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Antarctic Spore-Forming Microorganisms from Deception Island Inhibit the Growth of Various Bacterial Strains

(Mikroorganisma Pembentuk Spora Antartika dari Pulau Deception Merencat Pertumbuhan Pelbagai Strain Bakteria)

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ABSTRACT

Antarctic microbes have evolved and adapted unique strategies to survive in the harsh polar environment. Apart from the ability to adapt to the low nutrient soil content and extremely dry and cold polar environment, a particular strategy used by Antarctic bacteria is the production of antimicrobial compounds that can eliminate rivals in the same niche, giving them a competitive edge over other microbes. In contrast, it is unclear whether spore-forming microbes possess similar antimicrobial properties as one of their survival strategies, especially those from the Antarctic volcanic Deception island in the West Antarctic. Hence, this study aims to isolate and characterize the spore-forming microbes in Deception Island, Antarctica, as well as to identify the ones that are equipped with the ability to inhibit other microorganisms. Microbes were isolated using various growth media and were segregated into clusters based on their random amplified polymorphic DNA (RAPD) fingerprints. A total of 90 strains were isolated and clustered into 30 groups at a similarity of 60%. Representative strains from each cluster were assayed for antimicrobial activities against 13 Gram-positive and Gram-negative test bacteria comprising human pathogens. Twenty-five strains exhibited the ability to inhibit at least one test bacterium. The four strains, A60, Im31, Im32 and Im33 that showed the strongest inhibitory activities were subjected to 16S or 18S rDNA sequencing and analysis to identify them. They were identified as *Pseudogymnoascus, Bacillus, Leohumicola,* and *Talaromyces* spp. The ability of the aforementioned microbes to thrive in harsh environments and compete with fierce competitors for scarce nutrients is probably due to their ability to produce antimicrobial compounds that target and kill their rivals.

Keywords: *Bacillus; Leohumicola*; maritime Antarctic; *Pseudogymnoascus; Talaromyces*

ABSTRAK

Mikrob Antartika telah melalui evolusi dan dilengkapi dengan beberapa strategi adaptasi yang unik untuk bertahan hidup dalam persekitaran kutub yang amat mencabar. Selain daripada keupayaan untuk menyesuaikan diri dengan persekitaran kutub yang mempunyai kandungan nutrien tanah yang rendah, persekitaran yang kering dan sejuk melampau, satu strategi yang digunakan oleh bakteria Antartika ialah dengan penghasilan sebatian antimikrob yang boleh menghapuskan saingan dalam nic yang sama, memberikan mereka kelebihan daya saing berbanding mikrob lain. Namun begitu, tidak jelas sama ada mikrob pembentuk spora mempunyai sifat penghasilan antimikrob yang sama sebagai salah satu strategi kemandirian hidup mereka terutamanya di pulau gunung berapi Deception di Antartika Barat. Oleh itu, kajian ini bertujuan untuk memencilkan dan mencirikan mikrob pembentuk spora dari Pulau Deception, Antartika serta mengenal pasti mikrob yang dilengkapi dengan keupayaan untuk merencat mikroorganisma lain. Mikrob telah dipencilkan menggunakan pelbagai media pertumbuhan dan diasingkan ke dalam kelompok berdasarkan cap jari DNA polimorfik yang diamplifikasi secara rawak (RAPD). Sebanyak 90 strain telah

diasingkan dan dikelompokkan kepada 30 kumpulan dengan persamaan 60%. Strain perwakilan daripada setiap kluster telah disaring bagi aktiviti antimikrob terhadap 13 patogen Gram-positif dan Gram-negatif. Dua puluh lima strain menunjukkan keupayaan untuk merencat sekurang-kurangnya satu bakteria ujian. Empat strain, A60, Im31, Im32 dan Im33 yang menunjukkan aktiviti perencatan paling ketara telah dikenal pasti identiti mereka melalui proses penjujukan dan analisis rDNA 16S atau 18S mereka. Mereka adalah *Pseudogymnoascus, Bacillus, Leohumicola* dan *Talaromyces* spp. Keupayaan mikrob tersebut untuk hidup dengan berjaya dalam persekitaran yang mencabar dan bersaing dengan sengit dengan pencabar untuk mendapatkan nutrien yang terhad mungkin disebabkan oleh sebatian antimikrob yang dihasilkan oleh mereka untuk menyasarkan dan membunuh pesaing mereka.
Kata kunci: *Bacillus; Leohumicola*; maritim Antarctic; *Pseudogymnoascus; Talaromyces*

Introduction

Antarctica is home to a diverse range of microbial species that have adapted to harsh conditions such as frequent freeze-thaw and wet-dry cycles, desiccation, high ultraviolet radiation, and limited nutrients (Svahn et al. 2015; Wynn-Williams 1996; Zheng et al. 2016). The ability of these microorganisms to strive and maintain sustained growth has sparked the curiosity of researchers in studying microbial diversity and adaptation to extreme environments. There is a considerable amount of research on Antarctic bacterial survival abilities (Lo Giudice, Bruni & Michaud 2007; Lo Giudice et al. 2007; O'Brien et al. 2004; Wong et al. 2011), but there are few studies on Antarctic spore-forming microorganisms, especially those from volcanic soils.

Spore-forming microorganisms are a significant group of microorganisms isolated from Antarctic soils (Cowan et al. 2014; Dong et al. 2013). These microbes use spore formation as an adaptation strategy to escape unfavourable conditions and spread to new niches, but it is unclear how many of them also produce antimicrobial compounds, which provide an additional survival advantage. Actinobacteria, Firmicutes, and fungi are among the spore-forming microorganisms isolated from various locations in Antarctica (Logan et al. 2000; Nicolaus et al. 2001). Furthermore, the ability of Antarctic microorganisms to produce antimicrobial compounds has been proposed as a strategy for competing with their competitors for survival (Lo Giudice, Bruni & Michaud 2007; Lo Giudice et al. 2007; O'Brien et al. 2004; Wong et al. 2011).

Deception Island is a horseshoe-shaped volcanic island located at the southwestern end of the South Shetland Islands. Deception Island has a very unique and more varied ecosystem than Victoria Land because its geothermal habitats are lower in elevation (540 m) than other high-altitude (>2,400 m) continental volcanoes in Victoria Land (Held, Arenz & Blanchette 2011; Herbold, Mcdonald & Cary 2014). In the summer, soil temperatures on some parts of this island can reach up to 98 °C (Lezcano et al. 2019), despite the low surrounding air temperatures. There have been numerous reports of spore-forming microorganisms on Deception Island (Cheah et al. 2015; Gesheva 2012; Herbold, Mcdonald & Cary 2014). However, little was known about these microbes and their antimicrobial capabilities to outcompete others to maximize their survival in the nutrient-limiting harsh maritime Antarctic environments. Hence, the objectives of this study were to isolate, characterise, and identify spore-forming microbes on Deception Island that have strong inhibitory activities against other microorganisms. We hypothesized that some spore-forming microbes do produce antimicrobial compounds and probably use them as a survival strategy.

Materials and Methods

Sample collection, isolation and maintenance

Soil samples were collected from Deception Island, Antarctica, at a location with a temperature of 98 °C during the austral summer. The samples were pretreated with 6% yeast extract and 0.05% sodium dodecyl sulphate (SDS) in a 5 mM phosphate buffer at 40 °C for 20 min (Hayakawa & Nonomura 1989; Khanna et al. 2011). Serial dilutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were performed using a phosphate buffer (50 mM, pH 7). 100 µL of each soil suspension was spread onto the agar surface. Seven different selective isolation agar media: actinomycetes isolation agar (AIA, Difco), actinomycetes agar (ABA,

Difco), yeast extract-malt extract agar (YMA), oatmeal agar (OA), inorganic-salts-starch agar (ISSA) (Shirling & Gottlieb 1966), Gause modified medium I (IM2) (Hong et al. 2009) and *Streptomyces* agar (SA) were used. All culture media were supplemented with 50 mg/L cycloheximide and 50 mg/L kanamycin sulphate. The plates were incubated at 4 °C, 12 °C, 20 °C, 28 °C and 37 °C, respectively, for 10 days. Purified cultures were maintained on YMA slant agar media at the respective temperatures and as glycerol suspensions (20%, v/v) at -80 °C.

GENOMIC DNA EXTRACTION

Each strain was grown in 5 mL of yeast extract-malt extract broth (YMB) at the respective temperature with shaking at 180 rpm for 3-5 days. One mL of the broth culture was centrifuged at 7,500 rpm and the supernatant was discarded. The genomic DNA was extracted from the pellet using the DNeasy blood and tissue kit (Qiagen, Germany) according to the manufacturer's instructions with slight modifications. The pellet was crushed using a sterile micropestle after the addition of 180 µL of enzymatic lysis buffer. The DNA yield was assessed using 1% (w/v) agarose gel electrophoresis followed by DNA quantification using a Nanovue™ Plus spectrophotometer (GE Healthcare, United Kingdom).

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FINGERPRINTING

The PCR reaction mixture consisted of 20 ng bacterial genomic DNA, 5 µL of 5X PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 2 units of Taq polymerase (Promega, USA), 5 pmol primer AP4 (5'-AAGAGCCCGT-3') and sterile MilliQ water added to a final volume of 25 µL (El-Fiky et al. 2003). The PCR was performed with an initial denaturation step at 95 °C for 5 min, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C for extension, and a final extension at 72 °C for 5 min in an Eppendorf C1000 Thermal Cycler (Eppendorf, Germany) (El-Fiky et al. 2003). The amplified PCR products were separated on 1.2% (w/v) agarose gel and stained with ethidium bromide (EtBr) (0.5 µg/mL). The gel was viewed using the Alpha Image gel documentation system (Alpha Innotech, USA). All gel photographs were analysed using the Gel Compare program to calculate similarity indices and to generate a dendrogram by the unweighted pair group method using arithmetic averages (UPGMA) clustering to eliminate overlapping strains.

ANTIMICROBIAL ACTIVITY AGAINST BACTERIA

Antimicrobial activities of randomly selected strains were tested against 13 Gram-positive and Gram-negative bacteria (*Escherichia coli* O157:H7, *Escherichia coli* V517, *Escherichia coli* O125, *Salmonella* Paratyphi, *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Braenderup, *Klebsiella pneumoniae* 14x, *Enterobacter cloacae* 22x, *Bacillus cereus* K3, *Staphyloccus haemolyticus*, *Enterococcus faecalis,* and *Staphylococcus equorum*) according to the procedures described by Wong et al. (2011). These bacteria were provided by Professor Son Radu from Universiti Putra Malaysia, Malaysia.

16S or 18S rDNA AMPLIFICATION

The strains that inhibited five or more test bacteria were subjected to molecular identification. For the identification of prokaryotic strains, the 16S rDNA region was amplified using primers BSF8 (5'-AGAGTTTGATCCTGGCTCAG-3') and BSR1541 (5'-AAGGAGGTGATCCAG CCGCA-3') or p27f (5'-AGAGTTTGATCMTGGCTCAG-3') and p1525r (5'-AAGGAGGTGWTCCARCC-3'). For the identification of eukaryotic strains, the 18S rDNA regions were amplified using primers NS5F (5'-AACTTAAAGGAATTGACGGAAG-3') and NS8R (5'-TCCGCAGGTTCACCTACGGA-3'). Each PCR reaction mixture contained 200 ng genomic DNA, 25 µL TopTaq Master Mix (Qiagen, USA), 5 µL CoralLoad dye, 10 pmol of each primer and sterile MilliQ water was added to a final volume of 50 µL. PCR was performed with an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C for extension, and a final extension at 72 °C for 10 min in an Eppendorf C1000 Thermal Cycler (Eppendorf, Germany). The amplified PCR products were separated on 1% (w/v) agarose gels stained with ethidium bromide (0.5 µg/mL) and using the Alpha Image gel documentation system (Alpha Innotech, USA). The agarose gel portion containing the DNA fragment of interest was excised using a sterilized scalpel and purified using a QIAquick Gel Extraction kit (Qiagen, Germany) according to the manufacturer's instructions. The 16S or 18S rDNA amplicons were sequenced using a Sanger 8 capillaries sequencer. The DNA sequences were aligned using the NCBI Basic Local Alignment Search Tool (BLAST) and the phylogenetic tree was built using ClustalW in MEGA 11 (Molecular Evolutionary Genetics Analysis version 11: Tamura, Stecher & Kumar 2021).

CONSTRUCTION OF PHYLOGENETIC TREES

The construction of the phylogenetic tree involved several steps. Initially, sequence alignment was performed using MUSCLE within MEGA11 (Tamura, Stecher & Kumar 2021). The neighbour-joining clustering method was applied for alignment, while other settings remained at their default values. Subsequently, the aligned sequences underwent trimming using TrimAL with the '-gappyout' option to eliminate gap-containing regions (Capella-Gutiérrez, Silla-Martínez & Gabaldón 2009). Following alignment trimming, the phylogenetic tree was constructed using IQ-TREE software version 2.2.0.3 (Minh et al. 2020). The Modelfinder Plus algorithm was utilized to determine the best-fit substitution model (-m MFP) based on the trimmed aligned sequences. The tree construction process included bootstrap analysis, consisting of 1,000 SH-like approximate likelihood ratio tests and 1,000 bootstrap replicates. Finally, the consensus tree, displaying only bootstrap values, was generated from the bootstrap replicates.

RESULTS

ISOLATION OF ANTARCTIC MICROORGANISMS

A total of 90 strains were isolated from two soil samples. All strains were recovered from 10^{-1} to 10^{-3} diluted samples, but none were recovered from 10^{-4} and 10^{-5} diluted samples, indicating that the soil population was relatively small. Fifty-six strains were isolated from the agar plates incubated at 12 °C, 26 were isolated at 20 °C, seven were isolated at 4 °C and one was isolated at 28 °C (Table 1). Agar plates incubated at 37 °C yielded no isolates. Many of the strains isolated at 4 °C, 12 °C, and 20 °C formed slimy colonies. The colonies were white, creamy, yellow, pink, and black.

RAPD FINGERPRINTING

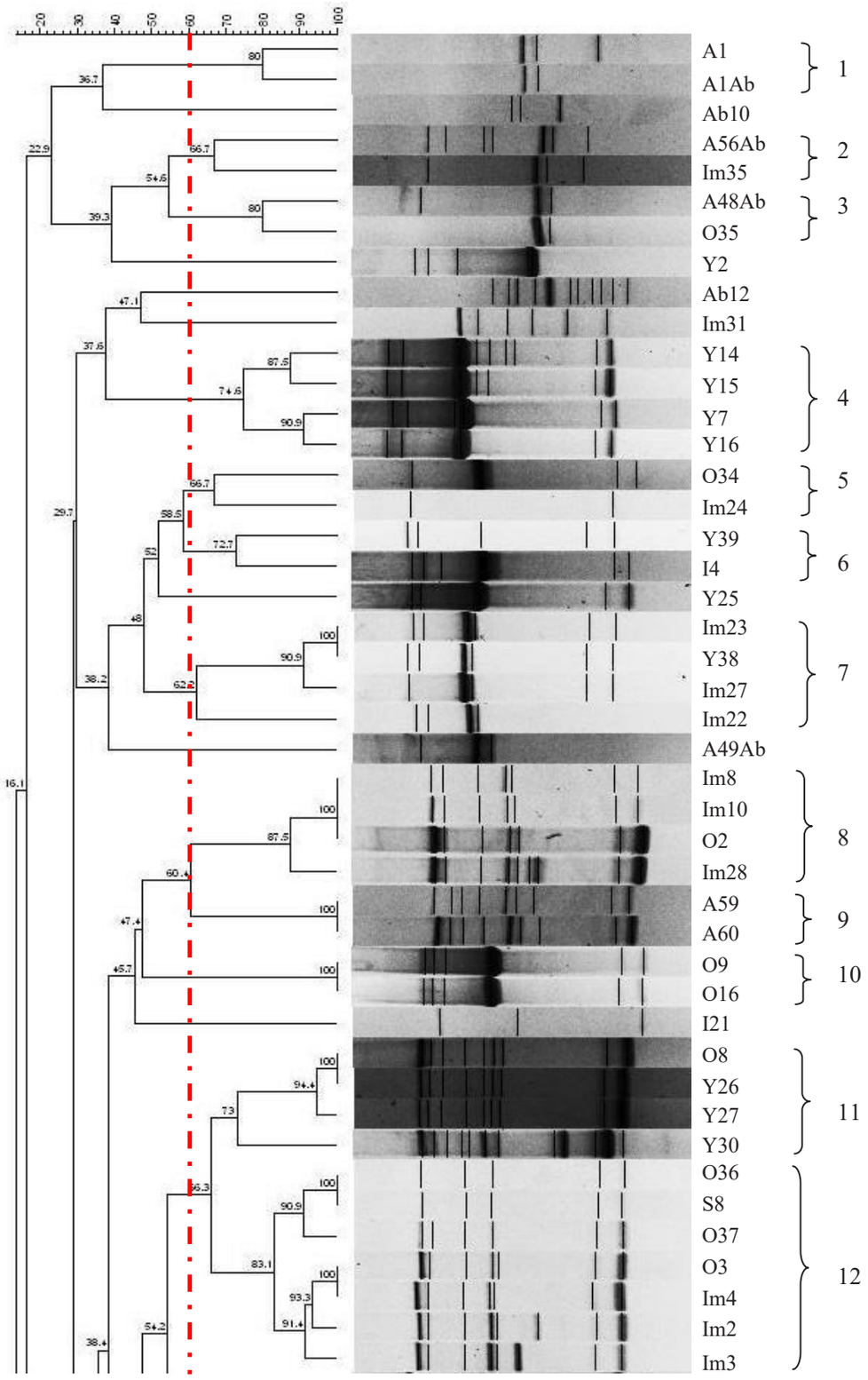
Amplification with primer AP4 was reproducible and produced clear discriminatory DNA banding patterns. A total of 77 different banding patterns were obtained (Figures 1(a) & 1(b)). The number of polymorphic DNA bands ranged from 2 to 14. At a similarity level of 60%, 79 out of 90 strains were clustered into 19 groups, while 11 strains (Ab10, Ab12, A49Ab, I21, Im31, Im32, Im33, O32, Y2, Y10 and Y25) were not classified into any groups (Table 2). One strain from each of the 19 groups (A1Ab, Im35, O35, Y14, O34, I4, Y38, Im28, A60, O16, O8, Im2, S5, Y28, I2, Y13, Y21, Y9 and I22) and the 11 strains that were not classified into any groups (Ab10, Ab12, A49Ab, I21, Im31, Im32, Im33, O32, Y2, Y10 and Y25) were subjected to testing for antimicrobial activities against the pathogens.

ANTIMICROBIAL ACTIVITIES AGAINST TEST PATHOGENS

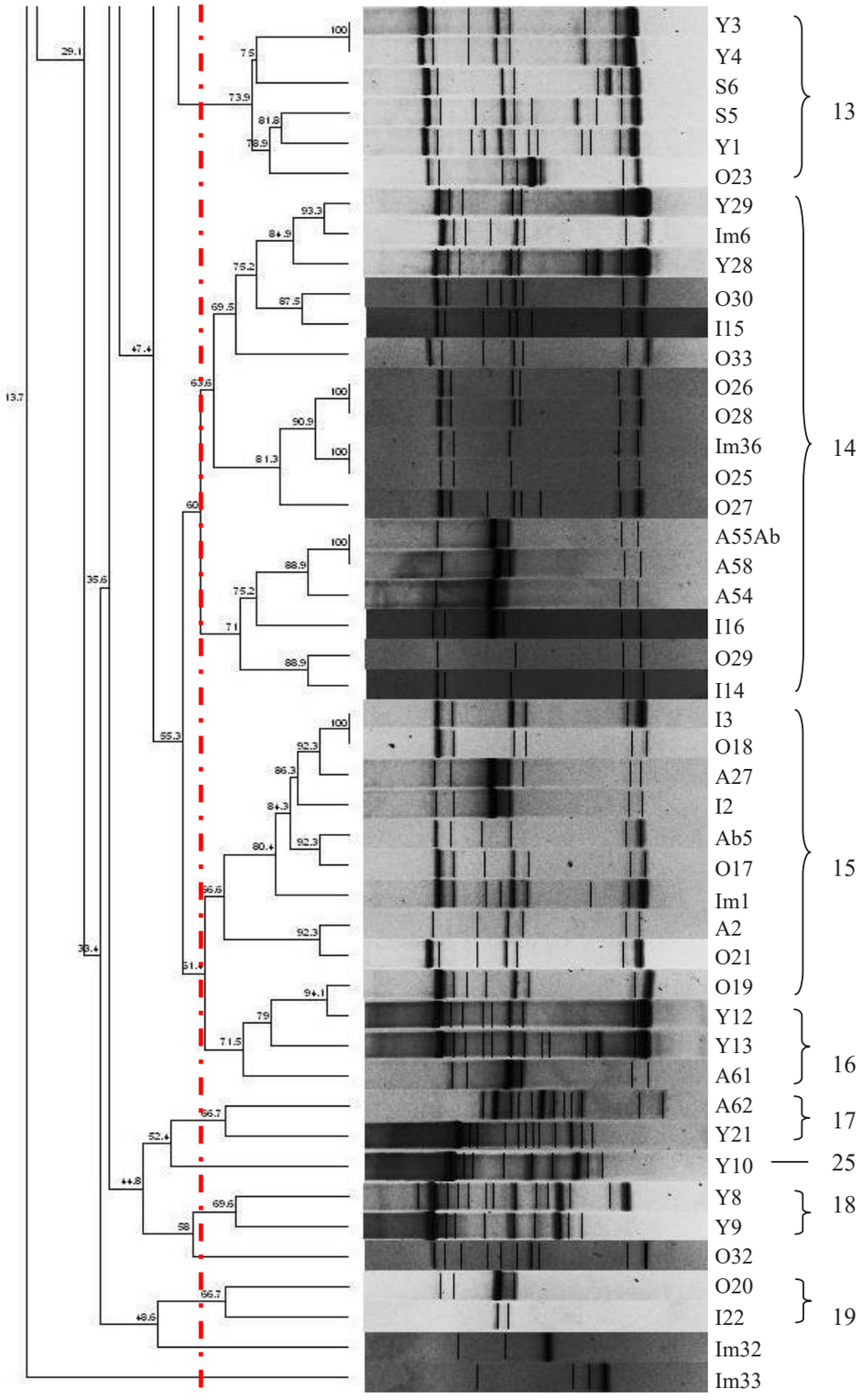
Twenty-five of the thirty strains had an inhibitory effect on one or more of the test pathogens. Out of the 13 tested, four strains, A60, Im31, Im32, and Im33, were found to inhibit five or more pathogens. The majority of the strains (21 out of 30) inhibited the growth of *S. equorum*. Im33 was the only strain that inhibited the growth of *E. coli* O125, *K. pneumoniae* 14x and *E. cloacae* 22x (Table 3). Representative agar plates demonstrating the inhibition activities of the strains against test pathogens are shown in Figures 2(a) and 2(b).

TABLE 1. Strains isolated at each temperature

Temperature (°C)	Strains
4	Ab10, Ab12, Im35, Im36, O37, Y38 and Y39
12	A1, A1Ab, A2, A27, Ab5, I2, I3, I4, I14, I15, I16, I21, I22, Im1, Im2, Im3, Im4, Im8, Im10, Im23, Im24, Im27, Im28, O2, O3, O8, O16, O17, O18, O19, O20, O21, O23, O25, O26, O27, O28, O29, O30, O32, O33, S5, S6, S8, Y1, Y2, Y3, Y4, Y7, Y21, Y25, Y26, Y27, Y28, Y29 and Y30
20	A48Ab, A49Ab, A54, A55Ab, A56Ab, A58, A59, A60, A61, A62, Im6, Im22, Im31, Im32, O9, O34, O35, O36, Y8, Y9, Y10, Y12, Y13, Y14, Y15, Y16
28	Im33


20
30
40
50
60
70
80
90
100
A1
A1Ab
Ab10
A56Ab
Im35
A48Ab
O35
Y2
Ab12
Im31
Y14
Y15
Y7
Y16
O34
Im24
Y39
I4
Y25
Im23
Y38
Im27
Im22
A49Ab
Im8
Im10
O2
Im28
A59
A60
O9
O16
I21
O8
Y26
Y27
Y30
O36
S8
O37
O3
Im4
Im2
Im3
1
2
3
4
5
6
7
8
9
10
11
12

(a)

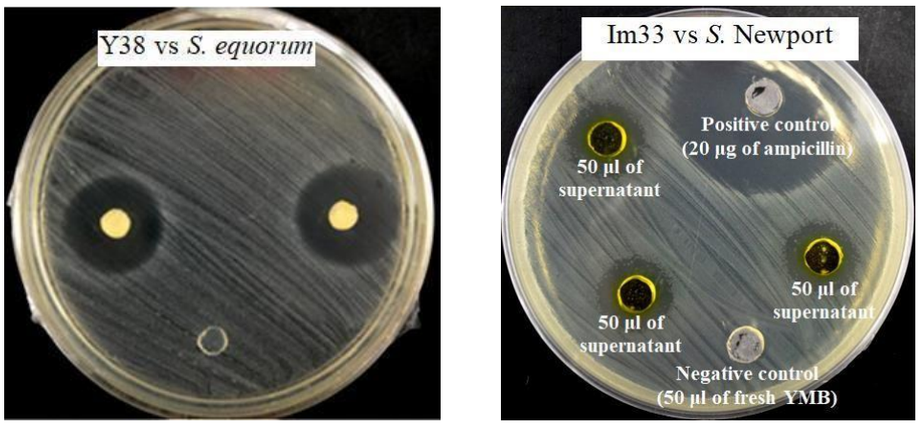


(b)

FIGURE 1. Dendrogram constructed using RAPD fingerprint profiles from (a) the first and (b) the second 45 of 90 strains

TABLE 2. Clustering of 79 out of 90 strains at a 60% similarity level. An asterisk denotes the strain subject to testing for antimicrobial activities against the test pathogens

Group No.	Strains	Group No.	Strains
1	A1 and *A1Ab	12	Im4, *Im2, Im3, O3, O36, O37 and S8
2	A56Ab and *Im35		
3	A48Ab and *O35	13	O23, *S5, S6, Y1, Y3 and Y4
4	Y7, *Y14, Y15 and Y16	14	A54, A55Ab, A58, I14, I15, I16, Im6, Im36, O25, O26, O27, O28, O29, O30, O33, *Y28 and Y29
5	*O34 and Im24		
6	*I4 and Y39		
7	Im22, Im23, Im27 and *Y38	15	A2, A27, Ab5, *I2, I3, Im1, O17, O18, O19 and O21
8	Im8, Im10, *Im28 and O2		
9	A59 and *A60	16	A61, Y12 and *Y13
10	O9 and *O16	17	A62 and *Y21
11	*O8, Y26, Y27 and Y30	18	Y8 and *Y9
		19	*I22 and O20



(a) (b)

FIGURE 2. Representative agar plates demonstrating the inhibition activities of the Antarctic strains against test pathogens using the (a) strain Y38 against *S. equorum* and (b) strain Im33 against *S.* Newport. Screening was performed by direct spore-forming microbe against a lawn of test pathogen on an agar plate, and by plating the culture medium of the spore-forming microbe against a lawn of test pathogen on an agar plate

TABLE 3. Inhibitory effect of 30 strains against 13 test bacteria during primary screening

Strain	Temp. (°C)	Diameter of inhibition zone (mm)												
		E. coli O157:H7	*E. coli* V517	*E. coli* O125	*S. paratyphi*	*S. typhimurium*	*S. newport*	*S. braenderup*	*K. pneumoniae* 14x	*E. cloacae* 22x	*B. cereus* K3	*S. haemolyticus*	*E. faecalis*	*S. equorum*
Ab10	4													19
Ab12							19							25
Im35											8	13		15
Y38														20
A1Ab	12													
I2														
I4						8								
I21														
I22														13
Im2														16
Im28														
O8														14
O16							8							18
O32														10
S5								8						9
Y2						8		8						13
Y21						7	7							
Y25							8							16
Y28														8
A49Ab	20													10
A60		7			8	7	11				7	10	7	22
Im31		18			24	20	14	20			10	29		41
Im32							10				9	23	26	16
O34			8				12						7	7
O35						7								7
Y9					7		20							
Y10						7	18							
Y13														8
Y14														
Im33	28	8	8	10	7	7	8	8	9	9	8	20	19	26

IDENTIFICATION OF STRAINS WITH ANTIMICROBIAL ACTIVITIES

Based on 16S or 18S rRNA gene sequence analysis, strains A60, Im31, Im32 and Im33 were identified as *Phacidium lacerum*, *B. subtilis., Leohumicola* sp. and *Talaromyces* sp. (Table 4) and they have been shown to be closest to A60, Im31, Im32 and Im33, respectively, in the phylogenetic trees (Figures 3 to 6).

TABLE 4. 16S or 18S rDNA sequence similarities of the strains to their closest neighbours

Strains	Closest neighbour	Max score	Total score	Query cover	E value	Similarity
A60	*Phacidium lacerum*	1105	1105	98%	0.0	99.51%
Im31	*Bacillus subtilis*	852	852	100%	0.0	100%
Im32	*Leohumicola* sp.	1140	1140	96%	0.0	97.88%
Im33	*Talaromyces* sp.	1162	1162	98%	0.0	99.34%

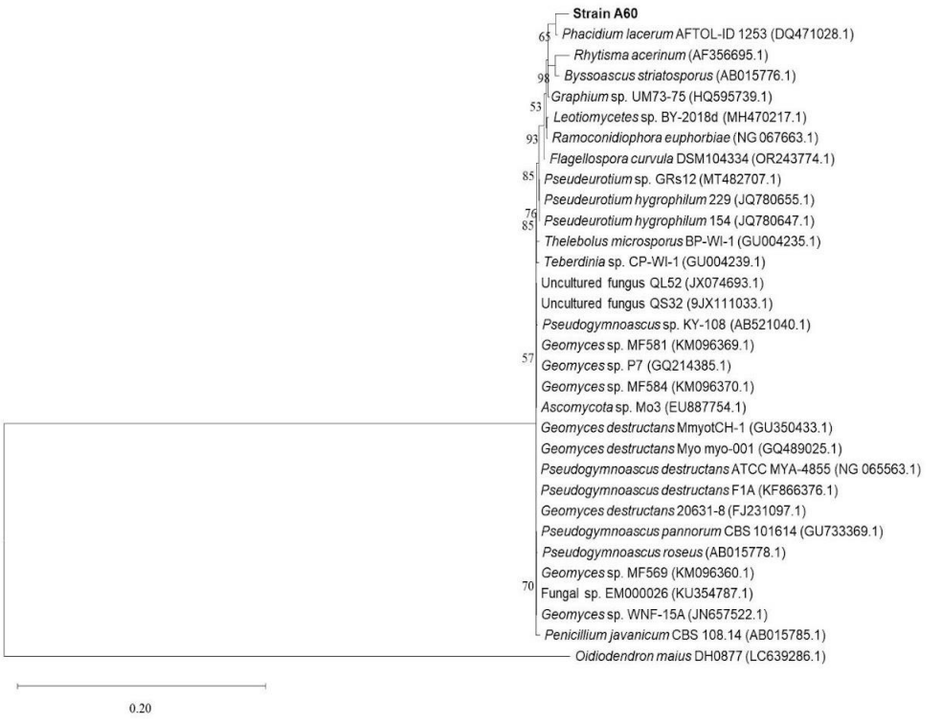


FIGURE 3. Phylogenetic tree of strain A60 and related species. A phylogenetic tree was generated using the K2P+FQ+R3 substitution model with 1,000 bootstrap replicates, *Oidiodendron maius* DH0877 serve as the outgroup. The accession numbers are provided in the parentheses. The bar represents 0.20 substitutions per nucleotide position

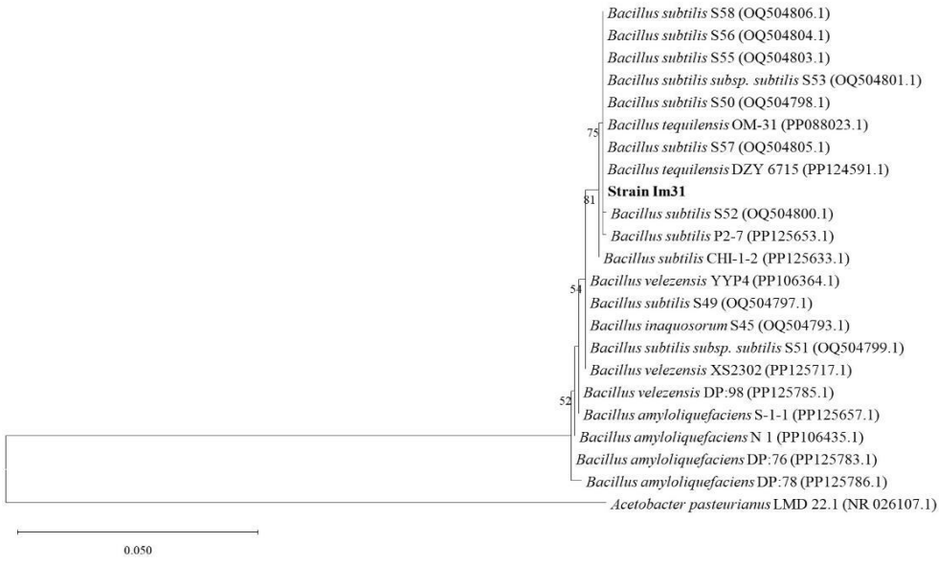


FIGURE 4. Phylogenetic tree of strain Im31 and related species. A phylogenetic tree was generated using the TN+F substitution model with 1,000 bootstrap replicates, *Acetobacter pasteurianus* LMD22.1 serve as the outgroup. The accession numbers are provided in the parentheses. The bar represents 0.050 substitutions per nucleotide position

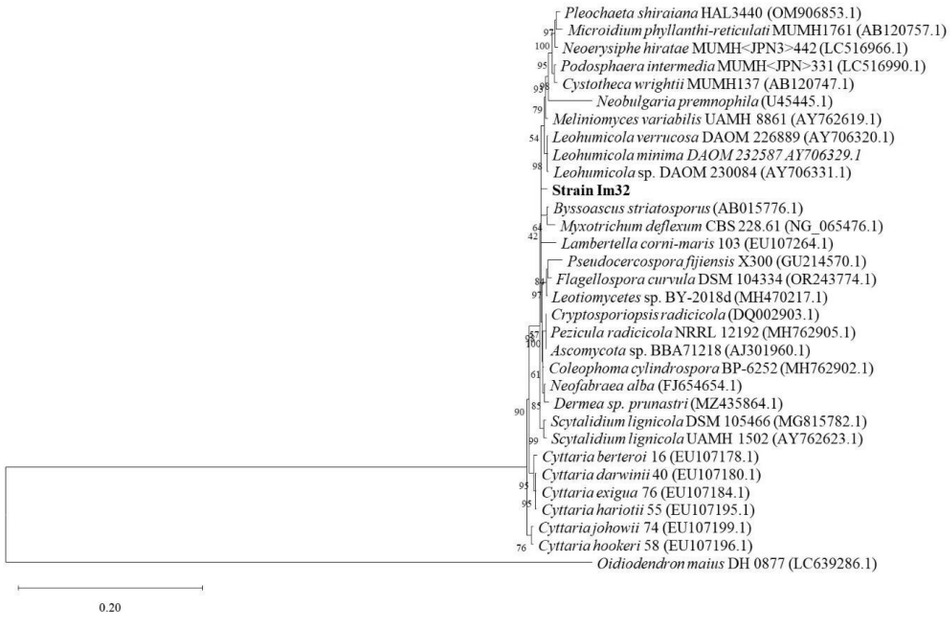


FIGURE 5. Phylogenetic tree of strain Im32 and related species. A phylogenetic tree was generated using the TN+F substitution model with 1,000 bootstrap replicates, *Oidiodendron maius* DH0877 serve as the outgroup. The accession numbers are provided in the parentheses. The bar represents 0.20 substitutions per nucleotide position

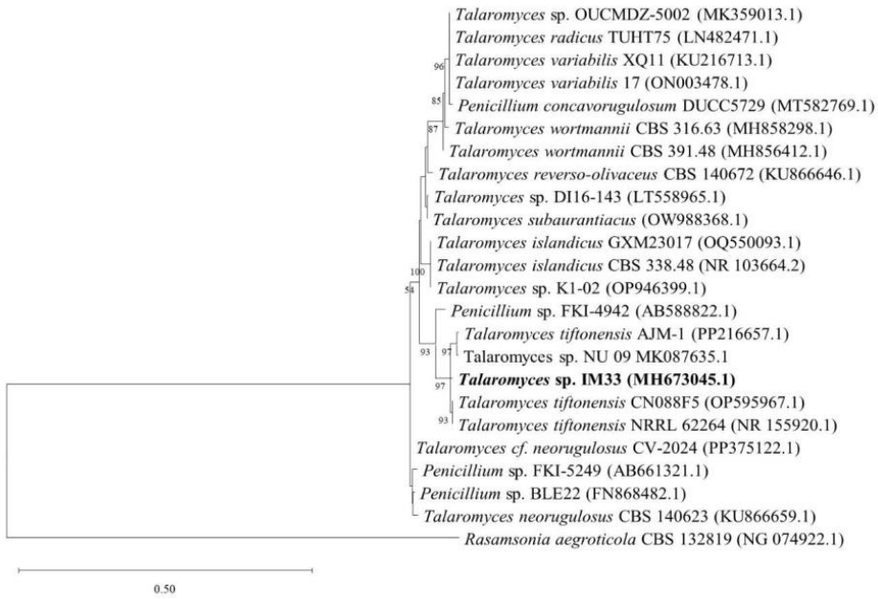


FIGURE 6. Phylogenetic tree of strain Im33 and related species. A phylogenetic tree was generated using the HKY+F+R2 substitution model with 1,000 bootstrap replicates, *Rasamsonia aegroticola* CBS132819 serve as the outgroup. The accession numbers are provided in the parentheses. The bar represents 0.50 substitutions per nucleotide position

DISCUSSION

In this study, 82 out of 90 strains were isolated at 12 °C and 20 °C (56 at 12 °C and 26 at 20 °C) (Table 1), which suggests that psychrotrophs constitute the majority of soil microbial communities on Deception Island. This was in agreement with other researchers who have also isolated abundant psychrotolerant microorganisms from soil samples collected from Deception Island (Carrión et al. 2011; Tomova et al. 2014). Cold-adapted microorganisms such as psychrophiles and psychrotrophs are believed to be the most predominant microorganisms in Antarctica due to their abilities to develop various structural and functional adaptations such as the expression of cold-shock and antifreeze proteins, accumulation of compatible solutes, production of cold-active enzymes and proteins, as well as increased genome plasticity and fluidity of the cellular membranes to survive in the harsh conditions (Casanueva et al. 2010). The low number of strains was isolated at 4 °C (Table 1), which was probably due to sample collection during the summer (ranging from -5.6 °C to 12.4 °C) that did not favour the growth of true psychrophiles (Bañón et al. 2013; Morita 1975). Interestingly, only one isolate, strain Im33, was isolated at 28 °C (Table 1), suggesting that the soil samples do not contain a large number of spore-forming microbes that prefer high temperatures. The fact that strain Im33 was able to survive in both moderately high and low temperatures on Deception Island suggests that it has effective adaptation strategies and competes or lives synergistically with other microbes.

Many isolated strains produced coloured pigments, which were consistent with the observation of O'Brien et al. (2004). According to Chattopadhyay and Jagannadham (2001), pigment production is one of the microbial strategies to protect themselves against the oxidative stresses imposed by low temperatures and strong ultraviolet (UV) radiation. Polar and non-polar carotenoids have been shown to play a role in the regulation of membrane fluidity in cold-adapted microorganisms in Antarctica (Chattopadhyay & Jagannadham 2001). Pigmented Antarctic microbes are also found to exhibit higher survival rates than their non-pigmented counterparts when they are exposed to 300 W/m^2 of solar radiation (Dieser, Greenwood & Foreman 2010). Moreover, the secretion of slime-like extracellular polymeric substance (EPS) is another way

to protect microorganisms against desiccation and strong UV radiation (Decho 1990), which explains why many strains appear as slimy colonies.

RAPD fingerprinting has been proven to be a simple, cost-effective, and rapid technique that has strong discriminatory power and good reproducibility to resolve the fingerprint of the 90 strains. It allows the classification and determination of genetic relationships among microorganisms so that genetically unique strains can be differentiated within closely related species without prior knowledge of the genome (Stefańska et al. 2022) and to identify overlapping strains that have been isolated multiple times in this work. As listed in Table 1, 79 out of 90 strains were clustered into 19 groups with at least one genetically related strain. The remaining 11 strains (Ab10, Ab12, A49Ab, I21, Im31, Im32, Im33, O32, Y2, Y10 and Y25) were genetically distinct and did not show similar fingerprinting patterns with others; hence, they were not clustered into any groups (Figure 1(a) and 1(b)). Twenty-five strains produced identical RAPD patterns (100% similarity) with each other (Im23 and Y38, Im8, Im10 and O2, A59 and A60, O9 and O16, O8 and Y26, O36 and S8, O3 and Im4, Y3 and Y4, O26 and O28, Im36 and O25, A55Ab and A58, as well as I3 and O18) (Figure 1(a) and 1(b)), indicating that they were the same strains.

In addition to the aforementioned cellular modifications, secondary metabolite production is believed to be a defence mechanism to improve the survival fitness of microbes. They produce secondary metabolites as chemical signals to communicate with the same or different species and/or inhibit the neighbouring competitors to reduce the competition for space and nutrients (Lo Giudice et al. 2007; Netzker et al. 2015). Antimicrobial compounds are among the secondary metabolites found in Antarctic spore-forming microbes. Cheah et al. (2015) investigated the antimicrobial activities of Deception volcanic soil Actinobacteria and isolated three strains that inhibited *S. paratyphi* A and *S. typhimurium*. Recently, Astudillo-Barraza et al. (2023) have partially purified some of the secondary metabolites from a spore-forming Antarctic *Streptomyces fildesensis* (INACH3013), which they reported to exhibit antimicrobial activities. They reported further that gene clusters that potentially carry out the biosynthesis of actinomycin-like antimicrobial compounds were found in the *S. fildesensis* (INACH3013) genome. Another Antarctic *Streptomyces* sp. So13.3 has also been reported to exhibit antibiotic activity against Gram-positive bacteria and growth reduction of Gram-negative pathogens (Núñez-Montero et al. 2019). In their genome survey, they found a large number of biosynthesis gene clusters that have not been reported before, suggesting that Antarctic *Streptomyces* strains as a promising source of novel antimicrobial compounds. These observations imply that the strains isolated from this study would likely exhibit similar traits.

In this study, 25 out of 30 strains (83%) demonstrated antimicrobial activities against the pathogens (Table 3). Ten strains (Ab10, A49Ab, I4, I22, Im2, O8, O32, Y13, Y28 and Y38) inhibited one pathogen, eight strains (Ab12, O16, O35, S5, Y9, Y10, Y21 and Y25) inhibited two pathogens, two strains (Im35 and Y2) inhibited three pathogens, one strain (O34) inhibited four pathogens, one strain, (Im32) inhibited five pathogens, two strains, (A60 and Im31) inhibited eight pathogens, and one strain, (Im33) inhibited 13 pathogens (Tables 3 & 4). Five strains (A1Ab, I2, I21, Im28 and Y14) did not inhibit any pathogen at all (Tables 3 & 4). Nonetheless, we cannot rule out the possibility that they can inhibit bacteria that are not used as test bacteria in this study. It was also noticed that strain Im33, which inhibited all pathogens, produced pigments that turned the supernatant and agar yellow (data not shown). The production of pigments might be related to their inhibitory effect against other microorganisms. Based upon the findings, spore-forming soil microbes on Deception Island were capable of secreting secondary metabolites that may probably outcompete other microorganisms and enhance their survival chances in harsh conditions (Astudillo-Barraza et al. 2023; Núñez-Montero et al. 2019).

Strains that inhibited five or more pathogens, A60, Im31, Im32 and Im33, were subsequently identified by 16S or 18S rRNA gene sequence analysis. As listed in Table 5, strain A60 was identified as *Phacidium lacerum* (99%), strain Im31 as *B. subtilis* (100%), strain Im32 as *Leohumicola* sp. (98%) and strain Im33 as *Talaromyces* sp. (99%). Members of the genera *Bacillus* and *Talaromyces* species have been isolated from the terrestrial and maritime habitats of the Fildes Peninsula, King George Island, Antarctica, as reported by Albores et al. (2018), dos Santos, Meyer and Sette (2020), Furbino et al. (2014) and Llarch et al. (1997). *Leohumicola minima* has also been previously isolated from Deception Island, Antarctica (de Menezes et al. 2019; Latter & Heal 1971; Purić et al. 2018) indicating that it is probably among the most commonly found dominant genus on this island. Additionally, members of the genus *Bacillus* and

Talaromyces are also found to be widespread in many other Antarctic ecosystems, such as from moss, wood, soil, seawater, and marine sediment samples (Albores et al. 2018; Purić et al. 2018).

Strain A60 clustered with several fungi species, namely *Phacidium sp.*, *Rhytisma* sp., and *Byssoascus* sp., but it was closest to *Phacidium lacerum* (Figure 3). *Phacidium lacerum* has not been isolated from the Antarctic but was reported to be a psychrotolerant fungus by Dix and Webster (1995). It was discovered in Chile and Washington State and linked to pre- or postharvest apple and pear rot (Díaz et al. 2019; Wiseman et al. 2016). Hence, this species was most likely introduced to this region and is not native to Deception Island. Regardless of whether it was alien in origin, its ability to produce antimicrobial chemicals most likely allowed it to survive and compete with bacteria in Antarctica.

The identity of strain Im31 has 100% identity with several *Bacillus subtilis* and is clustered with many other *B. subtilis* strains (Figure 4). *B. subtilis* is widespread around the world and is an antimicrobial-producing spore-forming bacterium. It possesses a rather large chemo-diversity within the *B. subtilis* group that is species- or clade-specific, as well as a few that are scattered within or between species based on an extensive analysis by Steinke et al. (2021). Hence, there is a possibility that *B. subtilis* strain Im31 might have antimicrobial compounds that are different from those *B. subtilis* found elsewhere, and this warrants further investigations in the future.

The phylogenetic tree showed that strain Im32 is closest to *Leohumicola minima* (Figure 5). Currently, there are only seven *Leohumicola* species have been reported, namely *L. atra, L. incrustata, L. lenta, L. levissima, L. minima, L. terminalis,* and *L. verrucosa* (Schoch et al. 2020). At the time of writing, the antimicrobial compounds of Antarctic *L. minima* have not been reported. Nevertheless, the crude extract of another species, *L. incrustata* shows the capability to kill pathogens (Adeoyo, Pletschke & Dames 2019). Hence, the findings of *Leohumicola* sp. Im32 in this work suggests that other species from this genus a likely to be antimicrobial compound producers as well. Hence, in the future, it will be interesting to test *L. atra, L. lenta, L. levissima, L. minima, L. terminalis,* and *L. verrucosa* for the presence of antimicrobial compounds too.

The identity of strain Im33 was also confirmed by Yong et al. (2023) to be *Talaromyces* sp. through the highly variable ITS1-5.8S-ITS2 region of DNA sequence analysis, which has been recognized as a more powerful genetic marker for species-level investigation of fungi (Schoch et al. 2012). It was observed that strain Im33 clustered together with several *Talaromyces* species, namely *T. neorugolosus, T. islandicus, T. reverso-olivaceus, T. wortmannii,* and *T. radicus* among others, but was closest to *T. tiftonensis* (Figure 6). However, there is very little information about *T. tiftonensis.* While no antibiotic activity has been reported for *T. tiftonensis*, one Antarctic *Talaromyces, T. cnidii*, has been discovered to produce antibacterial compounds (Cavalcante et al. 2024). Hence, it is intriguing that *T. tiftonensis* strain Im33 and Antarctic *T. cnidii* in Cavalcante et al. (2024) possess antibacterial properties.

Conclusions

In conclusion, spore-forming microorganisms were found to be abundant on Deception Island, and 83 percent of the representative strains tested inhibited at least one test pathogen. This suggests that the inhibitory properties most likely enhance the survivability of the spore-forming microorganisms on Deception Island, similar to their non-spore-forming bacterial counterparts. In addition to providing insight into how microbes adapt to harsh environments, this research could potentially lead to the discovery of microorganisms with medicinal and biotechnological uses, particularly those that produce antimicrobial compounds. These are much needed at a time when the emergence of multi-drug pathogens occurs far more quickly than the development of new antibiotics. Antarctic *Phacidium lacerum* strain A60, *B. subtilis* strain Im31, and *Talaromyces tiftonensis* strain Im33 reported in this study are potential sources of novel antibacterial compounds that warrant further detailed experimentation and analyses. Additionally, the discovery of *Leohumicola* sp. in Antarctica is noteworthy as it has not been previously reported, emphasizing the need for continued exploration and monitoring of microbial diversity, especially the spore-forming ones in extreme environments.

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