungi, with assay plates prepared in triplicate for each fungal isolate. Agar plugs (6 mm) were bored from the growing edge of fungal colonies using cork borer number 3 and inoculated into a well of the same size made at the centre of each assay agar plate, followed by incubation at 4, 10, 15, 20, 25, 30, 35 or 40°C. The plates were incubated for 10 d for polar isolates and 5 d for tropical isolates, in order to take into account the faster growth rates of the latter. The plates were then examined for the presence of a clear zone in the agar around the colony, indicating extracellular enzyme activity. *Antarctomyces psychrotrophicus* was used as a control since it has no activity for either of the enzymes screened. Amylase activities were confirmed by staining the plates with Lugol’s solution.

**Relative enzyme activity (RA)**

Fresh samples were used for enzyme screening in order to ensure that the enzyme activity was maximal (German et al. 2011). Each replicate was examined for the presence of a clear zone around the colony, and the diameter of the colony and of the clear zone (activity zone) were measured. The measurement was repeated in two mutually orthogonal dimensions, and the mean value calculated. The RA was calculated using the following formula:

$$Relative enzyme activity= \frac{Clear zone diameter-Colony diameter}{Colony diameter}$$

Isolates exhibiting an RA of >1.0 were classified as having ‘significant activity’ (following Bradner et al. (1999) and Duncan et al. (2008)). Isolates selected to include in further studies of amylase and cellulase activity under different temperatures are listed in Table 1. As some strains were amongst the top enzyme producers for both enzymes studied, a total of 28 strains were selected for further study.

Table 1 placed here

Strain AK07KGI1801 R5-2 Sp.2 (Unidentified sp. 11) and *Ramularia* sp. (Table 1) could not be revived from the source culture in the latter part of this study and, therefore, incubations were not carried out at 40°C for these two isolates for cellulase screening.

**Statistical Analyses**

Data obtained for amylase and cellulase screening from all 28 isolates from Antarctic, Arctic and tropical regions were analyzed using SPSS. Data for RA and colony size were examined for normality. As distributions were not normal, the data were compared using the non-parametric Kruskal-Wallis analysis of variance, with *post hoc* pairwise tests using the Mann-Whitney test. Correlations between RA and colony diameter were tested using the Pearson correlation test.

**Results**

**Amylase activity**

Kruskal-Wallis ANOVA indicated that temperature and species in combination had significant influences on the RA of amylase in the Antarctic isolates (H (47) = 136.36, P < 0.001). However, when the two factors were analysed separately, temperature had a significant effect on RA of amylase (H (7) = 99.99, P < 0.001) while that of species was not significant (H (5) = 9.39, P = 0.94). Pairwise comparisons using the Mann-Whitney test indicated that the amylase RA at 35°C was significantly different from those at all other temperatures (Figure 2a).

Analysis of Arctic strain data again illustrated that temperature and strain collectively had a significant influence on the RA (H (39) = 106.33, P < 0.001). Considered separately, as with the Antarctic strains, temperature had a significant effect on RA (H (7) = 59.26, P < 0.001) while strain had no significant effect. Pairwise comparisons using the Mann-Whitney test again showed that the values obtained at 35°C were significantly different from those at the other culture temperatures (Figure 2a).

For the tropical isolates, Kruskal-Wallis ANOVA indicated that the combined influence of temperature and strain as well as the two factors separately had significant effects on the RA of amylase (H (23) = 63.16, P < 0.001), H (2) = 16.30, P < 0.001, and H (7) = 15.66, P < 0.05, respectively). Pairwise comparisons indicated that the mean RA of tropical isolates was greatest at 40°C (Figure 2a).

An overall Kruskal-Wallis test was conducted including all the isolates from each region. This analysis demonstrated that temperature and species separately had significant effects on RA (H (13) = 142.70, P < 0.001, and H (7) = 95.29, P < 0.001, respectively). Pairwise Mann-Whitney tests showed that one isolate from the Antarctic (*Pseudogymnoascus* sp., AK07KGI1801 R1-2 Sp.2a) showed significantly greater amylase RA in comparison with all other strains, while the Arctic and Antarctic isolates showed significant differences from the tropical isolates (Figure 2b).

Antarctic isolates selected for this study were either *Geomyces* sp. or *Pseudogymnoascus* sp., as these recorded the highest RA in preliminary trials. The highest RA values were recorded at higher temperatures, above 25°C (Figure 3a-f). All isolates showed a trend of decreasing RA at temperatures approaching 40°C except for *Geomyces* sp. (AK07KGI1906 R1-1 Sp.3). Colony growth was maximum at lower temperatures, from 15°C-25°C (Figure 3a-f) and, at 35°C and above, no measurable growth was observed, with the colony not expanding beyond the initial plug.

Arctic isolates showed a similar pattern to those from the Antarctic, with higher RA observed at temperatures above 25°C (Figure 3g-k). All showed decreased RA when incubated at 40°C except for *Cosmospora viridescence* (HND16 R4-1 Sp.2). Colony sizes were again greatest between 15°C and 25°C (Figure 3g-k) and, as for the Antarctic isolates, most Arctic isolates did not grow at temperatures of 35°C or 40°C. *Geomyces* sp. (in 3i) did not show any radial extension at 30°C or above.

In contrast, RA values recorded from the tropical isolates varied little across the entire experimental temperature range, other than a very small increase in RA at 35°C, and this was not seen in AK14015 R5-2 Sp.5 (Figure 3l). Colony growth in these strains did not decrease at high temperatures (30°C-35°C), with some continuing to grow at 40°C (Figure 3l-n).

Correlation between RA for amylase and colony size was tested using the Pearson correlation test for the isolates from each region. A strong negative correlation was observed between the two variables tested for Antarctic (r = -0.658, n = 144, p < 0.001) and Arctic isolates (r = -0.649, n = 120, p < 0.001). This correlation was weaker and non-significant in the tropical isolates. Correlations between RA and colony diameter of each isolate are shown individually in Figure 4a-c.

**Celullase Activity**

Kruskal-Wallis ANOVA indicated that a combination of temperature and species had significant influence on the RA of cellulase in the Antarctic isolates (H (47) = 139.84, P < 0.001). Temperature as a single factor had a significant effect on cellulase RA (H (7) = 66.66, P < 0.001) while species did not have any significant effect (H (5) = 9.86, P = 0.79). A pairwise comparison using the Mann-Whitney test indicated that RAs at culture temperatures of 30 and 35°C were significantly different from the remaining temperatures (Figure 5a).

Similar to the Antarctic isolates, temperature and species had a significant effect on cellulase RA for Arctic strains (H (39) = 111.80, P < 0.001). Temperature alone again had a significant effect on cellulase RA of Arctic isolates (H (7) = 88.03, P < .001) while species did not have any significant effect (H (4) = 5.38, P = 0.250). Further pairwise comparisons using the Mann-Whitney test indicated that the RA at 35°C was significantly different from the remaining temperatures (Figure 5a).

In the tropical strains temperature and species in combination also had a significant effect on cellulase RA (H (23) = 65.25, P < 0.001), while species alone had no significant effect (H (2) = 1.59, P = 0.451) and temperature did (H (7) = 47.40, P < 0.001). The Mann-Whitney test confirmed that RAs at temperatures of 30, 35 and 40°C were significantly different from the lower temperatures (Figure 5a).

An overall Kruskal-Wallis ANOVA was conducted for all the isolates without segregating them according to their origin. Temperature and species separately had significant effects on the RA of cellulase, (H (7) = 164.72, P < 0.001 and H (13) = 76.81, P < 0.001). Pairwise comparisons using the Mann-Whitney test indicated that all polar isolate cellulase RAs were significantly greater than those from tropical isolates (Figure 5b).

Generally, Antarctic isolates exhibited good cellulase RA at temperatures of 25°C and above, with activity decreasing at 40°C (Figure 6a-f). Both *Pseudeurotium hygrophilum* isolates recorded the highest RAs, followed by two *Geomyces* sp. Most of the isolates showed largest growth at 20°C, except a *Geomyces* sp. isolate at 15°C (Figure 6e).

The RA for cellulase from Arctic fungi also increased at 25°C and above (Figure 6g-k). Two *Geomyces* sp. isolates recorded the highest RA values at 40°C (Figure 6 (h-i)), while all other isolates showed a decrease at 40°C. *Geomyces* sp. and *Pseudogymnoascus* sp. developed the largest colony diameter at 15°C and the remaining isolates at 20°C (Figure 6g-k). All the polar isolates showed no growth at 35°C and above.

RA for cellulase for tropical fungi was higher at temperatures of 30°C and above, although overall RAs (as with amylase) did not vary widely from the lowest to the highest culture temperatures. *Trichoderma spirale* recorded a decrease in RA at 40°C (Figure 6n). Colony growth in these tropical fungi was greatest at 25°C or above (Figure 6l-n).

RA for cellulase and the respective colony diameter showed a negative correlation in the Antarctic (r = -0.359, n = 144, p < 0.01) and Arctic (r = -0.513, n = 120, p < 0.01) isolates. Tropical isolates, in general exhibited a weak and non-significant positive correlation (r = 0.12, n = 72, p = 0.919). Correlations between RA and colony diameter in individual isolates are shown in Figure 7a-c.

**Discussion**

All of the Antarctic isolates selected for study of amylase were from the genera *Geomyces* or *Pseudogymnoascus*,which are closely related. Two of the Arctic strains were also *Geomyces* sp. According to http://www.indexfungorum.org/Names/Names.asp, an online fungal database, *G.* *pannorum* is correctly classified as *P.* *pannorum*. However, other than the reclassification of *G. pannorum*, the two genera should be considered as distinct (Minnis and Lindner 2013). Members of *Geomyces* have been reported from Arctic (Kochkina et al. 2007; Ali et al. 2013), Antarctic (Florczak, et al. 2013; Krishnan et al. 2016) and temperate regions (Baldrian et al. 2011; Minnis and Lindner 2013). Gao et al. (2016) reported that *G. pannorum* isolated from King George Island, (South Shetland Islands, Antarctica) was able to produce thermophilic α-amylase even though it is considered a psychrotolerant species. The current study similarly showed that a strain of *Geomyces* sp. had good RA of amylase when incubated at higher culture temperatures (35°C). In contrast, Zeng et al. (2004) reported that psychrotolerant species tend to have higher enzyme activities at temperatures below their optimum growth temperature.

*Pseudeurotium hygrophilum* exhibited considerable and significant cellulase RA in the current study. Studies on this species are very limited, originating from Russia and the Netherlands (Prenafeta-Boldu et a. 2005; Sogonov et al. 2005), and focusing on taxonomy and bioremediation aspects, respectively. Other than *P. hygrophilum*,two *Geomyces* sp. isolates were selected for cellulase study as they exhibited good activity. *Pseudogymnoascus* and *Geomyces* were good producers of both cellulase and amylase. In contrast to this finding, Gawas-Sakhalkar and Singh (2011) reported that two strains of *G. pannorum* from Ny Ålesund (Svalbard) did not produce amylase or cellulase. Previously cellulase was reported from soil microfungi from King George Island (Krishnan et al. 2016) and Livingstone Island (Gesheva and Vasileva-Tonkova 2012) from the Antarctic, Ny-Ålesund (Singh et al. 2012) from the Arctic, and India (Deshpande et al. 2009) and Malaysia (Rashid et al. 2009; Ibrahim et al. 2013) from tropical regions. As a majority of the selected polar strains for this study were representatives of *Geomyces*, they are likely to play an important role in the decomposition of starch from plant material and cellulose in the polar regions (Duncan et al. 2008; Arenz and Blanchette 2009).

In general, RA values obtained from Antarctic isolates for amylase or cellulase were greater than those from Arctic isolates, with both being much greater than those of tropical isolates. This pattern may relate to the natural environmental temperature variation experienced in the source regions. While standard air temperatures in the Antarctic region are chronically cold, the ground surface often experiences high temperature variation and, in a day, air temperature can fluctuate from -5°C to 35°C (Convey 1996) while, for the Arctic, there is no clear information about the range of temperatures experienced in summer (though see Coulson et al. (1995) and Convey et al. unpublished data (in review) for more general descriptions of Antarctic and Arctic soil temperature patterns over the seasons). However, Kleinteich et al. (2012) reported temperature shifts from 8-16°C during summer. In contrast, if shaded, tropical soils experience very low seasonal fluctuation in temperature (Janzen 1967; Huey 1976), although overall temperatures are 20-30°C higher than in the polar regions (Bonebrake and Mastrandrea 2010). The low temperature variation experienced by tropical species may mean they have less tolerance to thermal variation (Addo-Bediako et al. 2000; Ghalambor et al. 2006; Deutsch et al. 2008), which could underlie the very limited change in RA observed across the tested temperature range.

All polar strains examined exhibited a similar pattern of response for amylase and cellulase activity, with the highest RA being recorded at higher temperatures that are not representative of their natural environment. The isolates themselves showed maximum growth, in terms of colony size (radial growth) achieved, at lower temperatures (15-25°C). This may suggest a trade-off between growth and enzyme production. Lower temperatures are less stressful for polar fungi and they can therefore invest their energy and resources into growth rather than enzyme secretion. For these fungi, the stress level increases at higher temperatures. As temperature stress increases, they appear to invest more energy into EHE production and secretion rather than biomass production (i.e. growth) so that external carbon sources can be broken down and the product (glucose) absorbed by the cells in order to survive (Cunningham and Agard 2004).

Tropical isolates in general showed very low RA across the temperatures tested. In both amylase and cellulase screening, RA slightly increased at the higher culture temperatures, with RA for cellulase being consistently higher than that for amylase. However, when compared to those from the polar regions, RA values for tropical isolates were very low for both enzymes. One possible explanation for this is that the tropical soil samples may be rich in biologically available nutrients (Laudelot and Meyer 1954; Nye 1961; Zonn and Li 1962), thus reducing the requirement for EHE production. In contrast, the typical scarcity of available nutrients in many polar habitats (Convey et al. 2014) may be a pressure selecting for EHE production and use in polar fungi (Radwan 2008). However, while Waring et al. (2014) developed a model based on 17 different studies in tropical soils, showing that high temperature and low nutrient availability in tropical soil ecosystems could reduce the efficiency of microbial C utilization, it should be remembered that this model was developed based on tropical soils, where ‘low’ nutrient availability is still relatively rich in comparison with polar soils.

No previous studies have addressed the effect of culture (as a proxy for environmental) temperature on amylase and cellulase production by fungi sourced from different latitudes. A number of studies have examined metabolic enzyme temperature sensitivity at molecular level. It has been reported that, in crickets, enzymatic performances differ significantly across temperatures (Huestis et al. 2009), intertidal invertebrates obtained from different latitudes alter protein structure to be able to adapt to temperature difference (Dong and Somero 2009) and tropical damselfishes are genetically different to those from temperate regions (Johns and Somero 2004). More generally, temperature effects on soil organic matter have been studied across different latitudes from boreal forest to tropical rain forest (German et al. 2012), concluding that soil enzymes from higher latitudes and cooler climates were apparently more sensitive to changes in temperature. Cold functioningenzymes are also more sensitive than warm-functioning due to differences in protein structure causing the former to lose function more readily as temperature increases (Dong and Somero 2009; Koch et al. 2007; Somero 2004). These observations are consistent with the results of the present study.

Polar regions are generally considered to be sensitive indicators of climate change. The temperature record from the western North American Arctic has shown an increase of 0.1°C per year over the past 35 y (Anisimov et al. 2007). Climate change models have predicted that by 2080, Arctic ecosystems could face temperature increases of 4 to 7.5°C (Anisimov et al. 2001). There are also records of increased temperature causing increasing microbial activities in the Arctic region (Aerts 2006) and faster turnover of C in soils (Shaver et al. 2006; Rinnan et al. 2007; Biasi et al. 2008). In relation to the current research, in the event of future temperature increase polar soil microfungi could therefore become more active decomposers in their respective regions.

**Conclusions**

Polar fungi generally showed significant RA for externally secreted amylase and cellulase. Antarctic isolates showed higher RA in comparison with those from the Arctic, with both being higher than tropical isolates. At higher temperatures, the polar isolates appear to switch resource investment from growth into enzyme production, presumably to aid survival. Among polar fungi, *Geomyces* sp. and *Pseudogymnoascus* sp. exhibited significant RA for both amylase and cellulase production, which supports them playing an important role in decomposition processes in polar ecosystems. Tropical fungi in contrast showed low enzyme production at all temperatures examined. This means, in the event of continued regional temperature increase, polar soil fungi have the thermal capability to continue to function as decomposers, which may eventually increase the available soil C pool.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Figure captions

Fig 1 Sampling locations indicated on a global map

**Fig 2a** Effect of culture temperature on mean relative activity of amylase obtained from Antarctic, Arctic and tropical soil microfungi. \*\* significance level P < 0.001

**Fig 2b** Effect of species on mean relative activity of amylase obtained from Antarctic, Arctic and tropical soil microfungi. \*\* significance level P < 0.001

**Fig** 3 Relative activity and colony diameter during screening for amylase under different temperature treatments [a-f: Antarctic isolates; g-k: Arctic isolates; l-n- Tropical isolates]

**Fig 4** Correlation between relative activity and colony diameter during amylase screening

a: Antarctic isolates

b: Arctic isolates

c: Tropical isolates

**Fig 5a** Effect of temperature of mean relative activity of cellulase from Arctic, Antarctic and tropical soil microfungi. \*\* Significant at P < 0.001

**Fig 5b** Effect of species on mean relative activity of cellulase from Arctic, Antarctic and tropical soil microfungi. \*\* significant at P < 0.001

**Fig 6** Relative activity and colony diameter during screening for cellulase under various temperature treatments [a-f: Antarctic fungal isolates; g-k: Arctic fungal isolates; l-n: Tropical fungal isolates]

**Fig 7** Correlation between relative activity and colony diameter during cellulase screening

a: Antarctic isolates

b: Arctic isolates

c: Tropical isolates