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Temperature-dependent catabolic traits of cold-adapted soil yeasts *Goffeauzyma gilvescens* and *Naganishia albidosimilis* from Hornsund, Svalbard

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Running title: Catabolic diversity of Arctic soil yeast

Abstract: Studies on the diversity and catabolic functionality of fungi in Arctic soils are still scarce. This knowledge gap needs to be urgently addressed, as global warming may have unpredictable effects on cold-adapted fungal species. We isolated two yeast species of the family Filobasidiaceae: *Goffeauzyma gilvescens* and *Naganishia albidosimilis*, from soil in central Spitsbergen (Svalbard) and characterised their metabolic properties at two temperatures, 4°C and 20°C, in a laboratory test using Biolog® FF plates. The results of the Biolog® test, which followed the molecular identification of the strains, confirmed that the two species differed in their functional (catabolic) characteristics. The effect of temperature was also highly significant and the metabolic characteristics of two species varied between the two temperatures tested. As expected, among other chemical guilds of substrates on Biolog® plates, carbohydrates were the most used and also the most segregating of the strains studied, especially at 20°C. However, the relative use of almost all substrate groups changed between the two temperatures for both species tested, indicating that temperature can also affect soil microorganisms indirectly by altering their metabolic pathways. As soil microorganisms form a complex trophic and non-trophic network, such changes in metabolism under increasing temperature may also alter relationships between species and

between species and the environment. Our study contributes to a better understanding of the microbial ecology of the unique ecosystem of polar soils.

Keywords: Arctic, Wedel Jarlsberg Land, carbon utilization pattern, climate change, soil microorganisms.

Introduction

Polar regions have distinct characteristics that set them much apart from other ecosystems on our planet. These regions are characterised by simplicity in trophic networks, often consisting of a few primary producers which support relatively few consumers compared to more temperate regions (Gradinger 2009). Biodiversity in polar areas is significantly lower than anywhere else, which is a consequence of harsh environmental conditions, limited resources and dispersal limitations of many opportunistic taxa (Dziurzynski *et al.* 2023). The slow growth and development of organisms due to freezing temperatures further characterises these polar landscapes, where life persists against harsh conditions (Miller and Whyte 2011).

Microorganisms are ubiquitous in all ecosystems and that includes polar habitats. The adaptations of microorganisms reflect their importance in extreme ecosystems (Cavicchioli *et al.* 2019). In polar regions, microorganisms are highly specialized, with various adaptation strategies well developed to overcome direct and indirect life-endangering influence of low temperatures (Margesin *et al.* 2007; Kochhar *et al.* 2022). Despite our awareness of the importance of these polar microorganisms, our knowledge about their functioning remains limited (Cavicchioli *et al.* 2019). Arctic is a region of the world highly vulnerable to the progressive warming associated with global climate change (Yletyinen 2019; Rantanen *et al.* 2022). Therefore there is an urgency to study cold habitats microorganisms, especially in the face of climate change and anthropogenic disturbance at locations where they might disappear soon due to climate change (Boekhout *et al.* 2022).

Soil microorganisms are extremely diverse in their genetic and metabolic characteristics (Fierer 2017). In general, the diversity of the two major soil microbial groups, eukaryotic fungi and prokaryotes (bacteria and archaea), exhibits contrasting patterns across the latitudinal gradient. Fungal taxonomic diversity is known to decrease with increasing latitude, however their functional diversity has been shown to be the highest in tropics and also in boreal polar regions (Bahram *et al.* 2018). Arctic soils are exposed to extreme environmental conditions and have therefore developed unique microbial ecosystems, with microorganisms possessing specific adaptations that allow them to function in such a harsh climate (Robinson 2001;

Margesin *et al.* 2007; Kochhar *et al.* 2022). Sparse land in the Arctic enables soil development, where soil microorganisms play an essential role by contributing to the release of key nutrients from primary minerals and organic matter processing (Górniak *et al.* 2017).

A wide variety of species, including archaea, bacteria, yeasts, filamentous fungi, cyanobacteria, algae, and animals, are adapted to cold environments (Białkowska *et al.* 2017). Cold-adapted microorganisms can be classified into two groups based on their temperature tolerance: (*i*) psychrophiles, which can grow at temperatures up to 20°C, and (*ii*) psychrotolerants, which tolerate a broader range of temperatures, between 0°C and approximately 30°C (Morita 1975). Nevertheless, either psychrophilic or psychrotolerant fungi are characterized by physical adaptations that allow overcoming low temperatures or dryness by chlamydospores formation and mycelial thickening (Robinson 2001). The least is known about psychrotolerant yeasts despite the fact that they constitute a versatile group of eukaryotic microorganisms (Mašínová *et al.* 2018), characterized by diverse nutritional preferences and a surprising survival ability in extreme environments with widely varying physical and geochemical parameters. It has even been suggested that yeasts are better adapted to cold habitats than bacteria (Buzzini and Margesin 2014).

Yeasts have been repeatedly observed in a wide range of cold habitats, such as permafrost, snow, cold deserts, glacial ice, meltwater, sediments, deep sea, and in both frozen and refrigerated foods (Buzzini and Margesin 2014; Białkowska *et al.* 2017). To date, the greatest number of yeasts species has been isolated from soil, water, ice, and snow Arctic and Antarctic environment samples assigned to the genera *Candida* Berkhout 1923, *Dioszegia* Zsolt 1957, *Rhodotorula* F.C. Harrison 1927, *Mrakia* Y. Yamada and Komag. 1987, *Mrakiella* Margesin and Fell 2008, *Sporobolomyces* Kluyver and C.B. Niel 1924, *Glaciozyma* Turchetti, Connell, Thomas-Hall and Boekhout 2011, *Malassezia* Baill. 1889, *Saccharomyces* Meyen *ex* E.C. Hansen 1883, *Clavispora* Rodr. Mir. 1979, and *Cryptococcus* Vuill. 1901 (Białkowska *et al.* 2017). Both phenotypic and molecular methods are widely used for yeast identification. Phenotypic methods include the analysis of morphological, physiological and biochemical characteristics. On the other hand, molecular methods allow for rapid detection and identification of microorganisms, and also to establish taxonomic relationships among closely related genera and species as well as establishing taxonomic relationships between closely related genera and species (Białkowska *et al.* 2017). However, in some cases, phenotypic, culture-based approaches can yield cultures of species that were detected by direct sequencing of soil samples (Dziurzynski *et al.* 2023).

It is not clear how many yeast species occur in polar regions. In 2012, the Arctic yeast species list counted 46 species (Buzzini and Margesin 2014). Today, the yeasts.org database shows 75 polar-related yeast species, but without distinguishing between Arctic and Antarctic. It is worth noting that Arctic tundra soils represent the environment with the highest proportion of unidentified yeast species (Boekhout *et al.* 2022). Currently, research focus on cold-adapted yeasts include not only taxonomy, growth conditions, and their relationship with the abiotic environment but starts exploring their biotechnological potential, especially their enzymatic capabilities potentially valuable for industrial applications (Białkowska *et al.* 2017). Notably, lipases A and B, secreted by *Candida antarctica* (Goto, Sugiy. and Iizuka) Kurtzman, M.J. Smiley, C.J. Johnson and M.J. Hoffman 1983 (now *Moesziomyces antarcticus* (Goto, Sugiy. and Iizuka) Q.M. Wang, Begerow, F.Y. Bai and Boekhout 2015), are utilized in various biotransformations within the food, pharmaceutical, and cosmetic industries (Shivaji and Prasad 2009; Szczęsna-Antczak *et al.* 2014). Further research may mean the possibility of discovering unique microbial strains suitable for various biotechnological and medical applications. It is therefore valuable to study these organisms, even if they are common.

Yeasts are often considered as simple sugar-using organisms that are reside in soils or are only associated with nutrient-rich habitats, and the degradation of recalcitrant biopolymers is often neglected in the literature, as pointed out by Mašínová *et al.* (2018). However, a growing number of reports show that soil yeasts are characterised by broader metabolic properties (Yurkov 2018). In particular, psychrotolerant yeasts are a versatile group of eukaryotic microorganisms (Mašínová *et al.* 2018), characterized by diverse nutritional preferences and a surprising survival ability in extreme environments of widely varying physical and geochemical parameters.

The aim of this study was to identify and characterise the functional (catabolic) diversity of yeast strains isolated from soil samples collected in Spitsbergen, the largest island of the Svalbard archipelago. Traditionally, Svalbard has been treated as a natural laboratory for a wide range of cold research. Due to its specific location, about halfway between the northern coast of Norway and the North Pole, and based on many lines of evidence, global warming has led to noticeable climatic changes on Svalbard (ICPP 2022). We were particularly interested in metabolic differences between strains that could be expressed when grown at low and high temperatures (4°C and 20°C, respectively). To our knowledge, this is the first study of the metabolic characteristics of Svalbard soil yeasts using a standard tool, namely Biolog® plates. Changes in the use of carbon sources, common components of soil organic matter, by soil microorganisms with increasing temperature may lead to changes in the structure of stored soil

organic matter in polar regions, which may have far-reaching consequences for these regions in the coming global climate change.

Material and methods

Sampling. — Samples were collected during the Arctic summer of 2022, on 3rd July in central part of Spitsbergen (Svalbard). Twelve soil samples were collected in randomly selected locations in the vicinity of the Polish Polar Station Hornsund (coordinates of each sample are given in Table 1). Overall, the mean annual air temperature (MAAT) in the Hornsund area is -4.2°C and the total annual precipitation (TAP) is 450 mm (Marsz and Styszyńska 2007, 2013). Summer air temperatures range from 3°C to 7°C. Days with daily air temperatures of 8.2°C are considered extreme heat waves (Bednorz and Kolendowicz 2013). Soil samples were collected directly from the ground followed Sniegowski (2002) procedure, with and in a sterile falcon tube, up to the 10 mL and stored at a stable temperature of ~4°C for 3 weeks.

Laboratory phase. — For the direct plating method: 20 mL of water was added to each of the collected samples containing soil and vortexed at maximum speed for 1 minute. Then 1 mL of the sample was collected and plated on a Sniegowski solid plate (yeast extract 3 g, malt extract 3 g, bactopeptone 5 g, glucose 10 g, ethanol (99%) 80 mL, chloramphenicol 200 mg, HCl (1M) 1 mL, 1 l H_2O) (Sniegowski *et al.* 2002). Plates were incubated at 23°C for 3–5 days and colonies with a yeast-like shape (roundish and creamy, note: this may vary between strains) were selected. For enrichment method, equal amounts of samples (~3 g, or half the sample if a smaller amount was taken) were placed in 30 mL sterile flat bottom plastic tubes to which 20 mL of selective enrichment medium was added. The flasks were sealed tightly and incubated at 23°C without shaking. The tubes were regularly checked for signs of turbidity and gas formation (indicative of fermentation). The fermentation process was checked after 7 days and the tubes, which release fermentation gases when opened, were plated. For plating, 1 mL of liquid culture was taken if the culture showed no turbidity, otherwise 200 µL of culture was plated. Preliminary identification and selection of isolates was based on yeast-like colony morphology on Sniegowski selective media (Sniegowski *et al.* 2002). Bacterial colonies or moulds were discarded. Aqueous preparations of axenic strains were examined under a light microscope (Fig. 1). Strains were stored as glycerol stocks (1:1) at −70°C. Cultures were re-grown at 23°C on standard Yeast Peptone Dextrose (YPD) agar medium and liquid YPD medium was used for batch culture (YPD consisted of 2% dextrose, 1% yeast extract, 2% peptone, 2% agar).

Molecular identification of yeast isolates. — To confirm the purity of the culture, isolates were streaked for isolation on YPD agar plates. DNA extraction was performed by suspending a single colony in 25 µL on NaOH (30620, Sigma Aldrich, Saint-Louis, Missouri, USA) [20 mM] and incubating for 10 min at 100°C using the ProFlex PCR system (ThermoFisher, Waltham, MA, USA). The ITS region was amplified using the ITS1 forward primer 5'-TCCGTAGGTGAACCTGCGG-3' and the ITS2 reverse primer 5'-GCTGCGTTCTTCATCGATGC-3' (White *et al.* 1990). The amplification reaction was carried out under the following conditions A total volume of 25 µL was prepared with 2 µL DNA template, 7 µL distilled water, 6 µL nucleotide mix [1 mM], 5 µL 5× colourless GoTaq® Flexi Buffer (M8901, Promega, Madison, USA), 3 µL magnesium chloride solution, 1 µL of each primer [10 pmol/ml], 0.62 U Taq polymerase (M7808, Promega, Madison, USA). Using the ProFlex PCR system (ThermoFisher), the PCR mix was subjected to an initial denaturation cycle of 2 min at 95°C, followed by 35 cycles of 45 sec at 95°C, 45 sec at 57°C and 1 min at 72°C, with a final extension step of 5 min at 72°C. 2% TAE 1× agarose gel electrophoresis was performed on a PowerPac TM Basic (Bio-Rad) using a 3 µL aliquot of PCR product (30 min, 100 V). The gel was stained in an ethidium bromide bath for 30 minutes before imaging using Image Lab 3.0 software and the Gel Doc XR+ machine (Bio-Rad). ITS-PCR positive strains were sent to Eurofins Genomics for sequencing. Obtained sequences were submitted to GenBank under accession nos. PP976923-PP976927 The National Centre for Biotechnology Information (NCBI) BLASTN suite was used to identify the isolates. Sequences were compared using default settings.

Phylogenetics analysis. — Sequences were blasted against database of internal transcribed spacer region (ITS) from Fungi type and reference material (RefSeq, update date: 2024/02/14) using NCBI BLASTN algorithm (Zhang *et al.* 2000). In total, we extracted 21 hits with query coverage and percent identity higher than 80%. The highest e-value was equal to 5×10^{-38}. Next, all sequences were aligned using the ClustalW algorithm implemented in MEGA 7 software (Kumar *et al.* 2016) with default parameter settings. Maximum likelihood molecular phylogenetic analysis was performed in MEGA 7, based on the Tamura-Nei model (Tamura and Nei 1993), with 1000 bootstrap replicates. The analysis included 26 nucleotide sequences. A total of 157 positions were included in the final data set. All positions with gaps and missing data were eliminated.

The catabolic activity and functional diversity. — The catabolic activity and functional diversity of isolated yeasts were analysed using a commonly used method, Biolog® plates (Garland and Mills 1991; Stefanowicz 2006; Song *et al.* 2023). Biolog® FF plates, used

for the analysis of fungi, are prefabricated multiwell plates containing 95 carbon-only substrates and tetrazolium dye as a substrate utilisation indicator (http://www.biolog.com). Axenic cultures of yeast were first grown on YPD medium at 4°C and 20°C in the dark in 20 ml tubes for 5–10 days. The medium was then removed by several centrifugations with sterile 0.9% NaCl solution, retaining the pelleted yeast. The yeast pellet was then resuspended in sterile 0.9% NaCl solution. The OD of each suspension of yeast isolates was checked at 600 nm and adjusted to a range of 0.1 to 0.15. The Biolog® FF plates were then inoculated under a laminar flow chamber with the yeast isolates in 0.9% NaCl solution (100 µL per well) using a multichannel pipette and the plates were incubated at 4°C and 20°C (two replicates for each yeast isolate and temperature equals 24 plates used in the experiment). Substrate utilisation was measured as absorbance at 490 nm (µQuant spectrometer; BIO-TEK Instruments). The first measurement was made immediately after inoculation, and subsequent measurements were made at 24-hour intervals for 10 days. The absorbance value for each substrate was corrected by subtracting the value for the control well (position on plate: A1), which contained no substrate but only the yeast suspension. Absorbance changes below 0.06 (spectrometer detection limit) were considered as 0.

The general metabolic (catabolic) activity of the yeast isolates was calculated as the Area Under the Curve (AUC) using the following formula:

$$AUC = \sum_{i=1}^{N} \sum_{t=1}^{n-1} \left(\frac{A_n + A_{n+1}}{2} \right) \times \left(t_{n+1} - t_n \right)$$

where A_n and A_{n+1} are the absorbance of each individual substrate (well) in two consecutive measurements at times t_n and t_{n+1}; n represents specific measurements (scorings), and N represents the number of substrates on the plate (95). Substrate richness, *R* was simply calculated as the number of substrates used (s). The functional diversity index *H*', was derived from the Shannon-Wiener biodiversity index, which is based on the structure of substrate use:

$$H' = - \sum_{i=1}^{s} p_s \left(\log_{10} p_s \right)$$

was derived from the number of substrates degraded by yeast, and the proportion use of an individual substrate p_s was calculated as the absorbance for a given substrate (well) divided by the sum of absorbance for all substrates (wells). R_s and *H*' were compared to the same sample Average Well Color Development (AWCD) value of 0.2, calculated as the mean well absorbance, regardless of incubation time (Preston-Mafham *et al.* 2002). The absorbance values for individual substrates were standardised to 1 for each sample and these data were used to express strains' physiological profiles. In addition, carbon substrates were grouped into eight

substrate guilds: amines and amides, amino acids, carbohydrates, carboxylic acids, glycosides, polymers, polyols, and miscellaneous (Dobranic and Zak 1999).

Extracellular enzyme activities of isolated yeast strains. — Solid agar media (YPD and YPG) supplemented with a substrate (1–3) were spot inoculated with 2 µL of a standardised suspension of yeast cells. 1. Amylolytic activity; after eleven days of incubation, agar plates (YP) supplemented with 2% soluble starch were flooded with iodine solution. The amylolytic activity was estimated from the colouring of the medium to dark purple and the observation of clear zones around the colonies. 2. Pectinolytic activity; after 11 days of incubation, agar plates (YP) containing 2% apple pectin were flooded with 1% hexadecyltrimethylammonium bromide solution (Sigma-Aldrich, USA). Pectinolytic activity was indicated by a clear zone around the colonies. 3. Proteolytic activity: Agar plates (YPG) were supplemented with 2% skimmed milk and proteolytic activity was indicated by a clear halo around the colonies. Colonies on plates supplemented with the above substrates were measured. Feret diameters were obtained using ImageJ software (Schneider *et al.* 2012) from scans taken with a flatbed scanner at 300 dpi.

Statistical analysis. — The Biolog® test results were compared to identify the preferred carbon substrate utilisation pattern at the two temperatures. Two-way ANOVA and Tukey's post-hoc tests ($p<0.05$) were used to analyse differences between yeast strains and temperatures in AUC, R, H' and relative use of carbon substrates from different guilds. Clustering method, a method based on single linkage with Bray-Curtis distance, was used to detect similarity patterns between isolated strains, separately for carbon guilds and physiological profiles (individual substrates). The SIMPER (similarity percentage) method was used to determine which individual carbon substrates contributed most to the average dissimilarity between strains, separately at 4°C and 20°C. ANOVA analyses were performed using Statgraphics 18 (StatPoint Technologies Inc., Warrenton VA, USA) and other analyses with PAST4-Project software (Natural History Museum, University of Oslo, Norway).

Results

Two yeast species were successfully isolated. — Five yeast cultures were isolated from all collected soil samples, and were successfully identified. Clones 3.1, 5.2, 11.1, 11.2 are *Goffeauzyma gilvescens* (Chernov and Babeva) Xin Zhan Liu, F.Y. Bai, M. Groenew. and Boekhout 2015. Only one clone, 3.1 was isolated by the direct plating method. Clones 5.2, 11.1, 11.2 were isolated after seven days of incubation in the enrichment medium. Clone 5.1, isolated after seven days of incubation, matched *Naganishia albidosimilis* (Vishniac and Kurtzman) Xin

Zhan Liu, F.Y. Bai, M. Groenew. and Boekhout 2015. All isolated strains matched the NCBI sequences with 99–100% identity and 96–97% query coverage.

Isolated strains cluster with other closely related species. — The tree with the highest log likelihood (−651.63) is shown in Fig. S1. All our *G. gilvescens* sequences form a monophyletic group. The *G. gilvescens* sequence obtained from NCBI clusters together with *G. gastrica* (Reiersöl and Di Menna) Xin Zhan Liu, F. Y. Bai, M. Groenew. and Boekhout 2015 which are both basal to our *G. gilvescens* sequences. The *N. albidosimilis* sequence clustered together with *N. albidosimilis* and *N. liquefaciens* (Saito and M. Ota) Xin Zhan Liu, F.Y. Bai, M. Groenew. and Boekhout 2015 obtained from NCBI.

Effect of two temperatures on yeast isolates metabolic properties. — The activity index AUC values for yeast strains grown on Biolog® FF plates ranged from 124.9 to 313.5. The number of substrates R used by the yeast ranged from 60 to 87 (from all 95 substrates). The Shannon-Wiener Index of functional diversity H' ranged from 1.62 to 1.87. General activity and functional (catabolic) diversity indices differed significantly between temperatures and yeast isolates (both $p<0.05$) (Table 2). The strain *G. gilvescens* 3.1 was characterised by the highest AUC, R and H' values, whereas strain 5.2 showed the lowest values of these indices (Table 2). For each strain, AUC, R and H' were higher when plates were incubated at 20°C than at 4°C (Table 2), as there were no significant interactions between these two factors.

Substrate utilization varied between temperatures and strains, with carbohydrates most commonly utilized, while amides and polymers were least used. — Among the other chemical groups of substrates, carbohydrates and, to a lesser extent, carboxylic acids were used most frequently (55.7% and 15.0% on average, respectively; Fig. 2). Amides and polymers were the least used (1.3% and 1.7% on average, respectively; Fig. 3). According to the substrate usage pattern, *N. albidosimilis* was most distinct from all *G. gilvescens* strains tested at 4°C (Fig. 3), whereas at 20°C strains 5.1 and 3.1 were distant from the others (Fig. 3). The most similar strains were 11.1 and 11.2, isolated from the same site (Fig. 2). Yeast strains differed in their use of substrate chemical groups between temperatures (for each strain and substrate group $p < 0.05$). There were also significant interactions between these two factors, indicating that the strains differed in their physiological profiles between these two temperatures, as shown in Fig. 4 A–H. In particular, *G. gilvescens* strain 3.1 and *N. albidosimilis* strain 5.1 used relatively more amines and amino acids instead of carbohydrates at 20°C than at 4°C (Fig. 3. A–C). SIMPER analysis revealed that for individual carbon substrates, the largest contribution to the average dissimilarity in physiological profiles between strains at both 4°C and 20°C was from carbohydrate utilisation (Table 2). At 4°C, strains *N. albidosimilis* 5.1 and *G. gilvescens* 5.2 did

not degrade stachyose and D-raffinose and strains *G. gilvescens* 11.1 and 11.2 did not degrade gentiobiose (Table 3). At 20°C, all five of the most dissimilar substrates were degraded, but to different extents and their use was characterised by a higher variability (Table 3).

Isolated yeast strains grew on various substrates with better growth rates observed at 20 °C compared to 4°C, although no substrate clearing was observed. — All the isolated strains studied showed the ability to grow on pectin, soluble starch and skimmed milk enriched media. However, no substrate clearing (halo around colonies) was observed in any of the experimental plates. Colonies showed better growth rates at 20°C on all substrates tested - from 32 to 100% increase compared to colonies incubated at 4°C (Fig. 4). A more robust appearance was also observed at 20°C, whereas at 4°C they were smaller and flatter (data not shown).

Discussion

We isolated two closely related yeast species, *N. albidosimilis* and *G. gilvescens*. *Goffeauzyma. gilvescens* (formerly known as *C. gilvescens*) was identified as psychrophilic and found to be prevalent across all sampling sites (Chernov 1988). Like most *Cryptococcus* spp., *G. gilvescens* produces polysaccharide capsules and is commonly associated with Arctic environments (Ali *et al.* 2013; Białkowska *et al.* 2017; Ogórek *et al.* 2022).

Psychrotolerant *N. albidosimilis*, initially described in 1992 as *C. albidosimilis*, was isolated alongside *C. antarcticus* from soil samples in the Linnaeus Terrace of Antarctica (Vishniac and Kurtzman 1992). It has since been frequently reported in Arctic regions, such as soil samples from East Ongul Island in East Antarctica (Tsuji 2018) or as *C. albidosimilis* in ice core samples from the Midre Lovénbreen glacier in Svalbard (Singh *et al.* 2013). *Naganishia albidosimilis* shows promise as a producer of enzymes such as amylase or other starch, xylose, or cellobiose degrading enzymes (Vishniac and Kurtzman 1992; Białkowska *et al.* 2017).

The molecular data, coupled with metabolic profiles, solidified the distinction between the isolated strains. Within *G. gilvescens* strains, we discerned intraspecific diversification in catabolic preferences. Notably, strain 3.1 exhibited higher AUC, R, and H' values compared to its counterparts, underscoring its metabolic prowess. The substantial utilization of substrates by individual strains, averaging 76.6, further underscores the extensive metabolic potential of Arctic yeast. A high R value denote considerable repetition of degraded substrates across strains.

Our findings indicated a higher R value at 20°C compared to 4°C, hinting at a possibility of broader range of metabolizable substrates in natural environments. This elevated R value

likely correlates with increased metabolic activity, which saw an average 40% uptake between the tested temperatures. Such a temperature-induced boost in the catabolic potential of soil microorganisms could significantly impact decomposition rates in Arctic soils, potentially accelerating soil carbon loss in this climatically critical warming era. These may denote changes in soil organic matter structure stored in soils, as well as nutrients availability for plants and other far-reaching environmental consequences.

The modest 40% increase in AUC from 4°C to 20°C suggests the presence of potential limiting factors for yeast growth on Biolog® plates. Typically, for most soils, the rate of soil respiration rate doubles or more for each 10°C increase in temperature, with a more pronounced effect in colder regions of the world (Waldrop *et al.* 2010). Obviously, the metabolic response to temperature increase may vary between single-species laboratory isolates and soil microorganisms in their native environment. Complex trophic and non-trophic interactions within multi-species soil communities, alongside interactions between organisms and their environment, collectively shape the ultimate temperature response, reflected in emitted CO_2 levels.

It's widely acknowledged that unicellular yeasts, akin to bacteria, can only assimilate substrates within their immediate vicinity. Their ability to retain substrate degradation products for their own cells is facilitated by extracellular enzymes bound to their cell surfaces. Carbohydrates were used to a greater extent by all yeast strains, which is a common observation for soil fungi (Mašínová *et al.* 2018). Both carbohydrates and carboxylic acids are important components of soil organic matter, which is reflected in their high proportion among different carbon compounds on Biolog® plates. Although the carbon sources in these plates may not represent the dominant carbon sources found in specific locations, the oxidation of these substrates can serve as a proxy for understanding changes in utilisation patterns under different environmental conditions in soils.

Earlier observations have shown that common soil yeast species possess the capability to utilize L-arabinose, D-xylose, and cellobiose, carbohydrates derived from hydrolytic enzymes acting on lignocellulosic plant material (Botha 2011). These carbohydrates were consistently utilized across all yeast strains in our study. Interesting observation was that at 4°C strains 5.1 and 5.2 do not utilize stachyose and D-raffinose, both being a simple oligosaccharides, common components of plant tissues. However, strain 5.2 used D-raffinose in a very limited range at 20°C.

Relative increase in amino acids and amines use at 20°C than at 4°C denote increased microbial demand on nutrients, namely nitrogen, which is one of the most important element

for living organisms, which may strongly influence partitioning of assimilated carbon between respiration and growth and thus defines the soil-atmosphere C balance (Bruggeman *et al.* 2020; Cruz-Paredes and Rousk 2024). Some amino acids are essential and provide the building blocks for proteins (Kędra *et al.* 2021), however some amino acids are known to cause toxicity to yeast via specific mechanisms (Ruiz *et al.* 2020). Constraints in protein concentration limits strongly organisms growth rate, and polar soils are known to strongly nutrient limited (Poppeliers *et al.* 2022).

We found also that a greater proportion of metabolically resistant substrates, that is, polymers, were used by all the yeast strains tested at 20°C than at 4°C. Only three compounds represent polymers on Biolog® FF plates, and glycogen is especially interesting one, as this multibranched polysaccharide of glucose serves as a form of energy storage in living organisms (Wilson *et al.* 2010). In yeast, significant quantities of glycogen are synthesized and degraded as diploid yeast cells undergo the sporulation process, and nonsporulating cells exhibited little or no glycogen catabolism (Colonna and Magee 1978). The increase in glycogen consumption at 20°C compared to 4°C may be related to intense cell proliferation. However, neither the number of yeast cells nor their physiological state (cells in active or resting state) was measured, as the endpoint of the Biolog® method is only the colour change of the dye, which can be driven by a variety of microbial population characteristics that may differ in each individual well (substrate) on the plate.

In general, the ability to switch between metabolic pathways to maximise the use of resources by a microbial cell in a given environment is an adaptive trait to specific, harsh climatic conditions that maximises its fitness. Temperature is a critical environmental feature for organisms living in polar ecosystems, and it also manifests seasonality (Poppeliers *et al.* 2022). It can be therefore expected that for a given cosmopolitan soil microbial species, the metabolic response to temperature increase will be greater in polar ecosystems than in warmer, *i.e.*, temperate, ecosystems. Taking this speculation further, in the long term, as the climate warms in a given region of the Earth, a reduction in the temperature responsiveness of microorganisms can be expected.

Understanding the prevalence and functionality of microfungi in polar ecosystems remains limited, hence the identification and characterization of yeasts in Arctic soil samples yield valuable insights. Such information not only enriches our fundamental understanding but also holds significant importance in the context of ongoing climate and anthropogenic changes. There is an urgent need to study microorganisms in cold habitats, especially in areas where they may soon disappear due to man-made environment transformations (Yurkov 2018; Owczarek

et al. 2020; Boekhout *et al.* 2022). With the Arctic undergoing unprecedented habitat alterations due to temperature rise and human activities, monitoring microfungal communities becomes crucial for comprehending ecosystem dynamics both presently and in the future.

Conclusions

The isolated members of the Filobasidiaceae family, specifically *Goffeauzyma gilvescens* and *Naganishia albidosimilis*, exhibited distinct metabolic profiles, and temperature significantly influenced their substrate utilization patterns. Changes in metabolic profiles under temperature were strain dependent, but in general yeasts tended to use more substrates and a greater proportion of metabolically resistive substrates at the expense of carbohydrates at 20°C than at 4°C, although carbohydrates remained the most commonly used substrates. Changes in metabolic properties confirm the trophic elasticity of cold-adapted Arctic soil fungi, and suggest possible implications for soil organic matter decomposition processes under climate warming.

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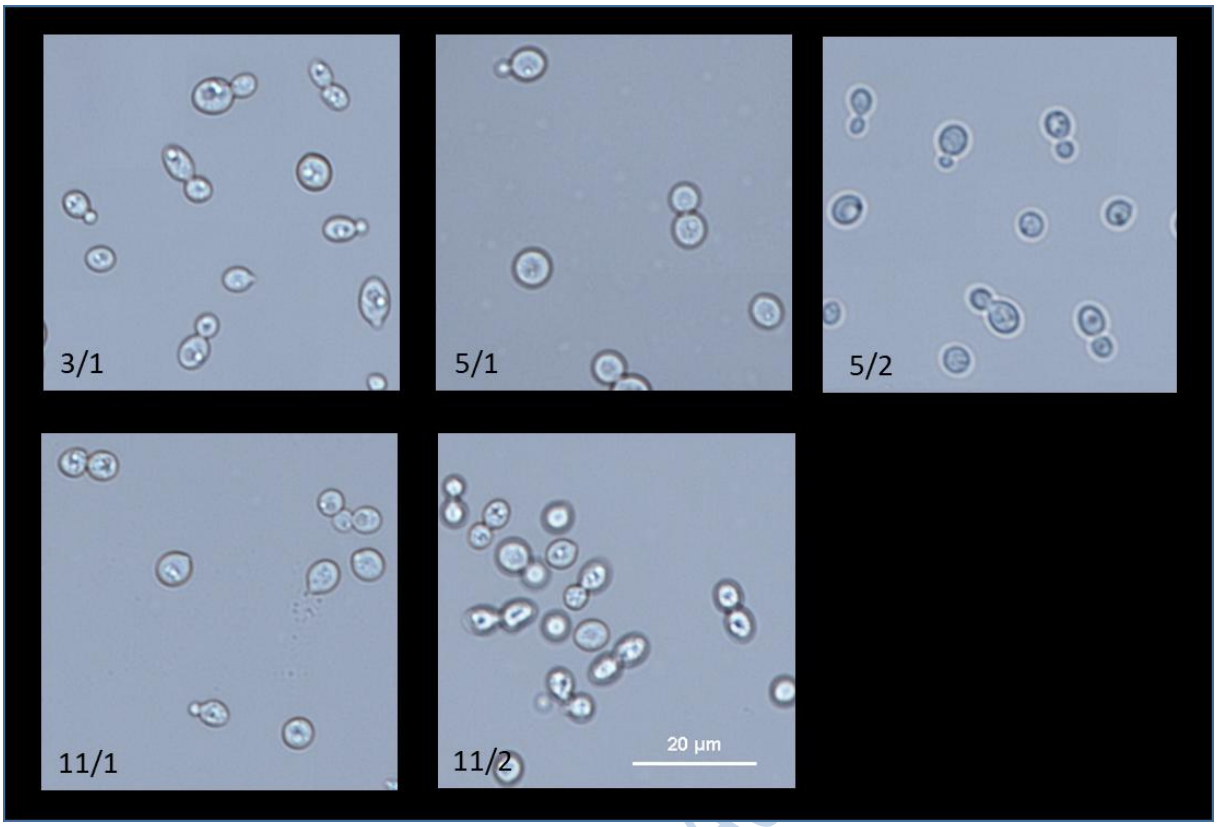

Fig. 1. Morphology of isolated yeast. Images were obtained with the usage of Nikon Eclipse 80i equipped with Nikon DS Ri2 camera. The same scale implemented with NIS elements image analysis software was used for all images.

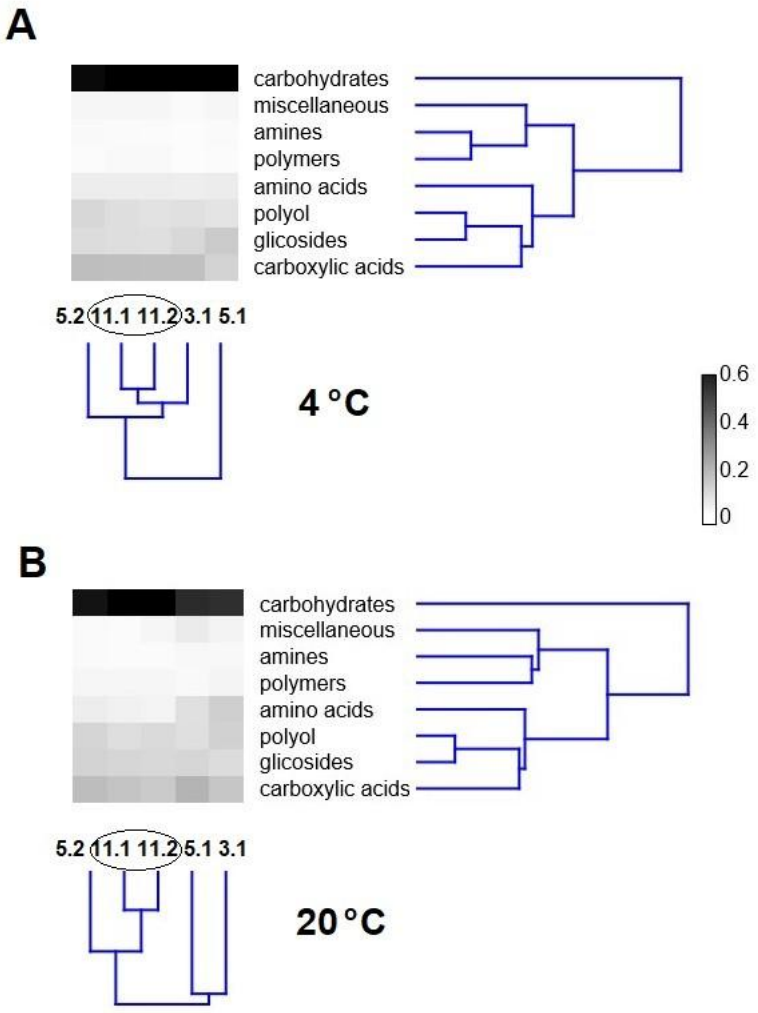

Fig. 2. Two-way clustering (single linkage algorithm, Bray-Curtis similarity index) for general metabolic preferences of isolated yeast strains (**A**) at 4 °C and (**B**) at 20 °C. Colour saturation indicates the relative proportions of substrates used from a given chemical guild. Two strains the most similar to each other, 11.1 and 11.2, were circled.

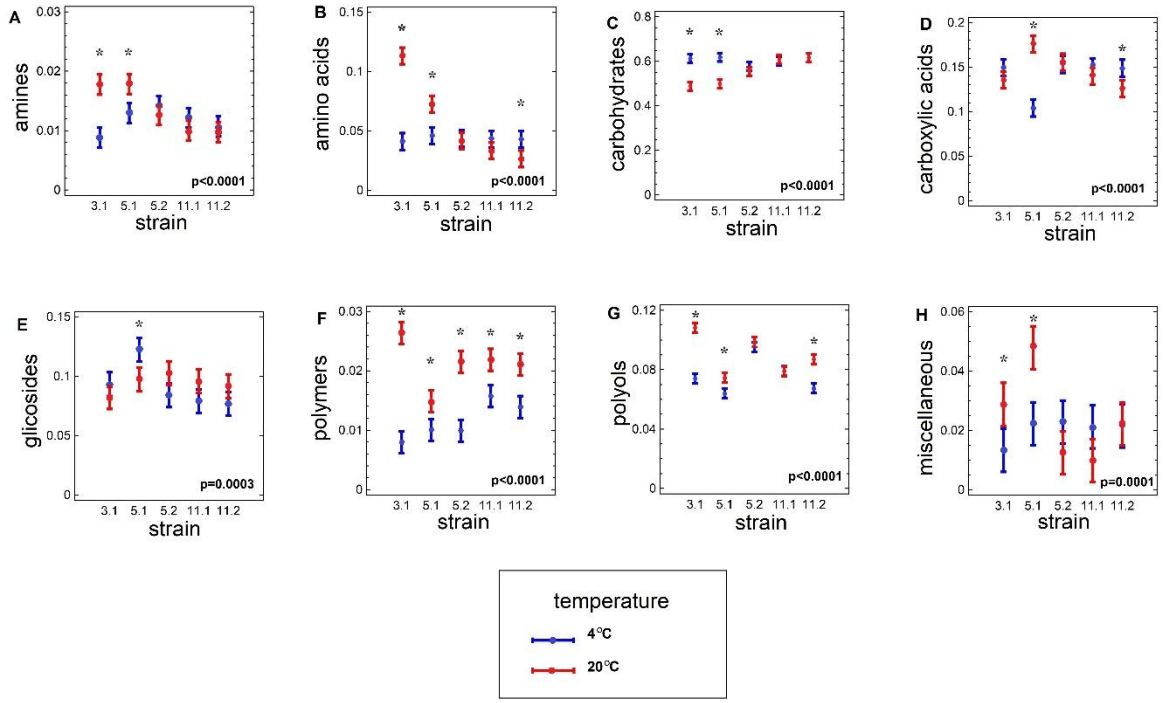

Fig. 3. Differences in substrate groups use by yeast strains (interaction plots for two-way ANOVA): **A**) amines, **B**) amino acids, **C**) carbohydrates, **D**) carboxylic acids, **E**) glicosides, **F**) polymers, **G**) polyols, **H**) miscellaneous. Blue lines present relative substrate usage at temperature 4°C and red lines present it for 20°C at Biolog® FF plates. The central point indicates the mean of the data, and the bars indicate 95% Tukey confidence intervals. An asterisk indicates statistically significant differences between temperatures for a given strain.

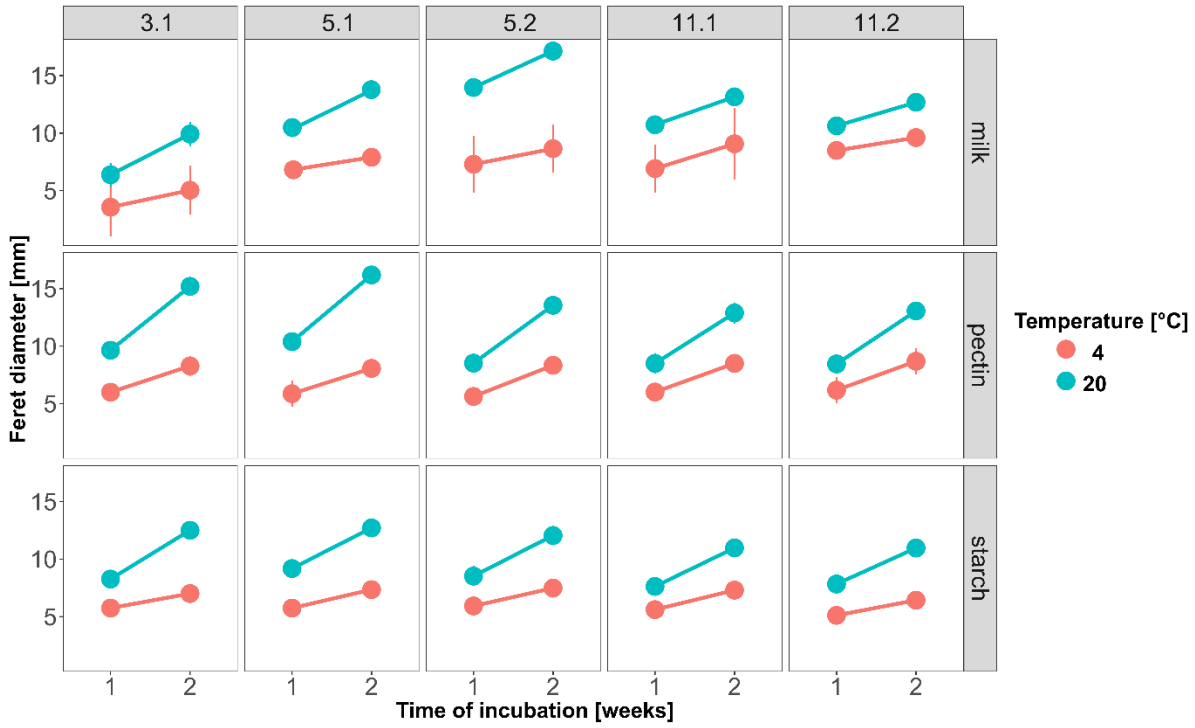

Fig. 4. Colony growth of the investigated strains on media amended with skim milk, pectin, and starch at two temperature regimes. Points indicate means of Feret diameters measured during two weeks of the experiment; vertical lines indicate standard deviations.

Table 1.

Location of the soil sampling sites. Norwegian Polar Institute digital elevation model with 5 meters resolution was used to obtain information on the altitude above the sea level (2014, https://doi.org/10.21334/npolar.2014.dce53a47).

No.	Latitude [°N]	Longitude [°E]	Altitude [m]	Clone	Species name
1	77.01996	15.58831	114	N/A	N/A
2	77.01996	15.58831	114	N/A	N/A
3	77.01996	15.58831	114	3.1	*Goffeauzyma gilvescens*
4	77.0065	15.3660	47	N/A	N/A
5	77.00242	15.36259	10	5.1	*Naganishia albidosimilis*
5	77.00242	15.36259	10	5.2	*Goffeauzyma gilvescens*
6	77.00325	15.36279	10	N/A	N/A
7	77.0070	15.3659	84	N/A	N/A
8	77.0043	15.3632	13	N/A	N/A
9	77.0056	15.3647	21	N/A	N/A
10	77.0165	15.4640	174	N/A	N/A
11	77.0165	15.44802	63	11.1	*Goffeauzyma gilvescens*
11	77.0165	15.44802	63	11.2	*Goffeauzyma gilvescens*
12	77.00531	15.48923	24	N/A	N/A

Table 2.

General activity (AUC) and functional diversity indices (R_s - substrate richness, and H' - Shannon-Wiener diversity index) for soil yeast strains grown at temperature 4°C and 20°C at Biolog® FF plates. Mean values (±SD, n=2) for individual parameters and results of two-way ANOVA test with corresponding P values at $p \leq 0.05$ are shown in the bottom of table. Different small letters (a, b, c) indicate statistically significant differences between strains, different large letters (A, B) indicate statistically significant differences between temperatures.

Temperature	Strain	Index		
		AUC	R	H'
4°C	3.1	219.6 (7.3) Ab	78.5 (0.7) Ab	1.74 (0.01) Ac
	5.1	194.3 (14.5) Aab	77.0 (8.5) Aab	1.70 (0.02) Ab
	5.2	143.0 (7.2) Aa	65.5 (7.8) Aa	1.65 (0.04) Aa
	11.1	144.9 (1.0) Aa	70.0 (4.2) Aab	1.70 (0.02) Aab
	11.2	164.1 (0.8) Aa	71.0 (1.4) Aab	1.68 (0.00) Aab
20°C	3.1	269.4 (62.4) Bb	85.0 (2.8) Bb	1.87 (0.01) Bc
	5.1	232.0 (0.4) Bab	82.5 (0.7) Bab	1.80 (0.01) Bb
	5.2	226.0 (8.4) Ba	77.5 (7.8) Ba	1.74 (0.02) Ba
	11.1	224.5 (4.7) Ba	77.0 (0.0) Bab	1.73 (0.00) Bab
	11.2	228.5 (2.4) Ba	81.0 (0.0) Bab	1.75 (0.00) Bab
ANOVA main effects				
Temperature		0.0046	0.0222	0.0001
Strain		<0.0001	<0.0001	<0.0001

Table 3.

Selected substrates use by soil yeast strains grown at temperature: 4°C and 20°C as measured with Biolog® FF plates. For the SIMPER analysis of community-level physiological profiles (CLPPs) in the soil yeast strains, only the first five substrates with the highest average dissimilarity and contribution were presented. Mean values (±SD, n=2) and corresponding P values of one-way ANOVA test are shown. More data can be found in Supplementary Material.

Temperature	Substrate	Substrate use [%]						SIMPER analysis parameters	
		P value	3.1	5.1	5.2	11.1	11.2	Average dissimilarity	Contribution (%)
4°C	Stachyose	< 0.0001	2.7 (0.0)	0.0 (0.0)	0.0 (0.0)	2.7 (0.2)	3.0 (0.2)	0.88	4.45
	D-Raffinose	< 0.0001	2.3 (0.1)	0.0 (0.0)	0.0 (0.0)	2.6 (0.1)	2.9 (0.1)	0.83	4.20
	Gentiobiose	< 0.0001	1.4 (0.0)	3.3 (0.2)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.80	4.07
	D-Galactose	< 0.0001	2.7 (0.0)	0.6 (0.1)	3.7 (0.1)	3.2 (0.0)	3.3 (0.1)	0.68	3.46
	Sucrose	< 0.0001	3.2 (0.0)	2.9 (0.0)	0.2 (0.2)	3.1 (0.3)	3.3 (0.0)	0.65	3.31
20°C	Sucrose	0.0004	1.6 (0.5)	2.0 (0.2)	0.1 (0.1)	3.6 (0.5)	3.9 (0.0)	0.97	4.04
	2-Keto-D-Gluconic Acid	0.0403	1.6 (0.1)	2.0 (0.3)	3.7 (2.0)	2.3 (0.1)	4.8 (0.4)	0.83	3.47
	D-Galactose	0.0001	1.6 (0.1)	0.8 (0.0)	2.6 (0.1)	3.5 (0.2)	3.8 (0.4)	0.80	3.31
	D-Mannose	< 0.0001	1.5 (0.1)	1.8 (0.2)	3.7 (0.3)	4.2 (0.1)	4.2 (0.1)	0.78	3.23
	D-Raffinose	< 0.0001	1.1 (0.0)	0.0 (0.0)	0.1 (0.1)	2.8 (0.2)	2.4 (0.1)	0.77	3.22

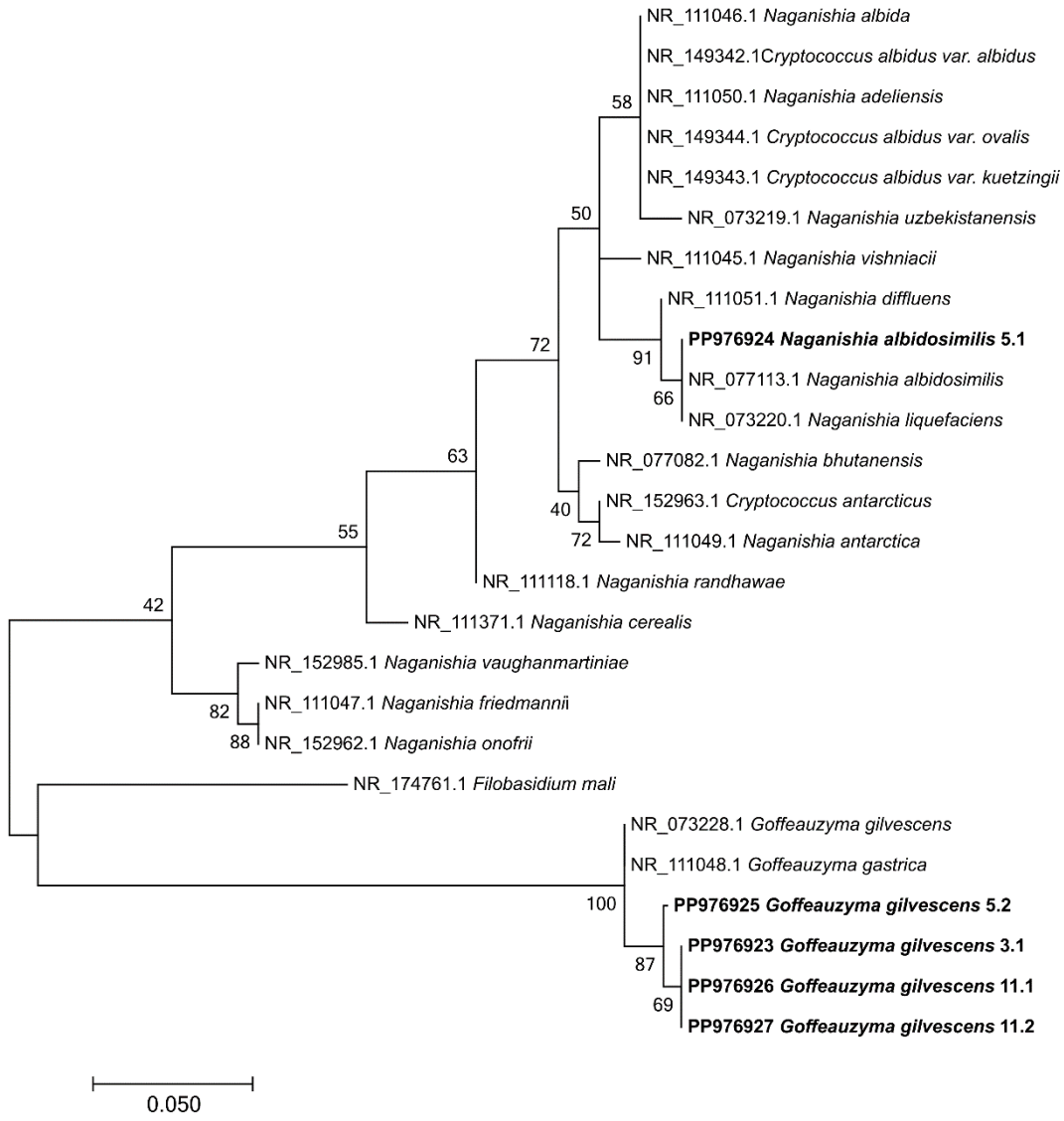

Fig. S1. Phylogenetic relationships between taxa inferred by Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequence captions begin with the GenBank accession numbers. Sequences obtained in this study are given in bold.