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RESEARCH

Revealing actinobacterial diversity inhabiting Malaysian Beach Ridges Interspersed with Swales (BRIS) soil : insights from culture-dependent and metagenomic approaches

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Abstract

The discovery of novel antibiotics remains a pressing global challenge as many known microorganisms continue to yield compounds already present in existing drugs. To overcome this limitation, bioprospecting in underexplored and extreme environments using both culture-dependent and culture-independent strategies has become essential. In this study, we investigated the microbial diversity of Beach Ridges Interspersed with Swales (BRIS) soil from Setiu, Terengganu, Malaysia—an environment characterized by poor nutrient retention, low water-holding capacity, and acidic conditions with lack information available on their microbial community composition. Therefore, this study was conducted with the main objectives to investigate actinomycetes community composition in BRIS soil using metagenomics and culture-dependent approaches. To address these objectives, a dual approach was employed: (i) culture-dependent isolation of actinomycetes using selective media, followed by morphological and 16S rRNA gene-based phylogenetic analysis, and (ii) culture-independent high-throughput sequencing of the 16S rRNA gene (Illumina MiSeq) to characterize the broader microbial community. Results from the selective isolation yielded 180 actinomycete isolates grouped into 69 colour-based categories, with 15 representatives identified by 16S rRNA sequencing as belonging predominantly to *Streptomyces*, alongside the rare genus *Dermacoccus*. In contrast, metagenomic analysis revealed a far richer microbial landscape comprising 4719 OTUs, 32 bacterial phyla, and 380 genera, including a high proportion of uncultured taxa. Notably, actinobacterial diversity was dominated by *Acidothermus*, whereas *Streptomyces* predominated in culture-dependent isolation, highlighting the complementary nature of both approaches. These findings confirm that BRIS soil harbours unique microbial communities shaped by its physicochemical conditions, with potential as a reservoir for rare actinomycetes and novel bioactive compounds. The study provides the first combined culture-dependent and metagenomic insight into BRIS soil microbiota and underscores its promise for future pharmaceutical and biotechnological exploration.

Keywords Microbial diversity · Actinomycetes · BRIS soil · Metagenomic · Culture-dependent

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Introduction

Soil microorganisms play important roles in their living environment by providing various ecological and physiological purposes such as organic matter decomposition, nitrogen fixation and nutrient regulation and their composition varies between habitats. Considering the estimation that less than 1% microorganisms can be cultured while the majority remains unculturable, information of the true microbial diversity is much needed to bring as much species out to be cultured in vitro for further investigation (Mhete et al. 2020; Idris et al. 2017). Advances in environmental biotechnology, particularly Next-Generation Sequencing (NGS), enable detailed characterization of microbial community structures, facilitating metagenomics-guided optimization strategies for targeted microbial isolation, as demonstrated by Jaswal et al. (2019). It also provides insights independent of our capacity to cultivate these species and co-culturing by replicating their specific environment in the laboratory (Bodor et al. 2020).

Given the rise of antimicrobial resistance, there is a critical need for new and effective antimicrobial agents to be discovered (WHO 2023; Trenozhnikova and Azizan 2018). However, the recurrence of existing antibiotics in newer microbial isolates complicates the bioprospecting for new drug producer (Schneider 2021). Thus, Subramani and Sipkema (2019) emphasized that rare actinomycetes especially the uncultured taxa should be the target of future isolation strategies and procedures with further application and exploration in genetic engineering and heterologous expression to find new drugs. Further research for these rare species of microorganisms can be done in an underexploited and extreme habitats where they could have the ability to produce novel compounds in order to survive in the extreme conditions (Helfer and Hassenruck 2021; Zhu et al. 2020; Amin et al. 2020).

The main aim of this study was to use a twin track approach for both culture-dependent and metagenomic to investigate the microbial diversity and actinobacterial abundance. They were investigated to find rare actinomycete isolates as potential compound producer from the unique environment of Beach Ridges Interspersed with Swales (BRIS) soil as outlined in the culture-dependent bioprospecting strategy by Goodfellow and Fiedler (2010). The application of both cultivable and non-cultivable approaches supports a complementary, multi-tier bioprospecting strategy in which cultivable diversity enables direct screening for antimicrobial metabolites and chemical characterization and downstream drug development while non-cultivable diversity reveals hidden biosynthetic gene clusters (BGCs) associated with secondary metabolite production (Gerard et al. 2024; Liu et al. 2022; Moreira et al. 2011). BRIS soil from Setiu, Terengganu was selected as environment of interest due to its unique properties with poorly structured, low water retention, acidic and considered as problematic soil in agriculture (Roslan et al. 2011; Ishaq et al. 2014). This study represents a significant advancement, as only limited research has been conducted on actinomycete isolates from BRIS soil to date (Hairi et al. 2025; Majhool 2020; Mustapha et al. 2017) and notably no studies have investigated the microbial diversity of BRIS environment using metagenomics approach.

Materials and methods

Soil sampling and physicochemical characterization

Soil samples were collected from a coastal area in Setiu, Terengganu. The soil samples were collected in duplicates from four series of BRIS soil which are Rhu Tapai, Rudua, Jambu and Baging at two different spots and four different depths which are 0–20 cm, 20–50 cm, 50–70 cm and 70–100 cm were collected from each spot with the supervision and assistance by experts from Department of Agriculture Malaysia (DOA). The coordinate, local temperature and soil pH were recorded in situ during sampling activity (Table 1) while 3 kg of each soil samples were sent to Department of Agriculture Laboratory, Kuala Lumpur. Physicochemical characteristics determination including percentage of organic carbon, total nitrogen, available phosphate and cation exchange capacity (Mustapha et al. 2017). Soil samples were kept in the sterile Falcon tubes and stored in ice then transported to the laboratory in Universiti Pendidikan Sultan Idris, Perak for further investigation.

Culture-dependent approach

Selective isolation of actinomycetes from BRIS soil

Actinomycetes were isolated using soil dilution plate technique where 1 g of dried soil was placed in 9 ml of ¼ strength of Ringer's solution, agitated for 15 min and then allowed the suspension to settle for 15 min prior to serial dilution up to 10^{-4}. 0.1 ml of each sample were spread onto yeast malt agar (ISP Medium No.2) (Shirling and Gottlieb 1966), Bennett`s agar (Jones 1949), Starch M-protein agar (Küster and Williams 1964), Actinomyces agar (Kornman and Loesche 1978) and Zobell marine agar (Zobell 1941) supplemented with nystatin, nalidixic acid and cycloheximide with 25 µg ml^{-1} concentration for each antibiotic

Table 1 Soil samples from Rhu Tapai, Rudua, Jambu and baging series

Soil Series	Soil Classification *(USDA Soil Taxonomy)	Coordinate	Temperature (°C)	pH	Sampling depth (cm)	Sample name
Rhu Tapai	Sandy, siliceous, isohyperthermic, Typic Haplorthods	5°29'37.37"N 102°59'42.867"E	27	4.13	0–20	RT1
			27	4.26	20–50	RT2
			28	4.52	50–70	RT3
Baging	Coated, siliceous, isohyperthermic, Typic Quartzipsamments	5°34'21.548"N 102°51'9.004"E	31.5	4.47	0–20	B1
			30.5	4.63	20–50	B2
			30.5	4.29	50–70	B3
			29.5	4.36	70–100	B4
Jambu	Uncoated, siliceous, isohyperthermic, Typic Quartzipsamments	5°32'32.637"N 102°53'12.327"E	32	4.21	0–20	J1
			30	4.45	20–50	J2
			29	4.48	50–70	J3
			29	4.57	70–100	J4
Rudua	Sandy, siliceous, isohyperthermic, Typic Haplorthods	5°29'25.723"N 102°59'19.956"E	29	4.55	0–20	RD1
			28	4.67	20–50	RD2
			28	4.18	50–70	RD3
			28	4.22	70–100	RD4

*USDA Soil Classification (Department of Agriculture (DOA) 2018).

was used as suggested by Majhool (2020). Isolation plates were incubated for 14 days at 28 °C prior to calculation of colony forming unit (CFU) and selected actinomycete colonies were subcultured onto ISP2 plates for purification and further investigation. Purified actinomycete isolates were subcultured onto the oatmeal agar (ISP 3) and peptone-yeast extract-iron agar (ISP 6) and incubated at 30 °C for 2 weeks and 4 days respectively. Morphology observation was done for each isolate, and they were assigned into colour groups based on their aerial spore mass, substrate mycelial, diffusible pigment and melanin production according to National Bureau of Standards (NBS) Colour Name Chart (Kelly 1958).

Strain identification

Colour group representatives were selected for identification based on 16S rRNA gene sequences. Selected isolates were grown in ISP2 broth (Shirling and Gottlieb 1966) for 3 to 7 days at 30 °C with shaking and the biomass were collected by centrifugation. Genomic DNA were extracted using GeneDireX Inc, DNA Extraction Kit (GeneDireX Inc., USA) according to the manufacturer's protocol and DNA amplification of 16S rRNA gene was carried out using universal forward primer 27f (5' – AGA GTT TGA TCC TGG CTC AG – 3') and reverse primer 1525r (5'- AAG GAG GTG ATC CAG CC – 3'), respectively (Lane 1991) and MyTaq HD Red Mix (Bioline Inc. UK). Polymerase chain reaction (PCR) was performed in a thermal cycler under the following conditions: initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 2 min followed by a final extension at 72°C for 5 min. The PCR products then were purified and sequenced by New Gene Sdn. Bhd., Malaysia according to the manufacturer's protocol where Sanger sequencing was implemented.

Partial 16S rRNA gene obtained sequences were compared with full sequences available in the GenBank database using BLAST and the pairwise sequence similarities were determined by using a web server known as EzTaxon (http://eztaxon-e.ezbiocloud.net; Kim et al. 2012). Based on the sequence data, the phylogenetic analysis then performed by using MEGA 11.0 (Tamura et al. 2021) across computing platform based on Neighbour Joining, Maximum Likelihood and Maximum Parsimony algorithms. Next, bootstrap values based on 1000 repeats will be generated referring to the evolutionary distance model by Jukes and Cantor (1969).

Metagenomics approach

16S rRNA gene-based metagenome sequencing

Community DNA were extracted directly from BRIS soil samples using SPINeasy DNA Kit for Soil (MP Biomedicals Asia Pacific Pte Ltd) following the manufacturer's protocol and the quality of DNA samples was analysed by a NanoDrop spectrophotometer. The DNA extracted from the soil samples was sent to GENEWIZ, Inc. in Suzhou, China, for Next-Generation Sequencing (NGS) library preparation and Illumina Miseq sequencing where V3 specific primers were employed to amplify the V3 hypervariable region of the 16S rDNA. For library preparation,

a 10ng DNA aliquot obtained from each BRIS soil samples were used to construct the V3 hypervariable region of the 16S rDNA genes. After the amplification, the amplicons underwent purification, and then they were evenly combined to form a sequencing library. To ensure data quality, the obtained sequences were rigorously subjected to quality control standards. Sequences that did not meet these criteria were excluded from further analysis, while the remaining sequences were utilized in subsequent analytical processes.

Bioinformatics analysis

Microbiome bioinformatics analysis was conducted using the Quantitative Insights into Microbial Ecology (QIIME) software (Tohamy et al. 2019) package version 1.9.1. To represent each OTU, the most abundant sequence was selected as the representative sequence, and then these sequences were subjected to taxonomic assignment using the RDP (Ribosomal Database Project) classification software. A bootstrap cutoff of 50% was employed to ensure the robustness of taxonomic assignments. The abundance of data of these representative sequences were normalized for each sample and log transformed. The relative abundance of microbial taxa was calculated from the phylum level down to the genus level for each collection method (Ding et al. 2021).

Microbial diversity analysis

Microbial diversity analysis that consists of alpha diversity and taxonomic composition were conducted where indices including rarefaction curve was calculated on rarefied OTUs tables using Mothur version 1.35.1 (Sakyi et al. 2020) and manual analysis was performed using the data from the Operational Taxonomic Unit (OTU) table. The findings from this analysis were then presented in the form of appropriate graphs using Microsoft Excel, providing a visual representation of the taxonomic composition of the microbial communities in each BRIS soil samples (Idris 2016).

Results and discussion

Physicochemical properties of BRIS soil series

The physicochemical characteristics of Rhu Tapai, Baging, Jambu and Rudua soil series determined by soil analysis are summarised in Table 2. It is apparent from this Table that the daylight temperature is set between 27 and 32 °C with the soils are acidic between pH 4.34

Table 2 Physicochemical properties of Rhu Tapai, Baging, Jambu and Rudua BRIS soil series

BRIS Soil Series	Sample Name	Temperature (°C)	pH	Percentage		C/N Ratio	Available P	Cation Exchange Capacity				
				Organic C	Total N			CEC	Ca	Mg	K	Na
Rhu Tapai	RT1	27	4.34	9.77	0.7	18.57	7.48	4.71	0.18	0.11	0.1	0.05
	RT2	27	4.48	6.12	0.36	22.61	6.4	4.83	0.15	0.11	0.04	0.05
	RT3	28	4.55	8.02	0.35	30.49	3.9	4.92	0.08	0.06	0.03	0.04
Baging	B1	32	4.41	2.56	0.16	21.31	3.61	0.64	0.12	0.14	0.03	0.04
	B2	32	4.53	1.17	0.05	31	2.45	0.27	0.07	0.07	0.02	0.02
	B3	30	4.59	0.67	0.03	29.67	2.23	0.19	0.05	0.05	0.02	0.02
	B4	30	4.67	0.54	0.02	36	2.01	0.26	0.04	0.03	0.01	0
Jambu	J1	32	4.47	5.31	0.23	30.7	3.6	2.96	0.8	0.26	0.03	0.05
	J2	30	4.48	1.22	0.04	40.5	1.91	0.44	0.22	0.07	0.01	0.02
	J3	29	4.55	0.82	0.03	36.33	1.73	0.21	0.08	0.03	0.01	0.01
	J4	29	4.58	0.7	0.03	31	1.78	0.16	0.06	0.02	0	0
Rudua	RD1	29	4.43	20.45	0.88	30.91	6.86	3.26	0.28	0.26	0.03	0.05
	RD2	28	4.67	4.51	0.19	31.58	2.5	0.33	0.07	0.05	0.02	0.03
	RD3	28	4.72	2.92	0.11	35.36	1.79	0.19	0.06	0.04	0.01	0.02
	RD4	28	4.62	11.46	0.44	34.64	3.76	2.59	0.04	0.03	0.02	0.03

to 4.72. The percentage of carbon and nitrogen as well as cation exchange capacity showed low value especially in the subsurface soil samples. The findings of this current study are consistent with those of Roslan et al. (2011), Ishaq et al. (2014) and Mustapha et al. (2017) who reported the acidic properties of BRIS soil and other parameters are classified as very low to low. It is suggested that the most limiting factors that classify BRIS as unhealthy soil are their soil texture and nutrient retention that were influenced by movement (Ishaq et al. 2014).

Culture-dependent approach

Estimation of culturable bacteria

Viable bacteria that consist of actinomycetes and other bacterial strains were successfully isolated in a selective isolation experiment portrayed by the CFU number ranging from 2.0×10^3 to 1.44×10^6 on ISP2, Bennet's, Actinomyces, Starch M protein and Marine medium used as shown in Fig. 1. Among four BRIS soil series studied, Baging showed the highest CFU number particularly on ISP2 agar with 1.44×10^6 CFU/g followed by Jambu series with the highest of 7.6×10^5 CFU/g. Rudua and Ru Tapai series showed a relatively low number of bacteria obtained with the highest CFU number of 1.3×10^5 CFU/g by Rhu Tapai and 2.4×10^5 by Rudua series.

Information obtained from CFU number of each BRIS soil series indicate the presence of microbial community and the potential diversity of culturable bacteria inhabiting the soil. This study produced results which corroborate the findings of previous selective isolation for actinomycetes experiment conducted by Majhool (2020) that reported CFU number between 3.1×10^4 to 3.1×10^5. In comparison with CFU number of other type of soil namely sandy soil (2.5×10^6), organic soil (4.2×10^6), forest soil (1.1×10^6 to 2.5×10^6), eucalyptus soil (5.25×10^6 to 2.0×10^6), sorghum soil (1.0×10^6 to 1.25×10^6), sandy soil (2.5×10^6), organic soil (4.2×10^6), tropical soil

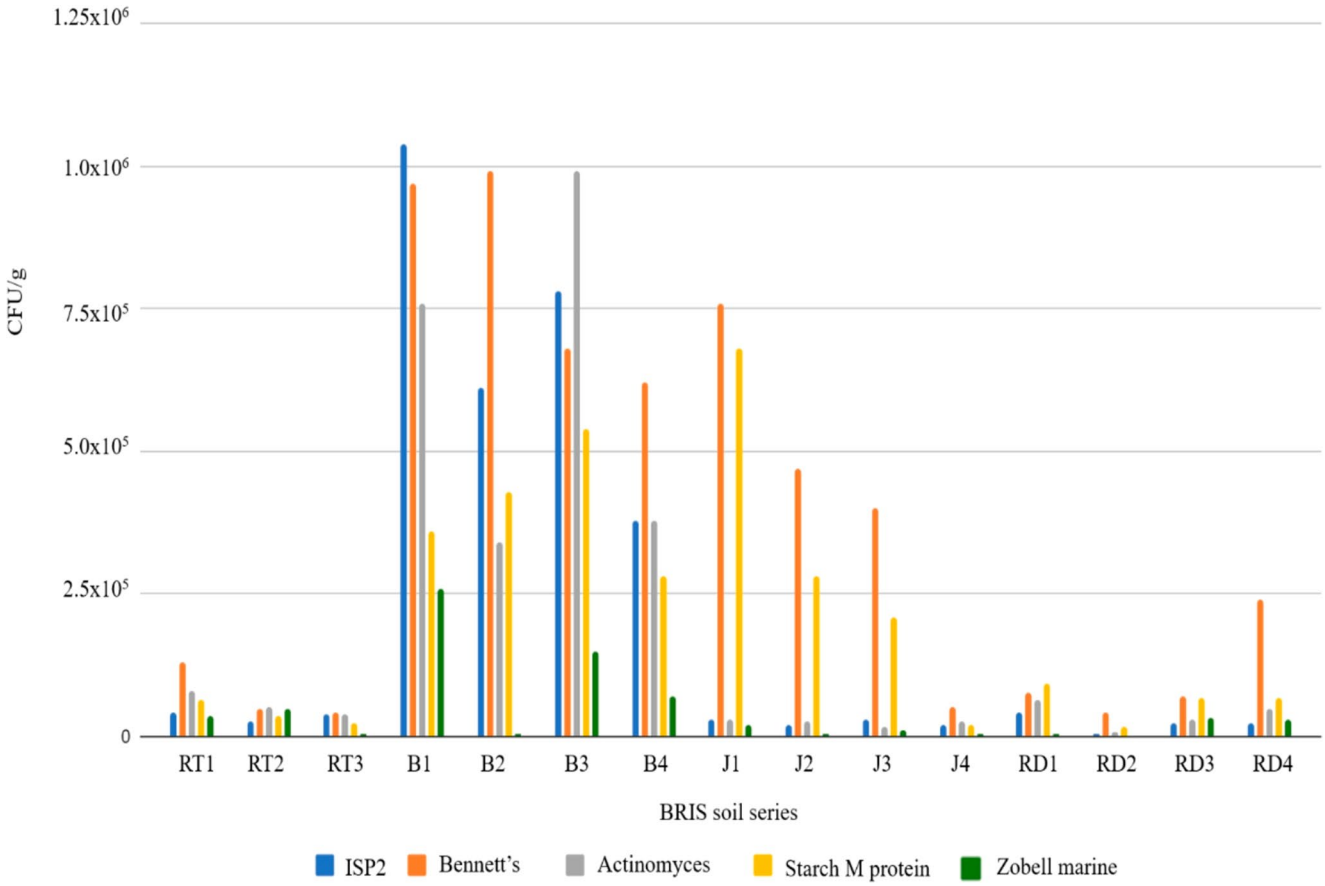

Fig. 1 Colony forming unit (CFU/g) of bacteria isolated from Rhu Tapai, Baging, Jambu and Rudua series on different isolation medium

(6.5×10^7 to 1.28×10^8) and subtropical soil (7.2×10^7 to 9.8×10^7) it can be seen that the CFU number of isolated bacteria from BRIS soil rather low (Singh and Tripathi 2020; Vieira and Nahas 2005; Øvreås and Torsvik 1998). However, despite being considered as problematic in agriculture due to its physical characteristics and presence of nutrients in small quantities that limit the ability in promoting crop growth (Norhayati et al. 2019; Mustapha et al. 2017; Ishaq et al. 2014; Roslan 2012; Roslan et al. 2011), it is evident that BRIS soil holds a potential to harbours their own unique culturable microbial diversity yet to be explored.

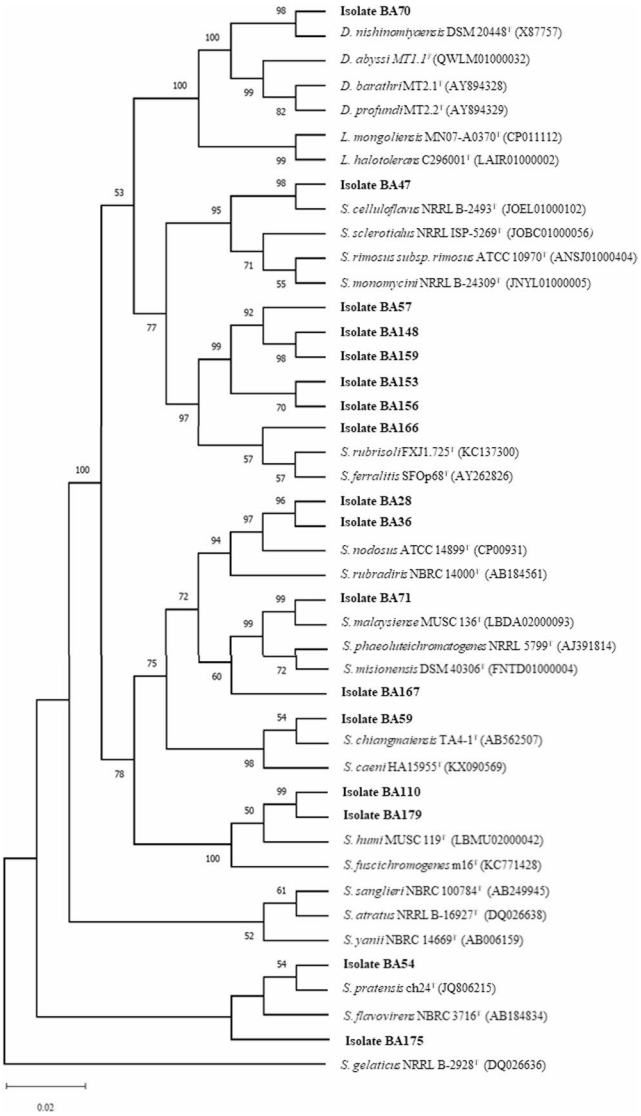

Fig. 2 Neighbour-joining tree based on the 16S rRNA gene sequences showing the relationship of 15 actinomycete isolates, between them and related species from *Dermacoccus*, *Luteipulveratus* and *Streptomyces* genera. Number at the nodes indicate levels of bootstrap support based on 1000 resampled datasets; only values above 50% is shown. The scale bar indicates 0.02 substitutions per nucleotide position

Colour group assignment of actinomycete isolates

Based on colony morphological characteristics on ISP3 medium, it is found that majority isolates showed filamentous actinomycete characteristics with ability to produce spores. All 180 actinomycete isolates were obtained from the 4 series of BRIS soil samples were assigned into 69 colour groups that consist of 34 single membered and 35 multi membered colour groups which are in accordance with previous study by Majhool (2020) where from 113 isolates, 72 colour groups were formed with 31 single membered and 41 multi membered. Pridham (1965) reported that colour systems are the most practically effective to allow placement is actinomycete isolates into well-defined categories prior to further exploration using various expand systems and methods. As each colour group indicate presumably different type of actinomycete genera based on colony morphology and melanin production, the information derived from this experiment are critical as the reliable indicators of diverse actinomycetes genera diversity in BRIS soil microbial community (Idris 2016; Antony-Babu and Goodfellow 2008).

Phylogenetic analysis of 16S rRNA gene sequences

A total of 15 colour group representatives were selected for identification based on 16S rRNA gene sequences. Analysis revealed that 14 of the isolates belong to genus *Streptomyces* while 1 isolate belongs to genus *Dermacoccus* (Fig. 2). It seems that majority of the identified isolates belong to genus *Streptomyces* which is in agreement with Goodfellow et al. (2012) in Bergey's Manual of Determinative Microbiology that highlighted *Streptomyces* as the largest genus in Actinomycetales family. This is due to the fact that they have the ability to form extensively branched substrate mycelia covered by abundant aerial spore mass and their ability to survive in numerous different environment (Donald et al. 2022). On the contrary, identification of isolate BA70 as a member of genus *Dermacoccus*, one of actinomycete rare genus. According to Parte et al. (2020) to date, there are only 4 species described that belong to *Dermacoccus* genus which are *D. abyssi*, *D. barathi*, *D. nishinomiyaensis* and *D. profundi* since it was first introduced in 1995 (Pathom-aree et al. 2006a, b; Stackebrandt et al. 1995). This finding, while preliminary, suggests that exposed BRIS soil as the habitat of rare actinomycete genera with the potential of finding novel species and new drug leads.

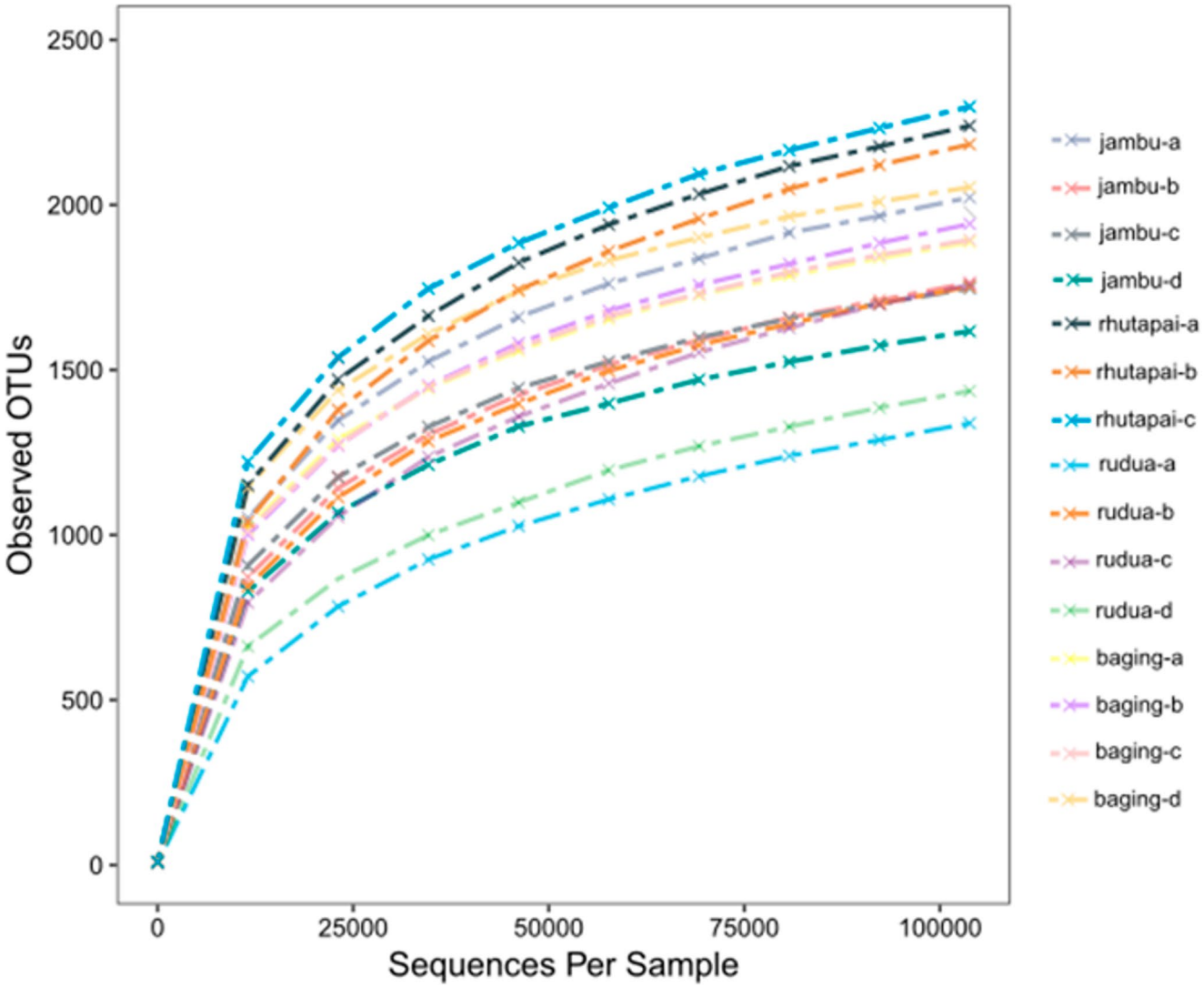

Fig. 3 Rarefaction curve of BRIS OTUs showing relation between the increase in bacterial OTUs and the number of randomly sampled sequence in the BRIS soil samples

Metagenomics approach

Metagenomic sequencing data

Based on the data obtained from MiSeq-Illumina technology, 2,108,260 raw reads were acquired from 15 samples of the 4 BRIS soil series and a total number of reads passing quality filtering was 1,958,604 high quality sequences. The refraction analysis conducted to enable comparison between taxonomic richness and estimate the extent to which total diversity has been recovered showed that all the BRIS soil samples reached asymptote which indicates the number of sampled sequences was sufficient with the total number OTU of 4719 (Fig. 3).

Taxonomic composition of actinomycetes community in BRIS soil

In this study, analysis of OTUs from BRIS soil samples on different taxonomic units unveiled microbial community composition that consist of 4719 species, 380 genera, 247 families, 166 orders, 76 classes, and 32 phyla bacteria. From 380 genera detected, 105 (28%) genera were assigned as 'uncultured' which indicate the presence of unclassified or unknown bacteria present in the studied soil samples. Classification of these microbial community members as uncultured due to their genomic sequences that match with available information in genome database that were obtained using metagenomic approach (Arikawa

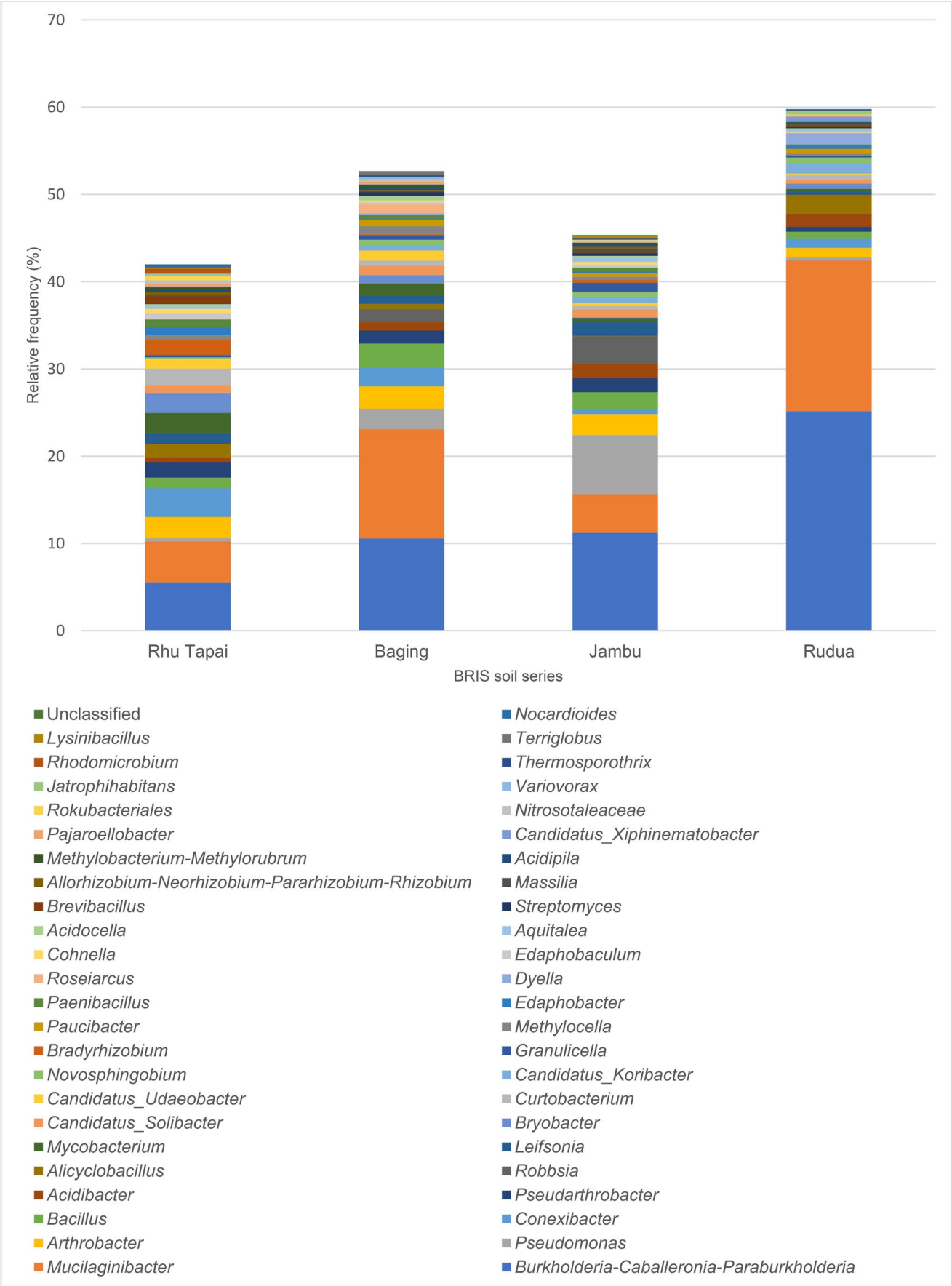
70
60
50
40
30
20
10
0
Relative frequency (%)
Rhu Tapai
Baging
Jambu
Rudua
BRIS soil series
Unclassified
Lysinibacillus
Rhodomicrobium
Jatrophihabitans
Rokubacteriales
Pajaroellobacter
Methylobacterium-Methylorubrum
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Brevibacillus
Acidocella
Cohnella
Roseiarcus
Paenibacillus
Paucibacter
Bradyrhizobium
Novosphingobium
Candidatus_Udaeobacter
Candidatus_Solibacter
Mycobacterium
Alicyclobacillus
Acidibacter
Bacillus
Arthrobacter
Mucilaginibacter
Nocardioides
Terriglobus
Thermosporothrix
Variovorax
Nitrosotaleaceae
Candidatus_Xiphinematobacter
Acidipila
Massilia
Streptomyces
Aquitalea
Edaphobaculum
Dyella
Edaphobacter
Methylocella
Granulicella
Candidatus_Koribacter
Curtobacterium
Bryobacter
Leifsonia
Robbsia
Pseudarthrobacter
Conexibacter
Pseudomonas
Burkholderia-Caballeronia-Paraburkholderia

Fig. 4 Relative abundance of microbial community detected in BRIS soil series at genus level

and Hasokawa 2023). Figure 4 presents relative abundance of the top 100 bacterial genera detected in all four BRIS soil series where genus *Burkholderia-Caballeronia-Paraburkholderia* is found to be the highest OTU detected followed by *Mucilaginibacter*. *Burkholderia-Caballeronia-Paraburkholderia* species show broad environmental adaptability and an advantage in acidic soils. For example, studies have demonstrated genus-wide acid tolerance and competitive advantage in low pH soils, suggesting acid tolerance is a general trait of *Burkholderia-Caballeronia-Paraburkholderia* that influences its occurrence in different environments (Dludlu et al. 2017; Stopnisek et al. 2014). *Mucilaginibacter*, on the other hand, is frequently isolated from acidic soils and plant-associated environments and is characterized by the production of extracellular polysaccharides, which may confer protection against low pH stress. While these genera are not exclusively acidophilic, their repeated detection in acidic ecosystems suggests a degree of acid tolerance rather than incidental presence (Kumar et al. 2022, 2025).

From the figure, it can be seen that Rhu Tapai has the lowest microbial genera composition followed by Baging, Jambu and Rudua. Further investigation on actinobacterial diversity at genus level revealed that from all genera detected, 83 of them are actinomycetes in which 47 of them are culturable actinomycetes and the remaining 36 are uncultured. To investigate the distribution of actinomycetes in Rhu Tapai, Baging, Jambu and Rudua series, relative abundance analysis is set out in Fig. 5. The most striking result emerge from this data is that the highest relative abundance of actinomycete genera across Rhu Tapai, Baging and Jambu soil series consist of *Acidothermus* while *Arthrobacter* was found highest in Rudua soil series. Although, these results differ from previous studies that found abundance of *Streptomyces* in different type of soils as reported by Idris et al. (2017), Manikkam et al. (2020) and Girao et al. (2024), they correlate with the acidic characteristic of BRIS soil and the findings by Mhete et al. (2020). On top of that, this analysis revealed the abundance of uncultured actinomycetes in all soil series that corresponds with concept of unexplored habitat harbour rare species of microorganisms with unique characteristics as suggested by Goodfellow and Fiedler (2010). Arikawa and Hasokawa (2023) pointed out that despite the growing numbers of uncultured prokaryotic genomic data available in the database, limited information is known beyond their DNA sequences.

Actinobacterial diversity in BRIS soil

Taken together, identification results at genus level provide important insights into the real actinobacterial community composition that populate in Rhu Tapai, Baging, Jambu and Rudua soil series as a rare genus of *Acidothermus* was found to be in highest abundance from metagenomic approach. In contradiction, *Streptomyces* was dominantly obtained in selective isolation experiment based on colour group and phylogenetic analysis. According to Parte et al. (2020) currently there is only one *Acidothermus* species described called *Acidothermus cellulolyticus* by Mohagheghi et al. (1986) which was isolated from acidic hot spring. Though the results are not consistent in both approaches, they open a new direction for research in microbial ecology field particularly on BRIS soil and taxonomy and systematics of actinomycetes. Looking at the effect of soil pH that concur with actinobacterial genera abundance discovered using metagenomic method, it is apparent that physicochemical characteristics of BRIS soil had influenced their structure along with other environmental pressures of low level of nutrients as presented in Table 1. This is supported by Mhete et al. (2020) who pointed out that apart of pH, other physicochemical parameters influenced the influence of microbial community structure and diversity in different type of soils studied.

Conclusion

Despite being regarded as extreme soil environment, it is evident that BRIS soil has the ability to provide habitat for unique microbial communities. This study highlights the first investigation attempt in elucidating microbial diversity inhabiting BRIS soil using both culture-dependent and culture independent approaches. In culture-dependent approach, actinomycetes were successfully isolated that give some insights of the bacterial composition and diversity of culturable microbes while metagenomic analysis unveil the true microbial community composition and diversity to a greater extent that include both culturable and unculturable microbes information. Looking at the effect of soil pH that correlate with actinobacterial genera abundance discovered using metagenomic method, it is apparent that physicochemical characteristics of BRIS soil had influenced their structure along with other environmental pressure of low level of nutrients as showed by our data.

Considering the uniqueness of microbial composition and potential to harbour new actinomycete species, BRIS soils deserve some attention to be delve deeper as the groundbreaking findings in this study indicates

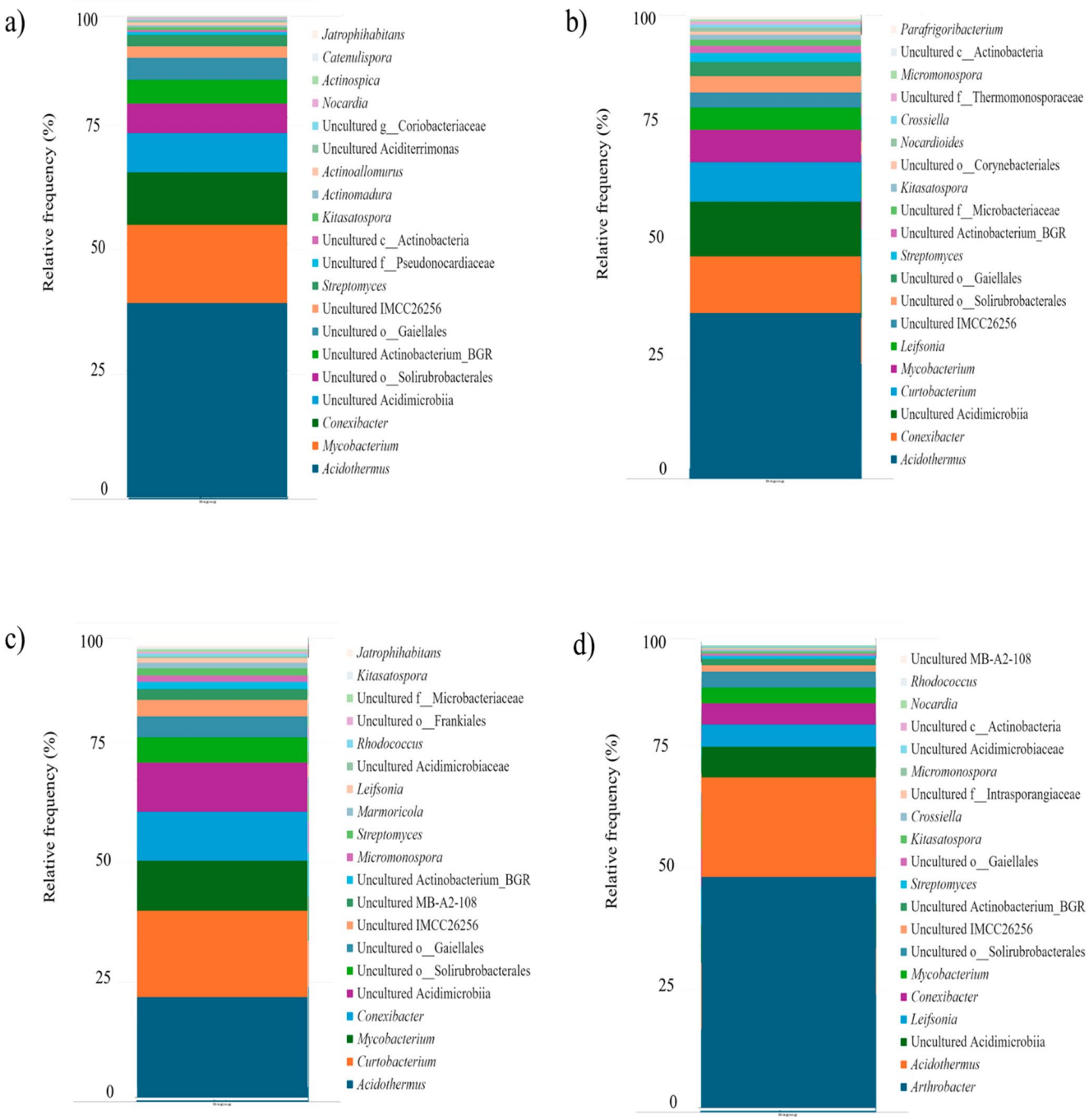

Fig. 5 Relative abundance of top 20 actinomycete genera detected in BRIS soil series: (**a**) Rhu Tapai (**b**) Baging (**c**) Jambu and (**d**) Rudua

the vast diversity of unidentified actinomycetes and potential of novel species awaiting to be discovered from BRIS soil environment. Four BRIS soil series were used in this study; thus, it is worthwhile for scientists to call attention to conduct investigation on other series and locations. With the aim to uncover more novel strains with biotechnological properties due to adaptation in BRIS soil environment, future work should be directed towards isolation and identification of more isolates which metagenomic data can be used in genomic-directed selective isolation of actinomycetes and other types of bacteria.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval The collection and analysis of BRIS soil samples was obtained in collaboration with the Department of Agriculture Malaysia (DOA), ensuring compliance with all environmental guidelines.

Consent for publication All authors approved the manuscript.

Competing interests The authors declare no competing interests.

References

Amin A, Ahmed I, Khalid N, Khan IU, Ali A, Dahlawi SM, Li WJ (2020) Insights on comparative bacterial diversity between different arid zones of Cholistan Desert, Pakistan. 3 Biotech 10(5):224

Antony-Babu S, Goodfellow M (2008) Biosystematics of alkaliphilic streptomycetes isolated from seven locations across a beach and dune sand system. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 94(4):581–591. https://doi.org/10.1007/s10482-008-9277-4

Arikawa K, Hosokawa M (2023) Uncultured prokaryotic genomes in the spotlight: an examination of publicly available data from metagenomics and single-cell genomics. Comput Struct Biotechnol J 21:4508–4518. https://doi.org/10.1016/j.csbj.2023.09.010

Bodor A, Bounedjoum N, Vincze GE, Kis AE, Laczi K, Bende G, Szilagyi A, Kovacs T, Perei K, Rakhely G (2020) Challenges of unculturable bacteria: environmental perspectives. Rev Environ Sci Biotechnol 19:1–22. https://doi.org/10.1007/s11157-020-09522-4

Department of Agriculture (DOA) (2018) Common soils of Peninsular Malaysia-Soil profile description and analytical data, 1st edn. Department of Agriculture, Ministry of Agriculture, Malaysia

Ding Z, Wang W, Zhang K, Ming F, Yangdai T, Xu T, Shi H, Bao Y, Yao H, Peng H, Han C, Jiang W, Liu J, Hou X, Lin R (2021) Novel scheme for non-invasive gut bioinformation acquisition with a magnetically controlled sampling capsule endoscope. Gut 2021 70:2297–2306

Dludlu MN, Chimphango SBM, Stirton C, Muasya AM (2017) Differential preference of *Burkholderia* and *Mesorhizobium* to pH and soil types in the Core Cape Subregion, South Africa. Genes (1):2. https://doi.org/10.3390/genes9010002

Donald L, Pipite A, Subramani R, Owen J, Keyzers RA, Taufa T (2022) Streptomyces: still the biggest producer of new natural secondary metabolites, a current perspective. Microbiol Res 13:418–465. https://doi.org/10.3390/microbiolres13030031

Gerard CMA, Cambiazo V, Gonzalez M (2024) Biosynthetic gene clusters from uncultivated soil bacteria of the Atacama Desert. mSphere 9(10):e0019224. https://doi.org/10.1128/msphere.00192-24

Girao M, Alexandrino DAM, Cao W, Costa I, Jia Z, Carvalho MF (2024) Unveiling the culturable and non-culturable actinobacterial diversity in two microalgae species from the Northers Portuguese Coast. Environ Microbiol e16620:1–15. https://doi.org/10.1111/1462-2920.16620

Goodfellow M, Fiedler HP (2010) A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek 98(2):119–142

Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki K, Ludwig W, Whitman WB (2012) Bergey's Manual of Systematic Bacteriology, 2nd edn. Springer, New York

Hairi HH, Ahmad A, Azman MAZ, Idris H, Abdullah MDD, Zin NM (2025) Actinobacterial diversity determination in Malaysian BRIS (beach ridges interspersed with swales) soil by culture-dependent approach. Jurnal Teknologi UTM 87(1). https://doi.org/10.11113/jurnalteknologi.v87.22358

Helfer V, Hassenruck C (2021) Microbial communities in mangrove sediments. Environ Sci. https://doi.org/10.1016/B978-0-12-816437-2.00003-3

Idris H (2016) Actinobacterial diversity in Atacama Desert habitat as a road map to biodiscovery. PhD. Thesis. Newcastle University, Newcastle Upon Tyne, United Kingdom

Idris H, Goodfellow M, Sanderson R, Asenjo JA, Bull AT (2017) Actinobacterial rare biospheres and dark matter revealed in habitats of the Chilean Atacama Desert. Sci Rep 7:8373

Ishaq UM, Umara B, Armanto HME, Adzemi MA (2014) BRIS soil suitability assessment on sweet potato in Merang-Terengganu region of Malaysia. J Biol Agric Healthc 4(7):1–19

Jaswal R, Pathak A, Edwards IIIB, Lewis IIIL, Seaman JC, Stothars P, Krivushin K, Blom J, Rupp O, Chauhan A (2019) Metagenomics-guided survey, isolation, and characterization of uranium resistant microbiota from the Savannah River site, USA. Genes 35:1–22

Jones KL (1949) Fresh isolates of actinomycetes in which the presence of sporogenous. J Bacteriol 57(2):141–145

Jukes TH & Cantor CR (1969) Evolution of protein molecules. Mammalian Protein Metabolism. Academic Press, New York, pp 21–132

Kelly KL (1958) Central notations for the revised iscc-nbs color-name blocks. J Res Natl Bur Stand 61(5):427

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing eztaxon-e: a prokaryotic 16s rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62(PART 3):716–721. https://doi.org/10.1099/ijs.0.038075-0

Kornman KS, Loesche WJ (1978) New medium for isolation of *Actinomyces viscosus* and *Actinomyces naeslundii* from dental plaque. J Clin Microbiol 7(6):514–518. https://doi.org/10.1128/jcm.7.6.514-518.1978

Kumar A, Mannisto MK, Patsi M, Kerkhof LJ, Haggblom MM (2025) Genome analysis reveals diverse novel psychrotolerant *Mucilaginibacter* species in Arctic tundra soils. ISME Commun. https://doi.org/10.1093/ismeco/ycaf071

Kumar A, Mukhia S, Kumar R (2022) Production, characterisation, and application of exopolysaccharide extracted from a glacier bacterium *Mucilaginibacter* sp. ERMR7:07. Process Biochem 113:27–36. https://doi.org/10.1016/j.procbio.2021.12.018

Küster E, Williams ST (1964) Selection of media for isolation of streptomycetes. Nature 202:928–929

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons, Chichester, United Kingdom, pp 115–175

Liu SW, Jadambaa N, Nikandrova AA, Osterman IA, Sun CH (2022) Exploring the diversity and antibacterial potentiality of cultivable actinobacteria from the soil of the saxaul forest in Southern Gobi Desert in Mongolia. Microorganisms. https://doi.org/10.3390/microorganisms10050989

Majhool AA (2020) Actinomycetes from BRIS soil and their secondary metabolites. MSc. Thesis. Universiti Pendidikan Sultan Idris, Tanjong Malim, Perak, Malaysia

Manikkam R, Imchen M, Kaari M, Angamuthu V, Venugopal G, Thangavel S, Joseph J, Ramasamy B, Kumavath R (2020) Metagenomic insights unveil the dominance of undescribed Actinobacteria in pond ecosystem of an Indian shrine. Meta Gene 23:100639. https://doi.org/10.1016/j.mgene.2019.100639

Mhete M, Eze PN, Rahube TO, Akinyemi FO (2020) Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. Sci Afr 7:e00246. https://doi.org/10.1016/j.sciaf.2019.e00246

Mohaghehi A, Grohmann K, Himmel M, Leighton L, Updegraff DM (1986) Isolation and characterization of *Acidothermus cellulolyticus* sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. Int J Syst Evol Microbiol 36(3):435–443. https://doi.org/10.1099/00207713-36-3-435

Moreira IV, Egas C, Nunes OC, Manaia CM (2011) Culture-dependent and culture-independent diversity surveys target different bacteria: a case study in a freshwater sample. Antonie Van Leeuwenhoek 100(2):245–257

Mustapha Z, Mat N, Othman R, Zakaria AJ (2017) Quantification of BRIS soil bacteria at Tembila, Besut Terengganu. AGRIVITA, Journal of Agricultural Science 39(3):252–256

Norhayati Y, Ng WH, Adzemi MA (2019) Effects of organic fertilizers on growth and yield of roselle (*Hibiscus sabdariffa l.*) on BRIS soil. Malays Appl Biol 48(1):177–184

Øvreås L, Torsvik V (1998) Microbial diversity and community structure in two different agricultural soil communities. Microb Ecol 36:303–315

Parte AC, Sardà JC, Meier-Kolthoff JP, Reimer LC, Göker M (2020) List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol MicroBiol 70:5607–5612

Pathom-aree W, Nogi Y, Sutcliffe IC, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006a) D*ermacoccus abyssi* sp. nov., a piezotolerant actinomycete isolated from the Mariana Trench. Int J Syst Evol Microbiol 56:1233–1237

Pathom-aree W, Nogi Y, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006b) *Dermacoccus barathri* sp. nov. and *Dermacoccus profundi* sp. nov., novel actinomycetes isolated from deep-sea mud of the Mariana Trench. Int J Syst Evol Microbiol 56:2303–2307

Pridham TG (1965) Color and streptomycetes. Report of an international workshop on determination of color of streptomycetes. Appl Microbiol 13(1):43–61

Roslan I (2012) Occurrence, properties and suitability of sandy beach ridges (BRIS) soils in Kelantan-Terengganu plains, Peninsular Malaysia. PhD thesis, University Putra Malaysia

Roslan I, Shamshuddin J, Fauziah CI, Anuar AR (2011) Fertility and suitability of the spodosols formed on sandy beach ridges interspersed with swales in the Kelantan-Terengganu plains of Malaysia for Kenaf production. Malaysian J Soil Sci 15(1):1–24

Sakyi ME, Cai J, Tang J, Abarike ED (2020) Effects of starvation and subsequent re-feeding on intestinal microbiota, and metabolic responses in Nile tilapia, *Oreochromis niloticus*. Aquacult Rep 17:1–2

Schneider YK (2021) Bacterial natural product drug discovery for new antibiotics: strategies for tackling the problem of antibiotic resistance by efficient bioprospecting. Antibiotics (Basel) 10(7):842. https://doi.org/10.3390/antibiotics10070842

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16(3):313–340

Singh NS, Tripathi SK (2020) Temporal changes in soil microbial diversity of tropical and sub-tropical forests of Mizoram, Northeast India. Environ Ecol 38(1):31–37

Stackebrandt E, Koch C, Gvozdiak O, Schumann P (1995) Taxonomic dissection of the genus micrococcus Kocuria gen. nov., Nesterenkonia gen. nov., Kytococcus gen. nov., dermacoccus gen. nov., and micrococcus Cohn 1872 gen. emend. Int J Syst Bacteriol 45:682–692

Stopnisek N, Bodenhausen N, Frey B, Fierer N, Eberl L, Weisskopf L (2014) Genus-wide acid tolerance accounts for the biogeographical distribution of soil burkholderia populations. Environ Microbiol 16(6):1503–1512. https://doi.org/10.1111/1462-2920.12211

Subramani R, Sipkema D (2019) Marine rare actinomycetes: a promising source of structurally diverse and unique novel natural products. Mar Drugs 17(5):249

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38(7):3022–3027

Tohamy ME, Azmy A, Muhammed R, Mahmoud EAB (2019) QIIME: quantitative insights into microbial ecology

Trenozhnikova L, Azizan A (2018) Discovery of actinomycetes from extreme environments with potential to produce novel antibiotics. Cent Asian J Glob Health 7(1):337. https://doi.org/10.5195/cajgh.2018.337

Vieira FCS, Nahas E (2005) Comparison of microbial numbers in solid by using various culture media and temperatures. Microbiol Res 160:197–202

WHO (2023) Antimicrobial resistance. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Zhu X, Jackson RD, DeLucia EH, Tiedje JM, Liang C (2020) The soil microbial carbon pump: from conceptual insights to empirical assessments. Glob Change Biol 26(11):6032–6039. https://doi.org/10.1111/gcb.15319

Zobell CE (1941) Studies on marine bacteria: the cultural requirements of heterotrophic aerobes. J Mar Res 4(1)