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1 **Antimicrobial properties and the influence of temperature on secondary metabolite**
2 **production in cold environment soil fungi**

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30 Abstract:

31 The Arctic and Antarctic share environmental extremes. To survive in such environments,
32 microbes such as soil fungi need to compete with or protect themselves effectively from other
33 soil microbiota and to obtain the often scarce nutrients available, and many use secondary
34 metabolites to facilitate this. We therefore (i) screened for antimicrobial properties of cold-
35 environment Arctic and Antarctic soil fungi, and (ii) identified changes in the secreted
36 secondary metabolite profiles of a subset of these strains in response to temperature variation.
37 A total of 40 polar soil fungal strains from King George Island, maritime Antarctic and
38 Hornsund, Svalbard, High Arctic, were obtained from the Malaysian National Antarctic
39 Research Centre culture collections. The plug assay technique was used to screen for
40 antimicrobial potential against Gram-positive and Gram-negative human pathogenic bacteria
41 (*Bacillus subtilis*, *B. cereus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and
42 *Escherichia coli*). About 45% of the tested fungal strains showed antimicrobial activity
43 against at least one tested microorganism. Three fungal isolates showed good bioactivity and
44 were subjected to secondary metabolite profiling at different temperatures (4, 10, 15 and
45 28°C). We observed a range of responses in fungal metabolite production when incubated at
46 varying temperatures, confirming an influence of environmental conditions such as
47 temperature on the production of secondary metabolites.

48

49 Keywords: Fungal metabolites, *Penicillium flavigenum*, *Pseudogymnoascus* sp., temperature
50 influence

51 1. Introduction

52 Fungi are remarkable microorganisms known to produce a diverse range of compounds
53 extracellularly, usually of low molecular weight, known as secondary metabolites (Keller et
54 al., 2005). Often these secondary metabolites are unique to particular microbial species
55 (Larsen et al., 2005). There is a general consensus that secondary metabolites are not
56 essential for growth, development or reproduction, even though they are produced by many
57 fungi (Madigan et al., 1997). Produced generally near the end of the active growth phase,
58 these compounds are synthesized from compounds that are themselves derived from primary
59 metabolic pathways (Davies, 1985). Although their ecological role often remains unclear,
60 many exhibit antifungal or antimicrobial activity (Peláez, 2006) and are therefore likely to
61 provide the producing microorganism with a competitive advantage facilitating survival in
62 their natural environment.

63 Although the systematic study of fungal secondary metabolites began in 1922 (Raistrick,
64 1985), it was not until the discovery of penicillin by Alexander Fleming in 1928 (Alharbi et
65 al., 2014; Ligon, 2004a,b) that the exploration of secondary metabolites started to intensify.
66 Over recent decades considerable efforts have been devoted to the study of these compounds.
67 This is, in part, driven by the rapidly increasing levels of resistance towards many of the
68 currently available antibiotics (Cooper and Shales, 2011).

69 It is widely accepted that differences in evolutionary pressures have led to some level of
70 specificity associated with ecological niches (Schutz, 2001). To date, the search for
71 pharmaceutically-active fungal strains has largely been focussed in the temperate and tropical
72 regions, and there have been relatively few studies in the cold and stressful environments of
73 the Arctic and Antarctic. Cold-adapted fungi may provide a valuable and currently untapped
74 source of novel metabolites. Their survival in the extreme polar environment requires high

75 adaptability and resistance against many stresses including low nutrient availability, high UV
76 radiation and prolonged exposure to both chronic cold and freezing temperatures and short
77 term thermal maxima during summer (Montiel, 2000; Madronich et al., 1998, Nishiyama,
78 1977; Des Marais, 1995). Tolerance of these stresses leads to the expectation of evolutionary
79 adaptations being developed in polar microbes enabling them to remain viable and function at
80 low temperatures.

81 Research on extreme environments started to gain momentum the middle of the 20th Century.
82 Margesin et al. (2007) reported that, by 2007, there were 30,000 studies published on
83 extremophiles, but also that two-thirds of these studies focused on thermophiles and studies
84 from cold areas remained rare. Recently, there has been greater emphasis on studies of cold
85 environment microbes. The production of extracellular cold-tolerant bioactive compounds
86 and enzymes by polar microbes has been a subject of increasing research interest, particularly
87 in the context of their potential for biotechnological and pharmaceutical application
88 (Groudieva et al., 2004; Priscu et al., 1999).

89 A number of secondary metabolites have been described from Arctic and Antarctic fungal
90 isolates that exhibit antimicrobial and antifungal activities (e.g. O'Brien et al., 2004). Li et al.
91 (2008) reported five novel bioactive asterric acid derivatives from an Antarctic ascomycete
92 (*Pseudogymnoascus* sp.). Some of these compounds displayed antifungal activity against
93 *Aspergillus fumigatus*, and some showed antimicrobial activity against Gram-positive and
94 Gram-negative bacteria. *Penicillium griseofulvum*, collected in Greenland, produced a range
95 of secondary metabolites including griseofulvin, fulvic acid, mycelianamide, roquefortine C,
96 roquefortine D, chanoclavine I and elymoclavine, all of which contributed to antimicrobial
97 activity (Frisvard et al., 2004). Niu (2014) reported isolation of new natural products with
98 unique structural features in *Spriromastix* sp. from deep sea sediments of the South Atlantic
99 Ocean.

100 Abiotic environmental stresses are particularly important in the terrestrial ecosystems of the
101 polar regions (Hogg et al., 2006; Tufto, 2000; Convey et al., 2014). Recent rapid changes in
102 global and regional climates have prompted a range of studies on factors that may influence
103 microbial interactions in these regions. Chwedorzewska (2010) stated that climate change
104 may affect polar terrestrial biota via three fundamental factors - temperature, water and solar
105 irradiance - and small shifts in these factors may result in greater biological impacts. The
106 recent Climate Change 2014 synthesis report by the Intergovernmental Panel on Climate
107 Change has predicted an increase of global average temperature likely to be in the range of
108 0.3°C to 0.7°C for the period 2016 to 2035 and projection for the end of the 21st Century
109 (2081-2100) likely to exceed 1.5°C, while the polar regions will continue to warm more
110 rapidly than the global mean (IPCC, 2014). However, the effects of temperature variation on
111 Antarctic organisms, as elsewhere, have largely not been studied in detail (Convey et al.,
112 2014). A number of temperature manipulation studies, particularly using methodologies such
113 as Open Top Chambers (OTCs), carried out in recent years have shed some light on the
114 response of native Antarctic soil arthropod, lichens and plant communities towards elements
115 of climatic change (Bokhorst et al., 2008, 2011, 2013). Although studies on temperature-
116 driven biodiversity shifts in polar microbial communities are available (Benhua et al., 2014;
117 Dennis et al., 2012; Newsham et al., 2015), limited data currently exist on the effects of
118 warming on secondary metabolism in soil microfungal communities.

119 Clearly one of the primary ecological roles of secondary metabolites is to help the producing
120 organisms function efficiently in their natural environment (Kliebenstein, 2004) and, often,
121 their production is influenced by environmental factors (Akula and Ravishankar 2011;
122 Alvarez et al., 2000). Various studies have examined the effects of external factors such as
123 temperature, UV-B radiation, soil composition, CO₂ and humidity on the production of
124 secondary metabolites (Abreau and Mazzafera, 2005; Eichholz et al., 2011; Rosa et al., 2001;

125 Szakiel and Henry, 2011). Here, we report an initial screening of the antimicrobial properties
126 of 40 polar soil fungal strains, and examine in detail the influence of temperature on the
127 overall secondary metabolite production profiles of three of the most active of these strains.

128 **2. Methodology**

129 **2.1. Fungal cultivation and maintenance**

130 A total of 40 polar fungal isolates were obtained from the National Antarctic Research Centre
131 Fungal Collection, held at the University of Malaya, Kuala Lumpur. These fungal strains
132 were originally isolated from soils collected at different locations in Hornsund, Svalbard
133 (High Arctic) and King George Island, South Shetland Islands (maritime Antarctic) during
134 the boreal summer of 2010 and austral summer of 2007/08, respectively. These fungal
135 isolates had previously been identified using BLAST searches (where sequence data existed)
136 and/or phylogenetic approaches, including the sequencing of type or voucher specimens. The
137 available Genbank accession numbers for the identified isolates are provided in
138 supplementary Table 1.

139 The 40 fungal strains were cultivated by transferring actively growing mycelia onto potato
140 dextrose agar plates (PDA; Difco Laboratories, Detroit, MI, USA), with incubation at 4°C.
141 PDA was prepared by suspending 19.5 g of potato dextrose agar powder in 500 ml of
142 distilled water and heating with frequent agitation for 1 min to dissolve the powder before
143 autoclaving at 121°C for 15 min. The autoclaved media was then poured into petri dishes and
144 allowed to cool. The prepared PDA had a pH of 5.6 ± 0.2 . Sub-samples of each strain were
145 preserved in individual universal bottles containing slants of PDA.

146 **2.2. Bioactivity screening**

147 **2.2.2. Preliminary screening for biological activity**

148 The antimicrobial activities of all 40 polar fungal isolates were tested in triplicate using the
149 plug assay technique (Hoskisson et al., 2001; Ezra et al., 2004) against five Gram-positive
150 and Gram-negative human pathogenic bacteria. The Gram-positive bacteria used were
151 *Bacillus subtilis* (Ehrenberg) Cohn. (ATCC 6051), *Enterococcus faecalis* (Andrewes and
152 Horder) Schleifer and Kilpper-Balz (ATCC 29212) and *B. cereus* Frankland and Frankland
153 (ATCC 11778). The Gram-negative bacteria were *Pseudomonas aeruginosa* (Schroeter)
154 Migula (ATCC 27853) and *Escherichia coli* (Migula) Castellani & Chalmers MTCC 443
155 (ATCC 25922). All the ATCC standard test human pathogenic bacteria were supplied by the
156 Microbiology Department, University of Malaya and were maintained on Luria Broth (LB)
157 agar at 4°C.

158 Fungal isolates were cultivated on sterile PDA for 14 d prior to carrying out the bioassay, and
159 bacterial test microorganisms were prepared in liquid suspension to provide a concentration
160 equivalent of 0.5 McFarland units. Plugs of fungal mycelium growing tangentially to the
161 edge of the colony were then cut and transferred using a 5 mm cork borer into bacterial-
162 pathogen-seeded Muller Hinton Agar (MHA) plates. Muller Hinton Agar was prepared by
163 suspending 19g of MHA powder (DifcoTM) in 500 ml of purified water, and the mixture was
164 then heated with frequent agitation. Next the mixture was autoclaved for 15 min at 122°C.
165 Once cool enough to handle (45-50°C), it was poured into sterile petri dishes and left to cool
166 to room temperature. The plates (n = 3 replicates per pathogen) were then incubated at 37°C
167 for 24 h. After the incubation period inhibition zones were observed and measured. The
168 degree of toxicity of the fungi on the test microorganisms was determined by the diameter of
169 the inhibition zone in millimetres (mm). Antimicrobial activities were classified based on the
170 diameter of the zone of inhibition, with < 7mm considered as no activity, 7-9 mm considered as
171 weak activity, 9-11 mm as moderate activity and > 11 mm as good activity. Only isolates
172 with good bioactivity were considered for further analysis.

173 The preparation of test pathogens involved streaking each test culture onto Trypticase Soy
174 Agar (TSA) and incubation overnight at 37°C. Trypticase Soy Agar was prepared by
175 suspending 20g of TSA powder (Difco™) in 500 ml of purified water, then heating with
176 frequent agitation, and autoclaving for 15 min at 122°C. Once the mixture was cool enough
177 to handle (45-50°C), it was poured into sterile petri dishes and left to cool to room
178 temperature. The test-pathogen-streaked TSA plates were incubated overnight at 37°C. After
179 24 h incubation, 4-5 single colonies were inoculated using a sterile loop into 15 ml universal
180 bottles containing sterile Mueller Hinton Broth (MHB) and shaken thoroughly. MHB was
181 prepared by suspending 21g of MHB powder (Difco™) in 1L of purified water, and again
182 heating with frequent agitation, followed by autoclaving for 15 min at 121°C. Once cool
183 enough to handle (45-50°C), the mixture was poured into 28 ml Universal Bottles and left to
184 cool to room temperature. The densities of the test pathogens in MHB were matched with 0.5
185 McFarland standards that were prepared by adding 0.5 ml of 0.048 M BaCl₂·2H₂O (1.17 %
186 wt/vol) into 99.5 ml of 0.18 M H₂SO₄ (1 % v/v). This standard is considered to equate to 1.5
187 x 10⁸ cfu/ml (Hendrickson and Krenz, 1991).

188 2.3. Disk diffusion assay

189 Inhibition of test bacterial growth by the polar fungal strains at different temperatures (4, 10,
190 15 and 28°C) was measured by the disk diffusion method. All experiments were repeated
191 three times. Prior to each experiment, two-week-old fungal plugs were transferred into 500
192 ml conical flasks containing 250 ml PDB and incubated at 4, 10, 15 or 28°C for 21 d. The
193 cultures were then centrifuged, filtered and extracted with equal volumes of ethyl acetate
194 (EtOAc). The organic layer was collected and dried using a rotary evaporator and the crude
195 extract was stored at 4°C until further use.

196 The test pathogens were prepared as described above and swabbed onto the surface of MHA
197 plates. A sterile 6 mm filter disk was loaded with 10 μ l of 1mg/mL crude extract and placed
198 onto the test microorganism seeded plates. As a positive control gentamycin was used and, as
199 a negative control, the solvent used to dissolve the extracts was included. The plates were
200 then incubated for 24 h at 37°C. The diameters (mm) of inhibition zones were then measured
201 and compared across the different incubation temperatures.

202 **2.4. Temperature manipulation studies**

203 Temperature manipulation studies of fungal strains with good bioactivity were carried out
204 following the in-house protocol MECSUS (Microtiter plate, Elicitors, Combination, Solid
205 phase extraction, UHPLC, Statistical analysis) (Rasha, 2013) with modifications. MECSUS is
206 a protocol for microbial metabolite studies that involves miniaturized parallel fermentations
207 in 96 well plates, parallel extraction and data analysis.

208 Three fungal strains that showed good bioactivity were selected for micro-scale cultivation at
209 four different temperatures, 4, 10, 15 or 28°C. Prior to Micro Titre Plate inoculation, fungal
210 strains were grown in 15 ml Falcon tubes containing sterile 70% Potato Dextrose Broth
211 (PDB). The growth medium for inoculation was prepared by adding 6 ml of 70% PDB into a
212 15 ml Falcon tube together with five small steel balls. The Falcon tubes were then covered
213 with cotton plugs and aluminium foil before being autoclaved at 121°C for 15 min.

214 Three 5 mm plugs of fungal mycelium were cut out from two-week-old fungal cultures using
215 a 5 mm cork borer and transferred into separate 15 ml Falcon tubes containing 70% PDB.
216 Inoculated tubes were then incubated at 4, 10, 15 or 28°C for 14 d.

217 At the end of 14 d incubation, the tubes containing fungal isolates were transferred into a
218 biosafety cabinet where the cotton wool packing and aluminium foil cover were replaced

219 with sterile screw caps. The closed tubes were then shaken in a Genogrinder[®] at 1500 rpm for
220 30 min in order to disrupt the fungal mycelia before cultivation on MT plates.

221 Cultivation in 2 mL square deep 96-well MTPs involved adding 900 μ l of 70% PDB into
222 each MTP well. The plates were then each covered with a silicon mat, sealed with a custom-
223 made clamp board, and autoclaved. Once the materials were cool enough to be handled, 100
224 μ l of disrupted mycelial solution of each of three fungal strains was pipetted into the MTP
225 wells. Each fungal strain was assigned individual rows of 12 wells. The MTPs were then
226 covered with sandwich covers (System Duetz[®], Germany) and a clamp board before being
227 incubated at 4, 10, 15 or 28°C for 21 d.

228 After incubation, the MTP plates were uncovered in a biosafety cabinet, sterile silica SPEX
229 beads added, and the cultures were then homogenised using a Genogrinder[®] SPEX[®]
230 (SamplePrep, New Jersey) at 1500 rpm for 20 min. This was followed by centrifuging the
231 plates at 4000 rpm for 30 min to allow the cell debris to settle at the bottom of the wells.
232 After this, 500 μ l of supernatant from the fungal culture from each well of a row
233 (corresponding to one fungal isolate) was collected in single a sterile glass tube, giving a total
234 volume of 6 ml (12 wells x 500 μ l) per fungal strain.

235 The collected supernatants were then subjected to extraction with an equal volume of EtOAc.
236 After shaking, the upper organic layer was transferred into a new and pre-weighed 30 ml vial.
237 This step was repeated three times and the organic layers were combined. The EtOAc was
238 evaporated to dryness using a rotary evaporator. The mass of each extract was recorded and
239 the extracts were then stored at 4°C prior to HPLC analysis.

240 **2.5. HPLC analysis**

241 Extracts were chromatographically analysed to identify changes in the overall secondary
242 metabolite profiles obtained at the different culture temperatures. Analytical HPLC was
243 performed on an Agilent 1260 system (Agilent Technologies, United States) equipped with a
244 column Synergy 4 μm Hydro-RP 80 \AA 150 \times 4.6 mm, 4 μm particle size (Phenomenex®,
245 USA). The HPLC system (Agilent Technologies, United States) consists of the following
246 components: quaternary pump G1311A, vacuum degasser pump G1322A, auto sampler
247 G1316A and diode detector (DAD) G1315B. As an internal standard (IS), 1 mL of 1 mg/mL
248 solution of 4-bromobiphenyl was added to the samples. Samples were prepared by diluting
249 extracted crude sample of unknown amount into 1 mL of HPLC grade methanol (MeOH).
250 The IS was then added to the sample. The mobile phase (H_2O /water) was prepared by
251 filtering acetonitrile (ACN) and deionized water on 0.45 μm nylon membrane filters. The
252 elution gradient was set as follows (solvent B, ACN): 10% at 0 min, 46% at 10 min, 70% at
253 15 min, 100% at 20 min, 100% at 30 min, 10% at 31 min, equilibration 10 min. The flow rate
254 was set to 1 ml/min. The injection volume was set at 10 μL . UV-vis data were recorded from
255 190 to 600 nm and displayed at 220 nm. A Chemstation® chromatography data system
256 (Agilent Technologies, United States), was used to control the chromatographic system and
257 process the chromatograms. Chromatographic peaks with intensity $>$ 50 mAU appearing
258 between retention times (R_t) of 3 and 26 min were considered. The variations in size of major
259 peaks were computed with reference to the IS.

260 **3. Results and Discussion**

261 **3.1. Antimicrobial activity of polar fungi**

262 Forty cold environment soil fungal isolates originating from various habitats were screened
263 for their antimicrobial properties using the plug assay method against five human pathogenic
264 bacteria, with their antimicrobial activity being classified according to the extent of the

265 inhibition zone produced. Forty-five percent of tested fungal isolates showed antimicrobial
266 activity against at least one of the five test pathogens. A majority of the fungi tested showed
267 high inhibition activity against the Gram-positive bacteria, *B. subtilis* and *B. cereus* (Table 1),
268 but only two isolates showed positive activity against the Gram-negative *E. coli* and *P.*
269 *aeruginosa*. None of the isolates showed activity against *E. faecalis*. Two fungal strains,
270 *Pseudogymnoascus* sp (AK 102 R1-4 sp 5) isolated from King George Island and *Penicillium*
271 *flavigenum* (HND 11 R8-1) isolated from Hornsund, exhibited broad spectrum activity. Of
272 the five *Pseudogymnoascus* sp. strains tested, three exhibited good activity. Of four
273 *Penicillium* sp. strains tested, three exhibited inhibition.

274 Most of the fungi tested showed some inhibition activity against Gram-positive bacteria,
275 comparable to the findings of Nedialkova and Naidenova (2005), who tested antimicrobial
276 activities in actinomycetes isolated from Antarctica. Previous studies have also indicated that
277 antibiotic agents of fungal origin most strongly inhibit Gram-positive rather than Gram-
278 negative bacteria (Kumar et al., 2005). The reason for the disparity in the inhibitory activity
279 between Gram-positive and Gram-negative bacteria relates to differences between the two
280 groups' cell wall structures, as Gram-negative bacteria are equipped with a cell wall barrier
281 that prevents the passage of large molecules and fat-soluble molecules, thus making them less
282 susceptible to the action of antibiotics in comparison with Gram-positive bacteria (Tortora et
283 al., 2001).

284 Two strains in this study displayed activity against both Gram-positive and Gram-negative
285 bacteria, *Pseudogymnoascus* sp (AK 102 R1-4 sp 5) and *Penicillium flavigenum*. (HND 11
286 R8-1). *Pseudogymnoascus* sp. showed broad activity against three test pathogens. This fungal
287 species is commonly encountered in cold environments around the world (e.g. Adams et al.,
288 2006; Singh, 2011), and various *Pseudogymnoascus* strains have been reported to be endemic
289 or indigenous to Antarctic and sub-Antarctic regions (Arenz et al., 2006, 2011; Hughes et al.,

290 2007). This genus belongs to the order Helotiales, which includes typically saprophytic fungi
291 playing an active role in nutrient recycling. Members of the genus *Pseudogymnoascus* have
292 been reported to produce a range of extracellular enzymes including amylase and cellulase
293 (Krishnan et al., 2014), keratinases (Mercantini et al., 1989), lipase, chitinase and urease
294 (Finotti et al., 1993, 1996). Li et al. (2008) reported that an Antarctic
295 *Pseudogymnoascus* strain exhibited antimicrobial activity against both Gram-positive and
296 Gram-negative bacteria, consistent with the current study. A study on Antarctic marine fungi
297 (Henriquez et al., 2014) also confirmed the antimicrobial and antitumoral potential of
298 *Pseudogymnoascus* sp.

299 A second fungal strain identified in the current study that exhibited broad spectrum
300 bioactivity was the “classic” antimicrobial compound producer *Penicillium flavigenum*
301 (HND11 R8-1), isolated from High Arctic Svalbard. Representatives of this genus are
302 globally distributed and clearly capable of thriving in various ecosystems. Sonjak et al.
303 (2006) reported that *Penicillium* species sourced from various habitats showed no or very few
304 differences in their secondary metabolite profiles. In the current study, isolate HND11 R8-1
305 exhibited good inhibition activity against both Gram-positive and Gram-negative bacteria,
306 unlike several other *Penicillium* strains tested here whose activity was limited to only one
307 pathogenic bacterium, *Bacillus subtilis*.

308 The bryophyte-associated fungus *Atradiidymella* sp. inhibited the growth of Gram-positive
309 bacteria. This species is also known to be able to infect mosses, and produces soluble
310 polyphenolic oxidases and cellulases (Davey et al., 2009), but no previous studies have
311 reported antimicrobial activity.

312 The three fungal strains mentioned above (*Pseudogymnoascus* sp., *Penicillium flavigenum*
313 and *Atradiidymella* sp. were selected for further temperature manipulation studies to examine

314 (a) their overall secondary metabolite production profiles at different temperatures, and (b)
315 their antimicrobial activity at different temperatures assessed via a disk diffusion assay.

316 **3.2. Temperature influence on secondary metabolite production profile**

317 Inspection of chromatograms and determination of the relative concentrations of major
318 compounds with reference to an internal standard revealed an overall increase in compound
319 concentrations for the major peaks at 4°C for *Atracidymella* sp. The other two isolates,
320 *Pseudogymnoascus* sp. and *Penicillium flavigenum*, differed with higher concentrations
321 obtained at 15°C. An overall reduction of concentrations produced by all strains was
322 observed at 10°C (Figure 1 a, b, c). These data confirm that culture temperature influences
323 the production of secondary metabolites in all three tested fungal strains, although not in a
324 simple fashion.

325 Environmental stressors such as temperature, ultra-violet radiation, water availability,
326 salinity, pH, micronutrient and water availability, and CO₂ concentration are important
327 factors that affect the physiology and metabolic pathways of fungi (Magan, 2007). These
328 factors play crucial roles in determining microbial community composition in soils (Castro et
329 al., 2010) and small shifts in these abiotic factors could have significant effects on the growth
330 and productivity of key microorganisms (Bell, 2013). Although the above-mentioned
331 environmental factors often do not act independently in polar soil ecosystems (Peck, 2006),
332 temperature is a critical environmental variable that affects soil ecology in the polar regions
333 (Convey et al., 2014), and seasonally changing temperatures influence the relative abundance
334 of microorganisms (Bell, 2013). Temperature is an important environmental factor that
335 influences physiological function of all organisms. This is particularly the case for
336 microorganisms as they are poikilotherms whose temperature follows that of their external
337 environment. Temperature variation in any given habitat is a natural phenomenon, taking

338 place over various timescales from the immediate and dynamic, through diurnal to seasonal
339 and longer, and these variations can have important impacts on the survival and evolution of
340 microorganisms (Convey et al., 2014; Davey et al., 1992; Peck et al., 2006). In polar soils,
341 microbial activities have been demonstrated at temperatures as low as -15°C (Steven, 2007).
342 Significant effects of microbial competition on biogeochemical flux were observed over the
343 summer where warmer temperatures led to an increase in overall activity (Bell, 2013). In the
344 current study, we have demonstrated that culture temperatures clearly affect the production of
345 secondary metabolites in the three polar soil fungal strains examined in more detail, but clear
346 and consistent patterns did not emerge and the fungal responses were complex, as also noted
347 by Shohael (2006).

348 **3.3 Effects of temperature on antimicrobial activity**

349 Microorganisms, particularly soil-dwelling fungi, are known to produce a number of
350 secondary metabolites with antimicrobial properties as a defence mechanism, thought to aid
351 in competition for resources (Gallo et al., 2004), and temperature has also been reported to
352 exert a profound effect on the antimicrobial production and activity of fungi (Himabindu and
353 Jetty, 2006). Castro (2010) demonstrated a substantial increase in antagonistic activity
354 between many Arctic soil bacterial isolates with increased temperature, proposing two
355 possible explanations: 1) increased production of antimicrobials or, 2) shifts in relative
356 growth rates. In the current study the antimicrobial activities of the selected
357 *Pseudogymnoascus* sp. strain tested varied at different temperatures (Table 2). After crude
358 extraction for the disk diffusion assay, the extracts obtained from incubations at 4°C or 15°C
359 inhibited four of the five tested human pathogens (*E. coli*, *B. subtilis*, *S. aureus* and *C.*
360 *albicans*). The widest diameters of the zones of inhibition, inferred to indicate the greatest
361 activity, were observed for extracts from cultures incubated at 15°C (Table 2). Only two test
362 organisms (*B. subtilis* and *C. albicans*) were inhibited at 10°C. All extracts were able to

363 inhibit the yeast *C. albicans*, although the diameter of the zone of inhibition reduced with
364 increasing temperature.

365 Three of the five test microorganisms were inhibited by *Penicillium flavigenum* crude
366 extracts obtained at 10 and 15°C. At 4°C two test microorganisms were inhibited and at 28°C
367 only *C. albicans* was inhibited. *S. aureus* was only inhibited by the crude extracts produced at
368 10 and 15°C. *P. aeruginosa* and *E. coli* were not inhibited by any of the crude extracts.
369 Although the ability to produce compounds that inhibited the test organisms appeared to be
370 retained over a wide range of culture temperatures, when exposed to the highest experimental
371 temperature the inhibitory ability reduced dramatically.

372 All crude extracts obtained from cultures at 10°C showed moderate biological activity (Table
373 2). Good inhibitory activity was visible at 4 and 28°C for *Pseudogymnoascus* sp. and
374 *Atradiidymella* sp. The crude extract of *P. flavigenum* exhibited moderate activity across all
375 culture temperatures. This suggests that the responses of the fungi tested here to temperature
376 were species-specific. More detailed studies of the optimal growth conditions and production
377 of secondary metabolites, along with formal identification of specific metabolites, of each of
378 these strains are required to further understand the relationship between growth dynamics and
379 secondary metabolite production at different temperatures.

380 4. Conclusions

381 This study investigated the antimicrobial properties of 40 cold-adapted soil fungi from the
382 Arctic and Antarctic. The data obtained showed that 45% of tested fungal cultures expressed
383 at least some antimicrobial activity towards at least one of the five human pathogenic bacteria
384 tested. *Pseudogymnoascus* sp., *P. flavigenum* and *Atradiidymella* sp. showed the strongest
385 antimicrobial activity. These taxa are found abundantly in polar soils and have also been
386 reported to exhibit antifungal activities and produce cold-adapted enzymes, consistent with

387 active roles in decomposition and nutrient recycling in the soil ecosystem. Our study provides
388 further confirmation that these species are rich producers of extracellular substances. Culture
389 temperature clearly influenced the production of secondary metabolites, including
390 compounds with antibacterial activity, in *Pseudogymnoascus* sp., *Penicillium flavigenum* and
391 *Atracidymella* sp. Further genomic and functional analyses of interspecific competition and
392 synergistic interactions in polar soil microbial communities are required to help identify the
393 effects of climate variability and warming on soil ecosystems.

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424 References

- 425
426 Adams, B. J., Bardgett, R. D., Ayres, E., Wall, D. H., Aislabie, J., Bamforth, S., & Stevens,
427 M. I. (2006). Diversity and distribution of Victoria Land biota. *Soil Biol. Biochem.*
428 38, 3003-3018.
- 429 Abreu, I. N., & Mazzafera, P. (2005). Effect of water and temperature stress on the content of
430 active constituents of *Hypericum brasiliense* Choisy. *Plant. Physiol. Bioch.* 43(3),
431 241-248.
- 432 Alharbi, S. A., Wainwright, M., Alahmadi, T. A., Salleeh, H. B., Faden, A. A., &
433 Chinnathambi, A. (2014). What if Fleming had not discovered penicillin? *Saudi. J.*
434 *Biol. Sci.* 21, 289-293.
- 435 Akula, R., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary
436 metabolites in plants. *Plant. Signal. Behav.* 6, 1720-1731.
- 437 Arenz, B. E., & Blanchette, R. A. (2011). Distribution and abundance of soil fungi in
438 Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys.
439 *Soil. Biol. Biochem.* 43, 308-315.
- 440 Bell, T. H., Callender, K. L., Whyte, L. G., & Greer, C. W. (2013). Microbial Competition in
441 Polar Soils: A Review of an Understudied but Potentially Important Control on
442 Productivity. *Biology*, 2(2), 533–554.
- 443 Benhua, S., Dennis, P. G., Laudicina, V. A., Ord, V. J., Rushton, S. P., O'Donnell, A. G.,
444 Hopkins, D. W. (2014). Biogeochemical responses to nutrient, moisture and

- 445 temperature manipulations of soil from Signy Island, South Orkney Islands in the
446 Maritime Antarctic. *Antarct. Sci.* 26, 513-520.
- 447 Bokhorst, S., Huiskes, A., Aerts, R., Convey, P., Cooper, E. J., Dalen, L., Dorrepaal, E.
448 (2013). Variable temperature effects of Open Top Chambers at polar and alpine sites
449 explained by irradiance and snow depth. *Glob. Change. Biol.* 19, 64-74.
- 450 Bokhorst, S., Huiskes, A., Convey, P., Sinclair, B., Lebouvier, M., Van de Vijver, B., &
451 Wall, D. (2011). Microclimate impacts of passive warming methods in Antarctica:
452 implications for climate change studies. *Polar. Biol.* 34, 1421-1435.
- 453 Bokhorst, S., Huiskes, A., Convey, P., Van Bodegom, P., & Aerts, R. (2008). Climate change
454 effects on soil arthropod communities from the Falkland Islands and the Maritime
455 Antarctic. *Soil. Biol. Biochem.* 40, 1547-1556.
- 456 Castro, H. F., Classen, A. T., Austin, E. E., Norby, R. J., & Schadt, C. W. (2010). Soil
457 microbial community responses to multiple experimental climate change drivers.
458 *Appl. Environ. Microb.* 76, 999-1007.
- 459 Chwedorzewska K. J. (2010). Recent rapid regional climate changes in Antarctic and their
460 influence on a low diversity ecosystems. *Pap. Glob.Change. IGBP.* 17, 17-30.
- 461 Convey, P., Chown, S. L., Clarke, A., Barnes, D. K. A., Bokhorst, S., Cummings, V., Wall,
462 D. H. (2014). The spatial structure of Antarctic biodiversity. *Ecol. Monogr.* 84, 203-
463 244.
- 464 Cooper, M. A., Shlaes, D., (2011). Fix the antibiotics pipeline. *Nature.* 472, 32-32.
- 465 Des, D.J., (1995). The biogeochemistry of hypersaline microbial mats. *Adv. Microb. Ecol.* 14,
466 251-274.

- 467 Davey, M., Pickup, J., & Block, W. (1992). Temperature variation and its biological
468 significance in fellfield habitats on a maritime Antarctic island. *Antarct. Sci.* 4, 383-
469 388.
- 470 Davey, M. L., Tsuneda, A., & Currah, R. S. (2009). Pathogenesis of bryophyte hosts by the
471 ascomycete *Atracidymella muscivora*. *Am. J. Bot.* 96, 1274-1280.
- 472 Davies, J. (1985). Recombinant DNA and the production of small molecules.
473 *Microbiology*.364-366
- 474 Dennis, P. G., Rushton, S. P., Newsham, K. K., Lauducina, V. A., Ord, V. J., Daniell, T. J.,
475 Hopkins, D. W. (2012). Soil fungal community composition does not alter along a
476 latitudinal gradient through the maritime and sub-Antarctic. *Fungal. Ecol.* 5, 403-408.
- 477 Eichholz, I., Huyskens-Keil, S., Keller, A., Ulrich, D., Kroh, L. W., & Rohn, S. (2011). UV-
478 B-induced changes of volatile metabolites and phenolic compounds in blueberries
479 (*Vaccinium corymbosum L.*). *Food. Chem.* 126, 60-64.
- 480 Finotti E., Moretto D., Marsella R., & Mercantini R. (1993). Temperature effects and fatty
481 acid patterns in *Geomyces* species isolated from Antarctic soil. *Polar. Biol.* 13, 127-
482 130.
- 483 Frisvad, J.C., Frank, J.M., Houbraeken, J.A.M.P., Kuijpers, A.F.A. & Samson, R.A., (2004).
484 New ochratoxin A producing species of *Aspergillus* section *Circumdati*. *Stud.*
485 *Mycol.*50, 23-43.
- 486 Gallo, M. L., Seldes, A. M., & Cabrera, G. M. (2004). Antibiotic long-chain and α,β -
487 unsaturated aldehydes from the culture of the marine fungus *Cladosporium* sp.
488 *Biochem. Syst. Ecol.* 32, 545-551.

- 489 Groudieva, T., Kambourova, M., Yusef, H., Royter, M., Grote, R., Trinks, H., & Antranikian,
490 G. (2004). Diversity and cold-active hydrolytic enzymes of culturable bacteria
491 associated with Arctic sea ice, Spitzbergen. *Extremophiles*. 8, 475-488.
- 492 Himabindu, M., & Jetty, A. (2006). Optimization of nutritional requirements for gentamicin
493 production by *Micromonospora echinospora*. *Indian. J. Exp. Bio.* 44, 842.
- 494 Henríquez, M., Vergara, K., Norambuena, J., Beiza, A., Maza, F., Ubilla, P., & Darias, J.
495 (2014). Diversity of cultivable fungi associated with Antarctic marine sponges and
496 screening for their antimicrobial, antitumoral and antioxidant potential. *World. J.*
497 *Microb. Biot.* 30, 65-76.
- 498 Hendrikson, D.A., & M.M. Krenz.(1991). Reagents and stains, p. 1289-1314. In A. Balows,
499 W.J. Hausler, Jr., K.L. Hermann, H.D. Isenberg, and H.J. Shadomy (ed), *Manual of*
500 *Clinical Microbiology*, Washington, DC.
- 501 Hogg, I.D., Cary, S. Craig., Convey P., Newsham K., O'Donnell G., Adams, B. J., Aislabie J.,
502 Frati F., Stevens M. I., & Wall, D. H. (2006) Biotic interactions in Antarctic terrestrial
503 ecosystems: are they a factor? *Soil. Biol. Biochem*, 38. 3035-3040.
- 504 Hughes, K. A., Bridge, P., & Clark, M. S. (2007). Tolerance of Antarctic soil fungi to
505 hydrocarbons. *Sci. Total. Environ.* 372, 539-548
- 506 IPCC, (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II*
507 *and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate*
508 *Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva,*
509 *Switzerland, 151 pp.*
- 510 Keller, N. P., Turner, G., & Bennett, J. W. (2005). Fungal secondary metabolism—from
511 biochemistry to genomics. *Nat. Rev. Microbiol.* 3, 937-947.

- 512 Kliebenstein, D. J., A. Figuth, & T. Mitchell-Olds. (2002). Genetic architecture of plastic
513 methyl jasmonate responses in *Arabidopsis thaliana*. *Genetics* 161, 1685–1696.
- 514 Krishnan, A., Convey, P., Gonzalez-Rocha, G., & Alias, S. (2014). Production of extracellular
515 hydrolase enzymes by fungi from King George Island. *Polar Biol.* 39, 65-76.
- 516 Kumar A.B.V., Varadaraj M.C., Gowda L.R., & Tharanathan R.N (2005). Characterization of
517 chito-oligosaccharides prepared by chitosan analysis with the aid of papain and pronase,
518 and their bactericidal action against *Bacillus cereus* and *Escherichia coli*. *Biochem. J.*
519 391, 167–175.
- 520 Larsen T.O., Smedsgaard J., Nielsen K.F., Hansen M.E., & Frisvad J.C. (2005). Phenotypic
521 taxonomy and metabolite profiling in microbial drug discovery. *Nat. Prod. Rep.* 22,
522 672-693.
- 523 Li, Y., Wadsö, L., & Larsson, L. (2009). Impact of temperature on growth and metabolic
524 efficiency of *Penicillium roqueforti*– correlations between produced heat, ergosterol
525 content and biomass. *J. Appl. Microbiol.* 106, 1494-1501.
- 526 Ligon, B. L. (2004). *Penicillin: its discovery and early development*. Paper presented at the
527 Seminars in Pediatric Infectious Diseases 15, 52-57. WB Saunders.
- 528 Ligon, B. L. (2004). Sir Alexander Fleming: Scottish researcher who discovered penicillin.
529 Seminars in Pediatric Infectious Diseases 15, 58-64.
- 530 Madigan, M. T., Martinko, J. M., Parker, J., & Brock, T. D. (1997). Brock biology of
531 microorganisms. Upper Saddle River, NJ: Prentice Hall.
- 532 Magan, N. (2007). Fungi in Extreme Environments. *Environmental and Microbial*
533 *Relationships* 4, 85.

- 534 Margesin, R., Schinner, F., Marx, J.-C., & Gerday, C. (2007). Psychrophiles: From
535 Biodiversity to Biotechnology. Springer Science & Business Media.
- 536 Mercantini, R., Marsella, R., & Cervellati, M.C. (1989). Keratinophilic fungi isolated from
537 Antarctic soil. *Mycopathologia* 106, 47–52.
- 538 Montiel P.O. (2000). Soluble carbohydrates (trehalose in particular) and cryoprotection in
539 polar biota. *Cryo Lett.* 21,83–90.
- 540 Madronich S., McKenzie R.L., Björn L.O., & Caldwell M.M. (1998). Changes in
541 biologically active ultraviolet radiation reaching the Earth's surface. *J. Photochem.*
542 *Photobiol. B.* 46,5–19.
- 543 Nedialkova, D., & Naidenova, M. (2005). Screening the antimicrobial activity of
544 actinomycetes strains isolated from Antarctica. *J. Cult. Collect.* 4, 29-35.
- 545 Newsham, K. K., Hopkins, D. W., Carvalhais, L. C., Fretwell, P. T., Rushton, S. P.,
546 O'Donnell, A. G., & Dennis, P. G. (2016). Relationship between soil fungal diversity
547 and temperature in the maritime Antarctic. *Nature Clim. Change* 6, 182-186.
- 548 Nishiyama, T. (1977). Studies on evaporite minerals from Dry Valley, Victoria Land,
549 Antarctica. *Antarct. Res.* 58, 171–185.
- 550 Niu, S., Liu, D., Hu, X., Proksch, P., Shao, Z., & Lin, W. (2014). Spiromastixones A–O,
551 antibacterial chlorodepsidones from a deep-sea-derived *Spiromastix* sp. *Fungus. J.*
552 *Nat. Prod.* 77, 1021-1030.
- 553 O'Brien, K., R. Leichenko, U. Kelkar, H. Venema, G. Aandahl, H. Tompkins, A. Javed, S.
554 Bhadwal, S. Barg, L. Nygaard & J. West. (2004). Mapping vulnerability to multiple

- 555 stressors: climate change and globalization in India. *Global. Environ. Change* 14, 303-
556 313.
- 557 Peck, L. S., Convey, P., & Barnes, D. K. A. (2006). Environmental constraints on life
558 histories in Antarctic ecosystems: tempos, timings and predictability. *Biol. Rev.* 81,
559 75-109.
- 560 Peláez, F., (2006). The historical delivery of antibiotics from microbial natural products—
561 Can history repeat? *Biochem. Pharmacol.* 71, 981-990.
- 562 Priscu, J. C., Adams, E. E., Lyons, W. B., Voytek, M. A., Mogk, D. W., Brown, R. L., Avci,
563 R. (1999). Geomicrobiology of subglacial ice above Lake Vostok. *Antarct. Sci.* 286,
564 2141-2144.
- 565 Raistrick, H. (1950). A region of biosynthesis. *Proceedings of the Royal Society of London.*
566 *Series B Biological Science* 136, 481-508.
- 567 Rasha, S. S., Kalavathy, S., Syed A., Cole A.L.J., Weber, J.F.F. (2013). Multifaceted
568 (Mecus) protocol for microbial metabolites studies, *Open. Conf. Proc. J.* 6, 287.
- 569 Rosa, T. M., Julkunen-Tiitto, R., Lehto, T., & Aphalo, P. J. (2001). Secondary metabolites
570 and nutrient concentrations in silver birch seedlings under five levels of daily UV-B
571 exposure and two relative nutrient addition rates. *New. Phytol.* 150, 121-131.
- 572 Singh, S., Puja, G., & Bhat, D. (2006). Psychrophilic fungi from Schirmacher Oasis, East
573 Antarctica. *Curr. Sci.* 90, 1388.
- 574 Sonjak, S., Frisvad, J. C., & Gunde-Cimerman, N. (2006). *Penicillium* mycobiota in Arctic
575 subglacial ice. *Microb. Ecol.* 52, 207-216.

576 Steven, B., Niederberger, T. D., Bottos, E. M., Dyen, M. R., & Whyte, L. G. (2007).
577 Development of a sensitive radiorespiration method for detecting microbial activity at
578 subzero temperatures. *J. Microbiol. Meth.* 71, 275-280.

579 Szakiel, A., Pączkowski, C., & Henry, M. (2011). Influence of environmental abiotic factors
580 on the content of saponins in plants. *Phytochem. Rev.* 10, 471-491.

581 Tufto, J. (2000). The evolution of plasticity and nonplastic spatial and temporal adaptations in
582 the presence of imperfect environmental cues. *Am. Nat.* 156, 121-130.

583 Tortora, G.J., Funke, B.R., Case, C.L., (2001). *Microbiology: An Introduction*. Benjamin
584 Cummings, San Francisco.

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Table 1. Bioactivity of fungal isolates against five test microorganisms.

Strain code	Test microorganism				
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia faecalis</i>
AK07KGI102 R2-3	-	-	+++	-	-
AK07KGI 301 R2-2 ASCO 6	++	-	-	-	-
AK07KGI 102 R2-3 SP 1	-	-	+++	-	-
AK07KGI 105 R3-2 SP 17	-	-	-	-	-
AK07KGI 1001 R1-1 SP 2	-	-	-	-	-
HND 10 R2-4	+	-	-	-	-
HND 9 R1-1 SP 34	+	-	-	-	-
HND 11 R2-2 SP 36	+	-	-	-	-
HND 10R1-3	+	-	-	-	-
HND 5 R5-3	-	-	-	-	-
HND 2 R8-2	-	-	-	-	-
HND 1 R7-2	-	-	-	-	-
HND 4 R7-3	++	-	-	-	-
AK07KGI 102 R1-4 SP 5	+++	-	++	+++	-
AK07KGI 102 R3-3 SP 30	-	-	+++	-	-
AK07KGI 102 R3-1 SP 16	-	-	-	-	-
AK07KGI 1001 R3-2 SP 1	++	-	-	-	-
AK07KGI 105 R3-1	-	-	-	-	-
HND 12 R8-1 SP 5	-	-	-	-	-
HND 2 R5-3	-	-	-	-	-
HND 1 R7-3	-	-	-	-	-

AK07KGI 102 R2-1 SP 18	++	-	-	-	-
HND 7 R1-1	-	-	-	-	-
HND 5 R6-2	-	-	-	-	-
AK07KGI 103 R2-1	+	-	-	-	-
HND 7 R6-3C	-	-	-	-	-
AK07KGI 102 R1-3 ASCO 2	++	-	-	-	-
AK07KGI 102 R3-2 ASCO 4	-	-	-	-	-
HND 11 R8-1	+++	+++	++	++	-
HND 2 R5-4	+++	-	-	-	-
HND 4 R5-1	-	-	-	-	-
AK07KGI 101 R2-3SP 1	-	-	-	-	-
AK07KGI 101 R3-1 SP 2	-	-	-	-	-
AK07KGI 1001 R2-1	-	-	-	-	-
AK07KGI 102 R3-1 SP 2	-	-	-	-	-
HND 12 R2-1	-	-	-	-	-
AK07KGI 1001 R1-1 SP 4	++	-	-	-	-
HND 12 R8-2(2)	-	-	-	-	-
AK07KGI 102 R1-4 SP 16	-	-	-	-	-
AK07KGI 2001 R2-1 SP 1	-	+	-	-	-

* Activities are classified as: - = no activity, weak activity = (+), moderate activity = (++)
high activity = (+++).

Table 2. Antimicrobial activity of *Atradiidymella* sp., *Pseudogymnoascus* sp., and *Penicillium flavigenum* crude extracts measured at different temperatures.

Isolates	Test organisms	mean \pm standard deviation diameter of inhibition zone (mm)			
		at different culture temperatures			
		4°C	10°C	15°C	28°C
HND 10 (<i>Atradiidymella</i> sp.)	<i>E. coli</i>	0.0	7.0 \pm 0.5	7.0 \pm 1.0	0.0
	<i>B. subtilis</i>	0.0	0.0	6.0	7.0 \pm 0.5
	<i>S. aureus</i>	15.0 \pm 0.5	9.0 \pm 0.5	7.0 \pm 1.0	9.0 \pm 1.73
	<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0
	<i>C. albicans</i>	11.0	13.0 \pm 0.5	15.0	15.0
AK 102 (<i>Pseudogymnoascus</i> sp.)	<i>E. coli</i>	23.0 \pm 1.7	0.0	24.0 \pm 1.5	0.0
	<i>B. subtilis</i>	16.0 \pm 1.0	6.0 \pm 0.5	17.0 \pm 1.7	7.0 \pm 0.5
	<i>S. aureus</i>	13.0 \pm 1.7	0.0	18.0 \pm 1.3	16.0 \pm 1.7
	<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0
	<i>C. albicans</i>	18.0 \pm 0.5	10.0 \pm 0.5	18.0	8.0
HND 11 (<i>Penicillium flavigenum</i>)	<i>E. coli</i>	0.0	0.0	0.0	0.0
	<i>B. subtilis</i>	7.0	7.0 \pm 1.5	7.0	0.0
	<i>S. aureus</i>	7.0	8.0 \pm 1.0	7.0 \pm 1.5	0.0
	<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0
	<i>C. albicans</i>	8.0	7.0 \pm 1.6	7.0	7.0

Strain ID	Collection Location	Thermal class	(Genebank Accession no)	Identity
AK07KGI 102 R2-1 SP18	King George Island	Psychrophillic	KY646431	<i>Pseudorotium</i> .sp
AK07KGI 2001 R2-1 SP1	King George Island	Psychrophillic	KY646433	<i>Pseudogymnoascus</i> .sp
AK07KGI 102 R1-4 SP5	King George Island	Psychrotolerant	KY623481	<i>Pseudogymnoascus</i> .sp
AK07KGI 1001 R3-2 SP 1	King George Island	Psychrophillic	KY646435	<i>Pseudogymnoascus</i> .sp
AK07KGI 105 R3-2 SP17	King George Island	Psychrophillic	n/a	n/a
AK07KGI 103 R2-1	King George Island	Psychrophillic	n/a	n/a
AK07KGI 102 R2-3 ASCO 2	King George Island	Psychrophillic	KY646438	<i>Penicillium commune</i>
AK07KGI 101 R2-3SP 1	King George Island	Psychrophillic	n/a	n/a
AK07KGI 102 R3-3 SP 30	King George Island	psychrotolerant	n/a	n/a
AK07KGI 301 R3-3 SP2	King George Island	psychrotolerant	KY646432	<i>Pseudogymnoascus</i> .sp
AK07KGI 102 R1-4 SP16	King George Island	Psychrotolerant	KY646441	<i>Tausonia pullulans</i>
AK07KGI 301 R2-2 ASCO 6	King George Island	Psychrophillic	n/a	n/a
AK07KGI 101 R3-1 SP 2	King George Island	Psychrophillic	KY623479	<i>Leuconeuospora</i> .sp
AK07KGI 102 R3-1 SP 2	King George Island	Psychrophillic	n/a	n/a
AK07KGI 102 R3-2 ASCO 4	King George Island	Psychrophillic	n/a	n/a
AK07KGI 1001 R1-1 SP4	King George Island	Psychrophillic	n/a	n/a
AK07KGI 102 R2-3 SP1	King George Island	Psychrotolerant	KY623480	<i>Penicillium commune</i>
AK07KGI 1001 R1-1 SP2	King George Island	Psychrophillic	KY646437	<i>Pseudgymnoascus</i> .sp
AK07KGI 1001 R2-1	King George Island	Psychrophillic	KY646434	<i>Pseudogymnoascus</i> .sp
AK07KGI 105 R3-1	King George Island	Psychrophillic	KY646436	<i>Pseudogymnoascus</i> .sp
AK07KGI 102 R3-1 SP 16	King George Island	Psychrotolerant	KY646440	<i>Tausonia pullulans</i>
HND11 R8-1	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrophillic	KY623482	<i>Penicillium flavigenum</i>
HND10 R2-4	Hyrneodden peninsula,	Psychrotolerant	KY934270	<i>Atradiymella</i> sp

	Mariesletta, Hornsund			
HND2 R5-4	Hyettevika,Hornsund	Psychrophillic	n/a	n/a
HND5 R6-2	Ralstranda, Hornsund	Psychrophillic	KY646429	<i>Isafaria farinosa</i>
HND5 R5-3	Ralstranda, Hornsund	Psychrophillic	KY623484	<i>Isafaria farinosa</i>
HND2 R8-2	Hyettevika,Hornsund	Psychrophillic	KY646428	<i>Isafaria farinosa</i>
HND2 R5-3	Hyettevika,Hornsund	Psychrophillic	KY646439	<i>Penicillium flavigenum</i>
HND1 R7-2	North-western slopes of Kvartsittknattane, Hornsund	Psychrophillic	KY646430	<i>Phialocephala</i> sp.
HND12 R8-1 SP 5	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrophillic	KY646427	<i>Isaria farinosa</i>
HND7 R6-3	North-western slopes of KvartsittknattaneH ornsund	Psychrophillic	n/a	n/a
HND4 R5-1	Revdalen, Hornsund	Psychrophillic	KY623483	<i>Tausonia pullulans</i>
HND4 R7-3	Revdalen, Hornsund	Psychrophillic	n/a	n/a
HND11 R2-2 SP 36	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrotolerant	n/a	n/a
HND9 R1-1 SP 34	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrotolerant	n/a	n/a
HND1 R7-3	North-western slopes of KvartsittknattaneH ornsund	Psychrophillic	n/a	n/a
HND12 R2-1	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrotolerant	n/a	n/a
HND12 R8-2(2)	Hyrneodden peninsula, Mariesletta, Hornsund	Psychrophillic	n/a	n/a
HND10 R1-3	Hyrneodden peninsula,	Psychrotolerant	n/a	n/a

	Mariesletta, Hornsund			
HND7 R1-1	Rotjesfjellet, Hornsund	Psychrophillic	n/a	n/a

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Figure 1 a. Changes in relative concentrations of major secondary metabolite peaks produced by *Atracidymella* sp. at 4, 10, 15 and 28°C.

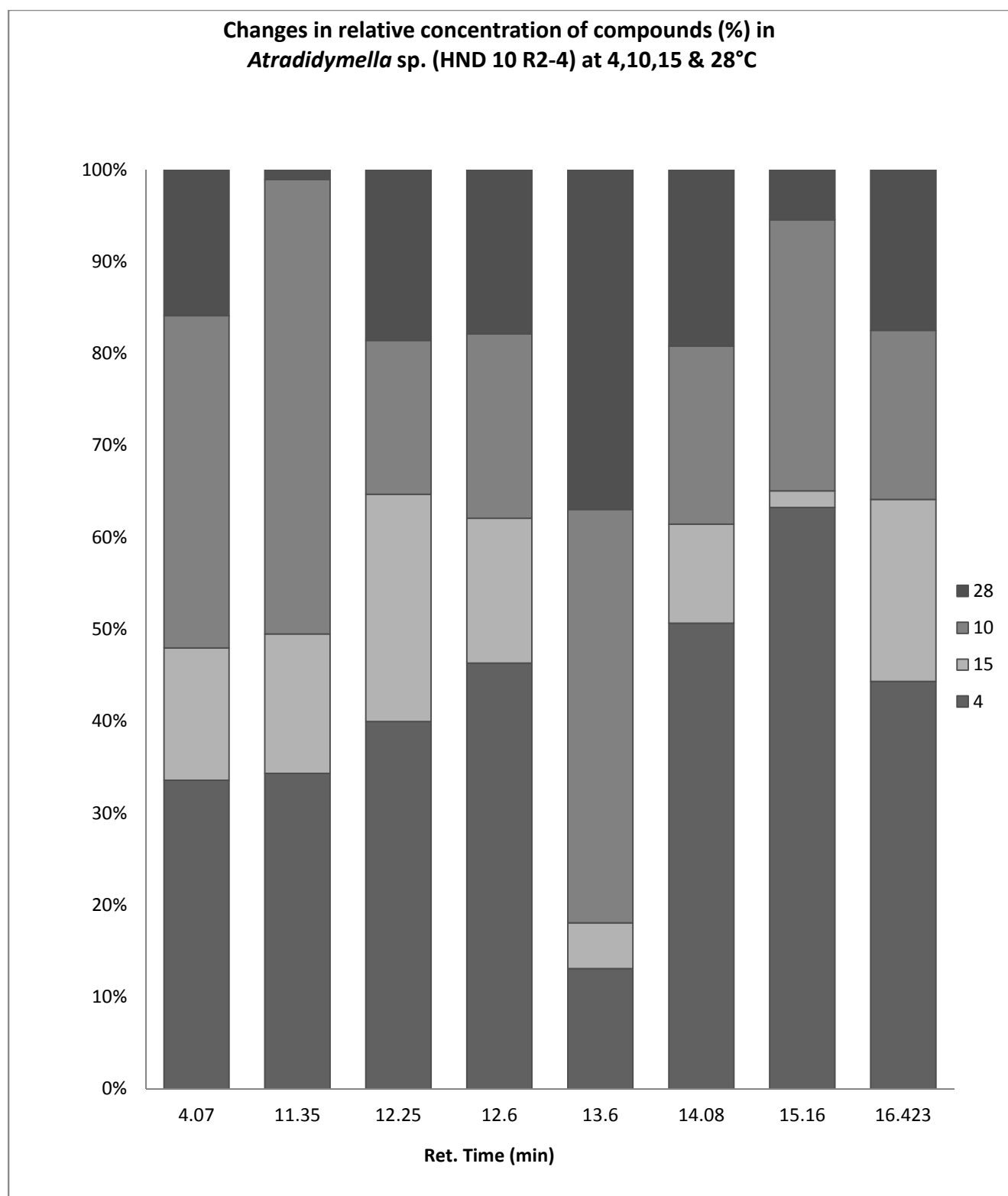


Figure 1 b. Changes in relative concentrations of major secondary metabolite peaks produced by *Pseudogymnoascus* sp. at 4, 10, 15 and 28°C.

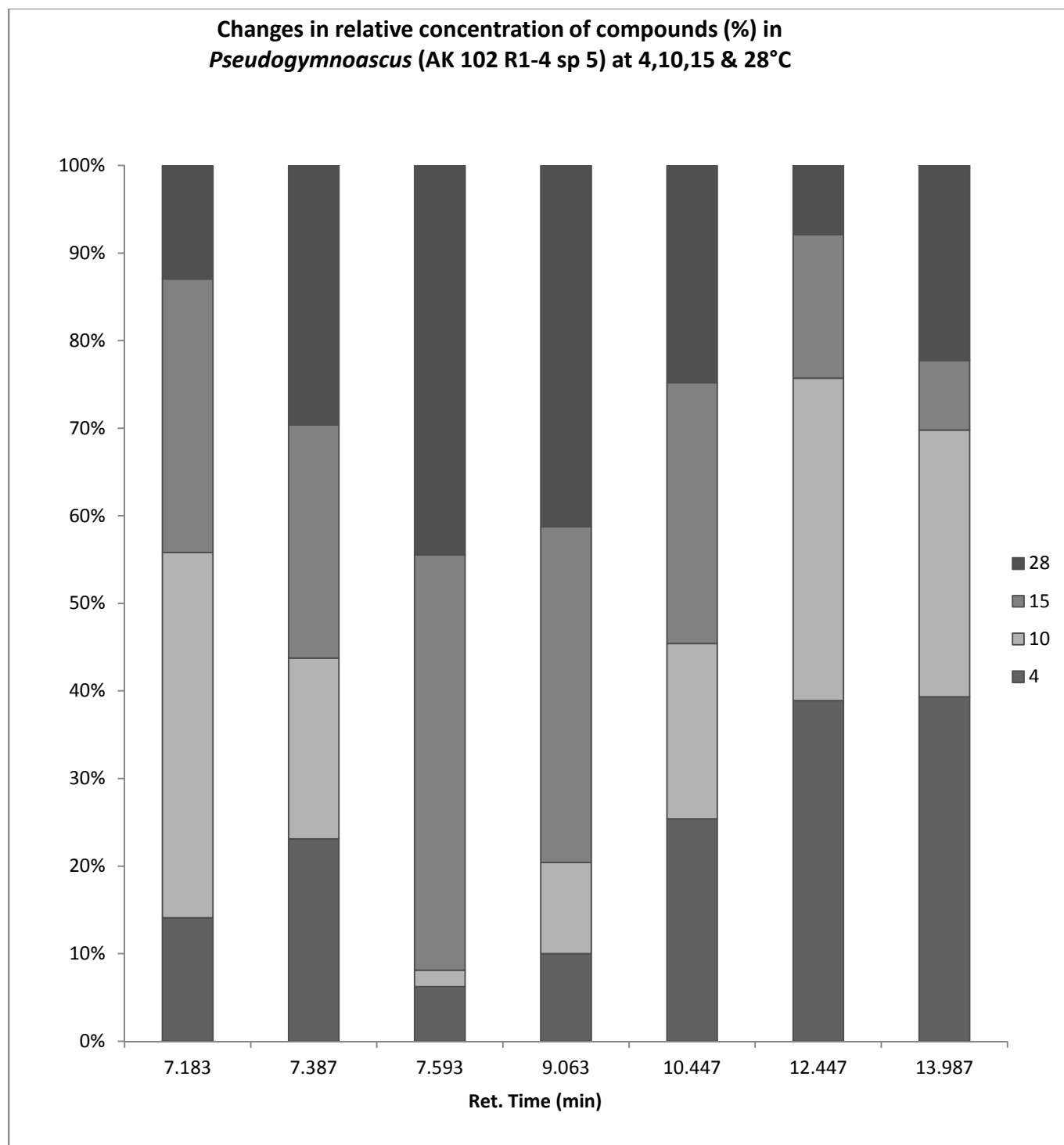


Figure 1 c. Changes in relative concentrations of major secondary metabolite peaks produced by *Penicillium flavigenum* at 4, 10, 15 and 28°C.

