

# Application of Principal Component Analysis (PCA) to the Evaluation and Screening of Multiactivity Fungi

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**Abstract** Continued innovation in screening methodologies remains important for the discovery of high-quality multiactive fungi, which have been of great significance to the development of new drugs. Mangrove-derived fungi, which are well recognized as prolific sources of natural products, are worth sustained attention and further study. In this study, 118 fungi, which mainly included *Aspergillus* spp. (34.62%) and *Penicillium* spp. (15.38%), were isolated from the mangrove ecosystem of the Maowei Sea, and 83.1% of the cultured fungi showed at least one bioactivity in four antibacterial and three antioxidant assays. To accurately evaluate the fungal bioactivities, the fungi with multiple bioactivities were successfully evaluated and screened by principal component analysis (PCA), and this analysis provided a dataset for comparing and selecting multibioactive fungi. Among the 118 mangrove-derived fungi tested in this study, *Aspergillus* spp. showed the best comprehensive activity. Fungi such as *A. clavatonanicus*, *A. flavipes* and *A. citrinoterreus*, which exhibited high comprehensive bioactivity as determined by the PCA, have great potential in the exploitation of natural products and the development of new drugs. This study demonstrated the first use of PCA as a time-saving, scientific method with a strong ability to evaluate and screen multiactive fungi, which indicated that this method can affect the discovery and development of new drugs.

**Key words** principal component analysis; biological activity; fungi; mangrove ecosystem; activity evaluation

## 1 Introduction

Fungi, as important compartments of microbial communities in mangroves, constitute the second largest part of marine fungi (Bhimba *et al.*, 2012). Mangrove-derived fungi show unique metabolic pathways, reproductive systems, and sensory and defense mechanisms because they have adapted to extreme environments with high concentrations of salt and moisture, frequent low and high tides and muddy and anaerobic conditions (Cheng *et al.*, 2009; Behera *et al.*, 2017; Guo *et al.*, 2018). Hence, mangrove-derived fungi are widely recognized as prolific sources of natural products with unique structures and high bioactivity. These natural products have been of great significance to the development of new drugs (Cui

*et al.*, 2017). Sun *et al.* 2018 reported that borrelidin H isolated from *Streptomyces rochei* SCSIO ZJ89, which originated from mangrove-derived sediment samples, has a therapeutic window superior to that of borrelidin A which has great potential as a new anticancer drug *in vitro* and could inhibit the migration of cancer cells. Bibi *et al.* (2020) concluded the articles published from 1990 to 2019, and observed that antimicrobial (48.9%) and antioxidant (12.2%) assays were identified as the two preferred assays for testing mangrove-derived fungi.

The identification of new fungi and compounds through increasing studies has become increasingly difficult (Wei *et al.*, 2017). To overcome this issue, the one-strain-many-compounds (OSMAC) strategy (Bode *et al.*, 2002; Scherlach *et al.*, 2010), co-culture (Bao *et al.*, 2017), precursor addition (Wang *et al.*, 2011) and genomics-based approaches (He *et al.*, 2018) are rapidly developing based on known active fungi for new natural product discovery. Using the OSMAC strategy, Meng *et al.*

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(2015, 2017) isolated twelve novel compounds with antimicrobial activity from the mangrove endophyte *Penicillium brocae* MA-231, which has been confirmed to be a bioactive fungus. Zhang *et al.* (2017) isolated five new compounds with  $\alpha$ -glucosidase inhibitory activity together with twelve known compounds through the co-cultivation of the mangrove endophytic fungus *Trichoderma* sp. 307 with the aquatic pathogenic bacterium *Acinetobacter johnsonii* B2. Oakley *et al.* (2017) isolated two novel compounds and an antibiotic not previously reported to be produced by *Aspergillus nidulans* by deleting the *mcrA* gene, which was generally overexpressed in *A. nidulans*. Although these approaches could be used to find more natural products produced by fungi, they were established based on known high-activity fungi. Hence, continued innovation in sampling and screening methodologies remains important for the discovery of high-activity fungi (Luo *et al.*, 2014).

It is obvious that high-quality multiactive fungi are crucial to the discovery of new natural products. Compared with the technologies developed for isolating compounds, the methodologies for activity screening are relatively weak. Most researchers have discovered fungi by evaluating their activity using only one indicator (Saito *et al.*, 2018), and this analysis could be considered incompletely. Abdalla *et al.* (2020) advanced this methodology to test fungi with potential antimicrobial, extracellular enzymatic and phosphate-solubilizing activities. However, few studies have performed a comprehensive evaluation of the activities of these fungi. The evaluation and comparison of the comprehensive activity of many multiactive fungi at the same time would be more difficult.

To resolve this issue, our study constitutes the firstly use of principal component analysis (PCA) for the screening of fungi with the highest comprehensive activity in scientific and expedient manner. PCA is a simple and effective statistical tool that is widely used in dimensionality reduction and factorial analysis of high-dimension datasets (Asante-Okyere *et al.*, 2020). Datasets with several correlated variables are decomposed into a smaller number of linearly independent variables by PCA (Mahmoudi *et al.*, 2021). Hence, we used PCA to evaluate multiactive fungi and compare their antibacterial and antioxidant activities with the aim of identifying the fungus with the highest comprehensive activity.

## 2 Materials and Methods

### 2.1 Isolates and Specimens

Mangrove ecosystem specimens were collected from Maowei Sea, Qinzhou City, Guangxi Province, P. R. China, and these samples included sediment, seawater, dead leaves and plant roots. The specimens were placed in sterilized zip-lock plastic bags on which the collection details were noted and transported to the laboratory under freezing conditions. The isolation media, potato dextrose agar (PDA) (Hernandez-Restrepo *et al.*, 2017) and malt

extract agar (MEA; Merck, Germany), are well suited (Chomnunti *et al.*, 2014) for the purification of cultured fungi after sterilization at 121°C for 20 min (Er *et al.*, 2015). Each specimen was placed on the surface of the media with a sterilized loop in triplicate and incubated in an incubator at 29°C. The mixture of cultured fungi was then transferred to new PDA or MEA plates for purification by picking up single colonies using a sterilized needle. All the strains were maintained at the Key Laboratory of Marine Chemistry Theory and Technology of Ocean University of China (Qingdao, Shandong, China).

### 2.2 Sequencing and Identification of Strains

Genomic DNA was extracted from fungal mycelium growing on PDA or MEA using the FastDNA kit (MP Biomedicals, CA, USA) following the manufacturer's protocols. Their identification was based on a molecular genetic analysis using the internal transcribed spacer (ITS) region (Al-Hindi *et al.*, 2017). The primers were forward ITS1 (TCCGTAGGTGAACCTGCGG) and reverse ITS4 (TCCTCCGCTTATTGATATGC). The PCR mixture consisted of 5  $\mu$ L of Ex Taq buffer, 2  $\mu$ L of dNTPs (2.5 mmol L<sup>-1</sup>), 0.2  $\mu$ L of TaKaRa Ex Taq, 1  $\mu$ L of genomic DNA as the template, 1  $\mu$ L (10  $\mu$ mol L<sup>-1</sup>) of the forward primer, 1  $\mu$ L (10  $\mu$ mol L<sup>-1</sup>) of the reverse primer and ddH<sub>2</sub>O to obtain a final volume of 50  $\mu$ L. The conditions for the PCR of ITS genes constituted of an initial denaturation step for 5 min at 94°C, 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 min, and a final extension step of 10 min at 72°C. The amplified PCR products were first visually examined in a 1% agarose gel stained with FloroSafe DNA stain using a gel documentation system (AlphaImager HP, CA, USA). The PCR products were sequenced using an ABI Prism 3730xl DNA Analyzer (Applied Biosystems, CA, USA) according to the manufacturer's instructions. The amplified DNA sequences were used to retrieve consensus sequences from GenBank, and the fungal species were confirmed after a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### 2.3 Metabolite Extraction

Each strain was cultured in 500-mL Erlenmeyer flasks containing 200 mL of liquid medium which consisted of 2 g of peptone, 2 g of malt extract, 10 g of glucose, 4 g of glucose and 1 L of distilled water. After fermentation for 21 days at 29°C, the broth and mycelia were separated by filtration using cotton gauze (Sheik and Chandrashekar, 2018). The fermentation broth was extracted twice with 100 mL of ethyl acetate. The ethyl acetate was then evaporated under reduced pressure to obtain a crude extract. A stock solution of the extract was prepared at a concentration of 1 mg mL<sup>-1</sup> in methanol for antioxidant and antibacterial assays. After lyophilization, mycelia were extracted twice with 25 mL of acetone:methanol (V:V = 1:1). The rest of the extraction process was the same as that used for the broth.

## 2.4 Antioxidant Assays

2,2-Diphenyl-2-picrylhydrazyl radical scavenging (DP PHRS) (Pripdeevech and Machan, 2011; Shekhar and Anju, 2014), hydroxide radical scavenging (HRS) (Bruins, 2002) and cupric reducing antioxidant capacity (CUP RAC) (Apak *et al.*, 2004) assays were used to evaluate the antioxidant activity of the crude extracts. All reactions were then performed in triplicate in 96-well microtiter plates and measured using a microplate reader (Multiskan FC, Thermo Scientific, China). Ascorbic acid (Vc) and methanol were used as the standard and blank, respectively.

### 2.4.1 DPPH radical scavenging assay

Extract stock solution (160  $\mu\text{L}$ ) and 40  $\mu\text{L}$  of a DPPH solution (0.15  $\text{mol L}^{-1}$ ) were added to a 96-well plate. The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance of the mixture was then measured at 517 nm (Sinurat *et al.*, 2018). The percent (%) radical scavenging capacity (RSC) was calculated using the following equation (Atiphasaworn *et al.*, 2017):

$$\text{RSC} = \frac{A-S}{A} \times 100\%, \quad (1)$$

where  $A$  is the absorbance of the control and  $S$  is the absorbance of the sample.

### 2.4.2 HRS assay

The HRS assay was conducted as follows: 50  $\mu\text{L}$  of  $\text{FeSO}_4$  (3  $\text{mmol L}^{-1}$ ) was reacted with 50  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  (2.5  $\text{mmol L}^{-1}$ ) to produce hydroxide radicals. Ten minutes later, 50  $\mu\text{L}$  of the sample extract was added to the mixture. To prepare the blank, the sample was replaced by methanol. After 10 min, 50  $\mu\text{L}$  of salicylic acid (3  $\text{mmol L}^{-1}$ ) was added to detect the residual hydroxide radicals. The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 520 nm. The HRS capacity was then calculated using the same method for determining the DPPH radical scavenging capacity.

### 2.4.3 CUPRAC assay

The CUPRAC assay was based on the reduction of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$  by the combined action of antioxidants (Yamashita *et al.*, 1998). Neocuproine (Nc, 2,9-dimethyl-1,10-phenanthroline hemihydrate) is a chromogenic and selective complex with  $\text{Cu}^+$  (maximum absorbance at 455 nm.) (Roohparvar *et al.*, 2018). The CUPRAC assay was conducted using the crude extracts with the method described by Apak *et al.* (2004). First, 50  $\mu\text{L}$  of ammonium acetate (1  $\text{mol L}^{-1}$ ) was added to the reaction system as a buffer to maintain a neutral pH, and then, 50  $\mu\text{L}$  of  $\text{CuCl}_2$  (2  $\text{mmol L}^{-1}$ ) and 50  $\mu\text{L}$  of stock solution were successively added. After 10 min, 50  $\mu\text{L}$  of Nc- methanol solution (1.5  $\text{mmol L}^{-1}$ ) was added to the system, and 30 min later, the solution absorbance was measured at 455 nm.

The absorbance was then converted to units of Vc using a standard curve (Fig.1), and the results are expressed as equivalents to the concentration of Vc.

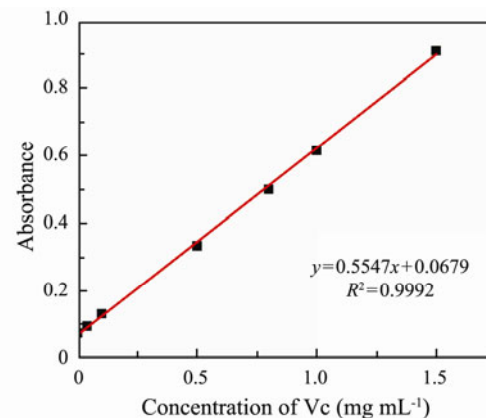


Fig.1 The typical standard curve in the CUPRAC assay.

## 2.5 Antibacterial Assay

Four human pathogenic bacteria, namely, gram-negative *Escherichia coli* (CMCC 44102), *Staphylococcus aureus* (CMCC(B) 26003), *Micrococcus luteus* (CMCC (B) 28001) and *Salmonella Paratyphi B* (CMCC(B) 50094), were used for bioassays based on the paper disk diffusion method (Mar and Pripdeevech, 2014). These bacteria were pre-cultured in sterile beef extract peptone broth (beef extract, 0.3 g; peptone, 1 g; NaCl, 0.5 g; distilled water, 100 mL; pH 7.4–7.6) at 37°C for 48 h. Sterilized 6-mm paper disks (Whatman<sup>TM</sup>, USA) were soaked in the extract solution for 24 h. Air-dried paper disks were placed in triplicate on beef extract peptone agar plates containing the bacteria. Paper disks saturated with methanol and kanamycin were used as negative controls and reference controls, respectively. To confirm the antibacterial activity, the diameter of the clear zone was measured after 24 h of incubation at 37°C.

## 2.6 Statistical Analysis

Each experiment was performed in triplicate. The data are expressed as the means  $\pm$  standard deviations. The comprehensive activity was evaluated by combining the antioxidant activity of the mycelial and broth extracts and the antibacterial activity of the mycelia. PCA was performed using SPSS.v23 (<https://www.ibm.com/analytics/spss-statistics-software>). The dependency matrix was analyzed using the Kaiser-Meyer-Olkin (KMO) test and Bartlett's method of sphericity. Data extraction was performed based on a normalized PCA using the quartimax rotation method with varimax rotation and Z-score normalization (Vandermarken *et al.*, 2018). A composite score ( $F$ ) was used to evaluate the comprehensive activity of the fungi and was calculated with the following equation:

$$F = \sum_{i=1}^n p_i \beta_i, \quad (2)$$

where  $p_i$  is the score of a principal component (PC) and

$\beta_i$  is the variance in the corresponding PC.

### 3 Results

#### 3.1 Fungal Diversity

A total of 118 different fungal species identified by colony morphology and molecular biology were separated, purified and successfully cultured. These belonged to 30 genera, 22 families, 11 orders, six classes and three phyla. The phyla Basidiomycota, Zygomycota and Ascomycota accounted for 2.54%, 9.32% and 88.14%, respectively (Fig.2). In the Basidiomycota phylum, only three fungal species were listed as belonging to one class,

two orders, two families and two genera. The Zygomycota phylum included 11 species that belonged to one class, one order, six families (one incertae sedis) and six genera (one incertae sedis). The Ascomycota phylum, which exhibited the maximum count and highest diversity, included four classes, eight orders, 14 families, 22 genera and 104 species. The *Aspergillus* genus recorded the highest count (34.62%), followed by the *Penicillium* genus (15.38%), *Fusarium* genus (9.62%), *Trichoderma* genus (9.62%) and *Talaromyces* genus (5.77%). The genera *Aspergillus*, *Penicillium* and *Talaromyces* constituted the largest class of Eurotiomycetes among all fungi isolated from mangrove ecosystems.

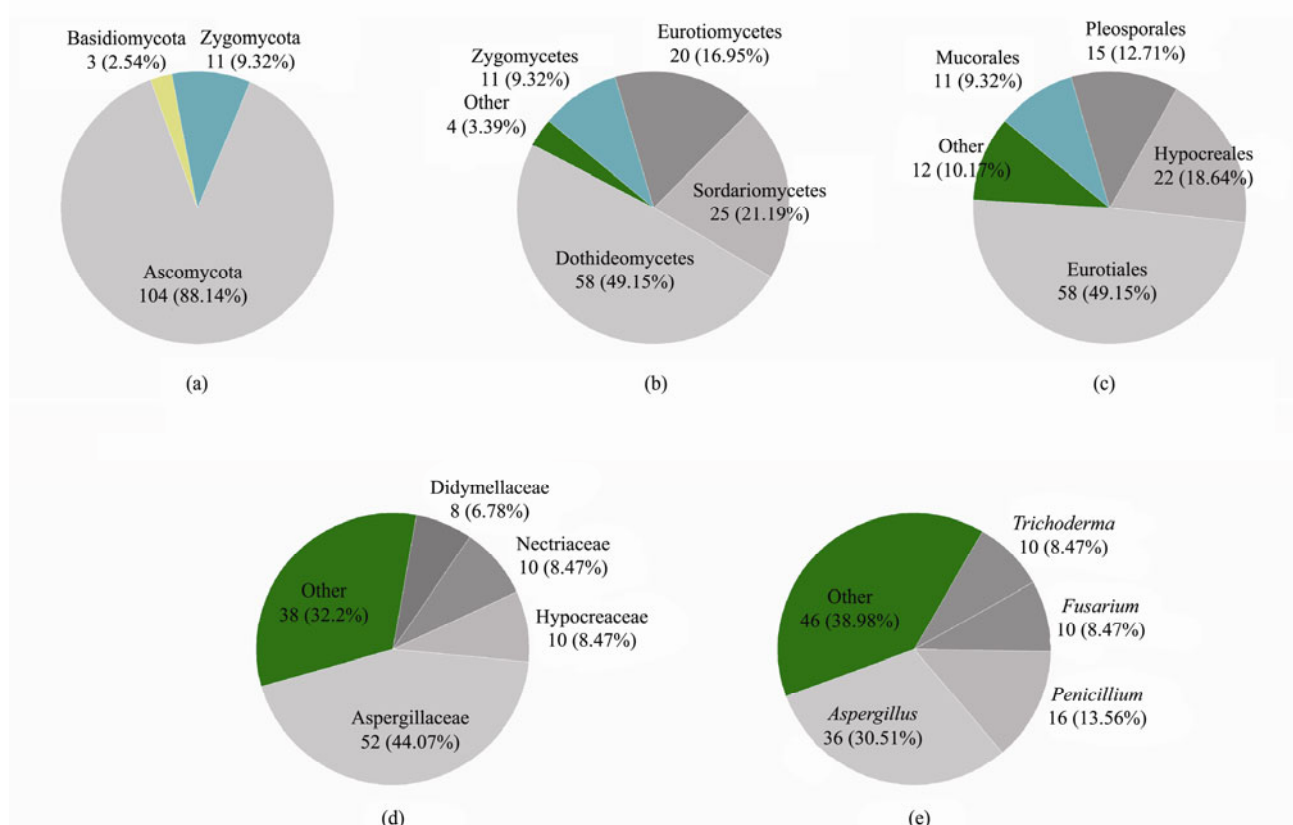


Fig.2 Numbers of dominant fungi at the phylum (a), class (b), order (c), family (d) and genus (e) levels.

#### 3.2 Statistical Analysis of Activity

Seven activity bioassays, namely, DPPHRS (A1), HRS (A2), CUPRAC (A3) assays and inhibition assays against *Salmonella Paratyphi B* (A4), *Escherichia coli* (A5), *Staphylococcus aureus* (A6) and *Micrococcus luteus* (A7), were selected to test the activities of crude broth and mycelial extracts from each fungus. Among the crude broth extracts, 69.5% and 26.3% extracts showed DPPH and hydroxide RSCs higher than 90%, respectively (Fig.3). Eight and 11 crude broth extracts could completely scavenge DPPH and hydroxide radicals, respectively, and 34.7% of fungal crude broth extracts ( $1.00 \text{ mg mL}^{-1}$ ), which showed better antioxidant capacity than the positive control, showed values equal to or higher than those of  $1.00 \text{ mg mL}^{-1}$  Vc. The number of crude mycelial extracts with antioxidant activity, particularly HRS activity,

was less than that of the crude broth extracts (Fig.3). Only three fungal crude mycelial extracts could scavenge hydroxide radicals, with values reaching 90%, and none could completely scavenge hydroxide radicals. In addition, 10.2% of the fungal crude mycelial extracts could scavenge DPPH radicals, with values reaching 90%. Only the crude mycelial extract from *Nectria haematococca* could completely scavenge DPPH radicals. In addition, 7.6% of the fungal crude mycelial extracts ( $1.00 \text{ mg mL}^{-1}$ ) showed better antioxidant capacity than Vc. The antibacterial activity results showed that 29.6% of the crude broth extracts were resistant to at least one of the tested bacteria, and no crude mycelial extracts showed antibacterial activity. The crude broth extracts of *Aspergillus terreus*, *Aspergillus clavatonanicus*, *Aspergillus clavatus* and *Aspergillus giganteus* showed broad-spectrum antibacterial activity against all four human pathogenic bacteria.

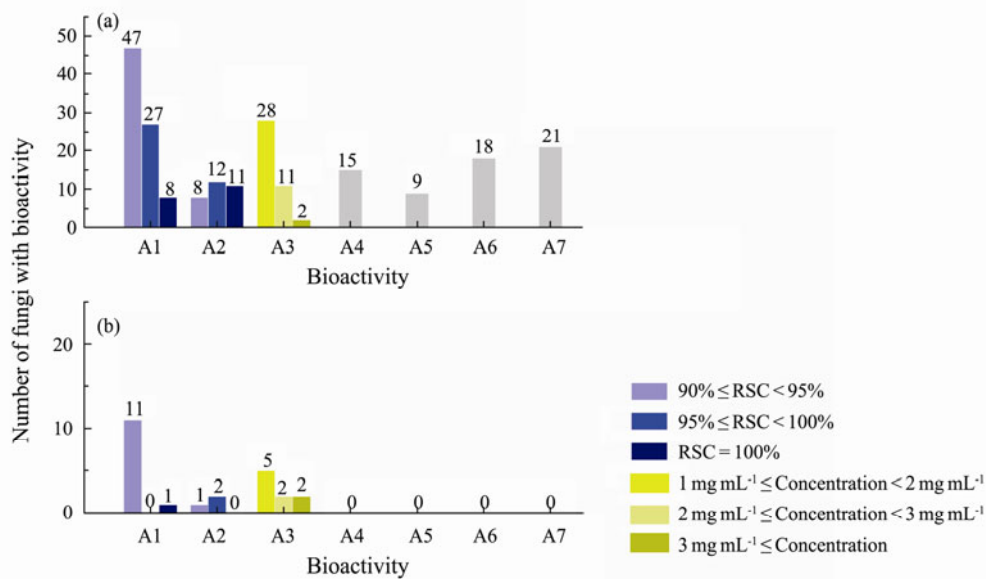


Fig.3 Number of fungi with bioactivity observed in the broth extracts (a) and mycelium extracts (b). A1–A3 represent the antioxidant activity analyses based on the DPPHRS, HRS and CUPRAC assays, respectively. The A1 and A2 results are represented as the percent (%) radical scavenging capacity (RSC). The A3 results are expressed as the concentration of Vc equivalent to 1.00 mg mL<sup>-1</sup> extract. Vc (A1=97%, A2=95%, A3=1.00 mg mL<sup>-1</sup>) and methanol (A1=0%, A2=0%, A3=0 mg mL<sup>-1</sup>) were used as the standard and blank, respectively. A4–A7 represent the analyses of the antibacterial activities against *Salmonella paratyphi B*, *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus luteus*. Paper disks saturated with methanol (A4=0 mm, A5=0 mm, A6=0 mm, A7=0 mm) and kanamycin (A4=27.8 mm, A5=15.2 mm, A6=25.0 mm, A7=18.9 mm) were used as negative and reference controls, respectively.

### 3.3 Principal Component Analysis

The dataset with ten variables (three variables for broth antioxidant activity, four variables for broth antibacterial

Table 1 Statistical results of the PCA and information of the first three PCs

Parameter	PC1	PC2	PC3
Eigenvalues	3.35	2.20	1.10
Variances	0.2706	0.2105	0.1835
Variance (cumulative)	0.2706	0.4811	0.6646
KMO		0.714	
Bartlett's sphericity test		P << 0.5	

Notes: The available PCA results were statistically tested using the Kaiser-Meyer-Olkin (KMO) test (>0.7) and Bartlett's sphericity test (P << 0.5).

activity and three variables for mycelium antioxidant activity) was decomposed into a smaller number of linearly independent variables by PCA (Fig.4). The first three PCs named PC1, PC2 and PC3 were selected as significant because their eigenvalues were larger than 1 (Kaiser, 1960). The dependency matrix was tested by the Kaiser-Meyer-Olkin test (KMO > 0.7) and Bartlett's method of sphericity (P << 0.05). The results showed that 66.46% of the dataset variance was explained by PC1, PC2 and PC3 (Table 1). The PC scores were calculated as the product of the input data matrix and the eigenvector matrix, which represents the strength of each PC for each of the observed points (Martini *et al.*, 2017). The comprehensive activity of a fungal strain was evaluated and represented by *F*, which was calculated using the following equations:

$$F = 0.2706P_1 + 0.2105P_2 + 0.1835P_3, \tag{3}$$

$$P_1 = -0.012A_{b1} + 0.201A_{b2} + 0.140A_{b3} + 0.750A_{b4} + 0.866A_{b5} + 0.836A_{b6} + 0.787A_{b7} + 0.081A_{m1} - 0.042A_{m2} + 0.091A_{m3}, \tag{4}$$

$$P_2 = 0.737A_{b1} + 0.531A_{b2} + 0.782A_{b3} - 0.070A_{b4} + 0.004A_{b5} + 0.180A_{b6} + 0.261A_{b7} + 0.506A_{m1} - 0.034A_{m2} + 0.552A_{m3}, \tag{5}$$

$$P_3 = -0.121A_{b1} + 0.272A_{b2} + 0.131A_{b3} + 0.201A_{b4} - 0.110A_{b5} + 0.020A_{b6} - 0.057A_{b7} + 0.728A_{m1} + 0.865A_{m2} + 0.628A_{m3}, \tag{6}$$

where *P*<sub>1</sub>, *P*<sub>2</sub> and *P*<sub>3</sub> represent the scores of PC1, PC2 and PC3, respectively, *A*<sub>b1</sub> to *A*<sub>b7</sub> represent the normalized results of the A1–A7 analyses of the crude broth extracts, and *A*<sub>m1</sub> to *A*<sub>m3</sub> represent the normalized results of the A1–A3 analyses of the crude mycelial extracts.

## 4 Discussion

This study demonstrated that mangrove-derived fungi

could be recognized as prolific sources of bioactive natural products and involved the first application of PCA to evaluate multiactive fungi and identify the fungi with the best comprehensive bioactivity. The screened fungi with



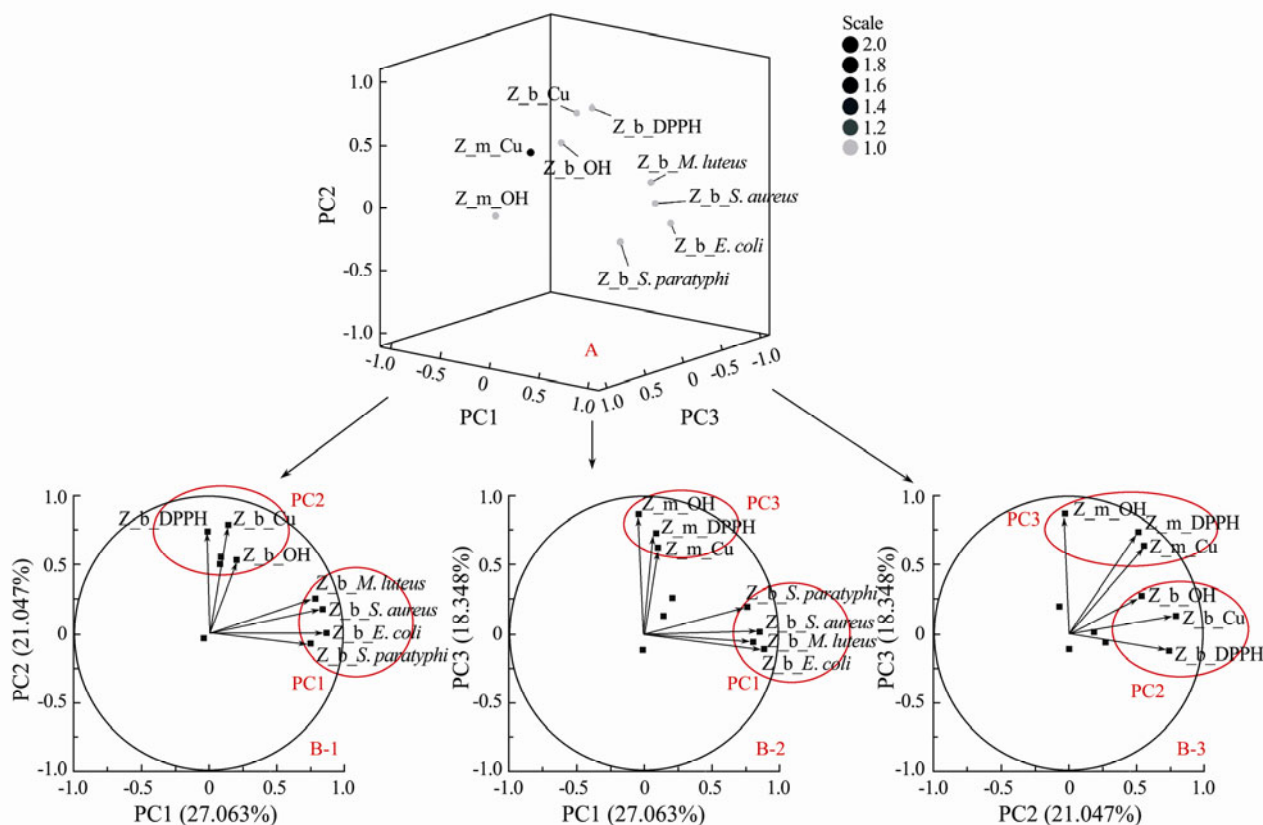


Fig.4 PCA loading plots obtained after varimax rotation of the whole dataset. The factor loadings represent the correlations between the original variables and the principal components. A shows the correlations in three dimensions. B-1, B-2 and B-3 show PC1 vs. PC2, PC1 vs. PC3 and PC2 vs. PC3 in two dimensions, respectively. The prefix Z represents the data normalized by the Z-score. The prefixes b and m represent the activities of the broth and mycelium, respectively. The further away a variable lies from the origin, the stronger its influence on the PCs. For example, the antibacterial activities of broth extracts against *S. paratyphi*, *E. coli*, *S. aureus* and *M. luteus* were strongly correlated with PC1. Therefore, PC1 could represent the antibacterial activities of broth extracts. Similarly, PC2 and PC3 represent the antioxidant activity of broth and mycelial extracts, respectively.

bioactivity could be used for the isolation of natural products with potential antioxidant and antibacterial activities. These fungi had great potential in the development of new drugs.

Most of the cultured mangrove-derived fungi showed high antioxidant and antibacterial activities and are worth further study. Ascomycota, which included the most abundant species, accounted for 88.14% of the 118 cultured fungal species isolated from mangrove ecosystems. Jones *et al.* (2009) tested 530 marine-derived fungi and reported that Ascomycota is the most dominant phylum in marine fungi. Monika and Rohit have also demonstrated that Ascomycota is the most dominant phylum and is found in various environments (Sharma and Sharma, 2016). Among the 118 fungi, the *Aspergillus* spp. (30.51%) was the most common, followed by the *Penicillium* spp. (13.56%). *Aspergillus* genus and *Penicillium* genus have been widely reported as the two major bioactive fungal genera in mangrove ecosystems (Kumaresan and Suryanarayanan, 2001; Ananda and Sridhar, 2002; Liu *et al.*, 2007). *Aspergillus* spp. have been widely reported to accumulate high amounts of organic acids and utilize a number of carbon sources in the natural environment (Brown *et al.*, 2013; Yang *et al.*, 2016). In addition,

lots of bioactive secondary metabolites produced by *Aspergillus* were used in industrial applications such as food additives, pharmaceuticals and detergents (Yang *et al.*, 2017). In *Penicillium* spp. study, there was a well known antibiotics group compounds of penicillin (Reschke and Schügerl, 1984). 29.6% of the crude broth extracts were found to exhibit resistance to at least one of the tested bacteria. However, no crude mycelia extracts showed antibacterial activity due to *in vitro* rejection (Huband *et al.*, 2015). Fungi can secrete secondary metabolites to face complicated environments and protect themselves from infection. Hence, the bioactivity of broth extracts was usually better than that of mycelium extracts (Fig.3). Among the 118 cultured fungi, only 20 fungal species did not show any bioactivity (the RSCs of DPPH and hydroxide were less than 90%, the crude extracts at a concentration of 1.00 mg mL<sup>-1</sup> were equivalent to Vc at a concentration lower than 1.00 mg mL<sup>-1</sup>, and the diameter of the clear zone was 0). In addition, 83.1% of the mangrove-derived fungi showed at least one bioactivity in the A1–A7 analyses. Rahmawati *et al.* (2019) confirmed that mangrove-derived fungi produce secondary metabolites with bioactivities similar to those of the original plants or even relatively high activities. Mangrove-derived fungi

could be recognized as prolific sources of bioactive natural products.

However, the evaluation and screening of fungi with the best comprehensive bioactivity were difficult. The extracts of *Penicillium simplicissimum* without any antioxidant activity showed high antibacterial activity against *S. Paratyphi B*, *E. coli* and *S. aureus* with clear zone diameters of  $(19.7 \pm 0.2)$  mm,  $(19.3 \pm 0.1)$  mm and  $(36.3 \pm 0.1)$  mm, respectively. *Penicillium camponotum* showed specific antibacterial activity against *S. Paratyphi B* with a clear zone diameter of  $(23.1 \pm 0.8)$  mm. *Aspergillus pseudonimus* showed specific antibacterial activity against *S. aureus* with a clear zone diameter of  $(15.8 \pm 0.2)$  mm. Broth extract of *Cochliobolus kusanoi* could completely scavenge DPPH radicals but did not show any other bioactivity. *Trichoderma harzianum* and *Cochliobolus lunatus* yielded the same results as *Cochliobolus kusanoi*. Although *Trichosporon faecale* showed better DPPHRS activity ( $95 \pm 1\%$ ) and improved CUPRAC (equivalent to  $(3.13 \pm 0.19)$  mg mL<sup>-1</sup> Vc) than *Aspergillus fumigatus* (DPP HRS:  $86\% \pm 1\%$ , CUPRAC: equivalent to  $(0.63 \pm 0.03)$  mg mL<sup>-1</sup> Vc), *A. fumigatus* showed better HRS activity ( $100 \pm 0\%$ ) than *T. faecale* (HRS:  $29\% \pm 7\%$ ). Furthermore, some fungi, such as *Aspergillus gorakhpurensis*, *Aspergillus tubingenis*, *Penicillium ochrochloron* and *Penicillium limosum*, only exhibited antioxidant activity in the broth extracts, whereas only the mycelial extracts of other fungi, such as *Mucor circinelloides* and *Penicillium citrioviride*, showed antioxidant activity. Zhou *et al.* (2018) and Chi *et al.* (2019) also reported that most mangrove-derived fungi show antioxidant and antibacterial activities but did not perform any comparisons or evaluations of fungal bioactivity. In short, the evaluation fungal bioactivity using only one activity indicator was imprecise. All activity indicators should be taken into consideration for the evaluation of fungal bioactivity and screening fungi with the best comprehensive bioactivity.

PCA, which has been used in dimensionality reduction and factorial analysis of high-dimension datasets, was found to be suitable for the evaluation of the comprehensive bioactivity of fungi and allow the comparison and ranking of fungal activity. The new distribution axes, which called principal components (PCs), preserved most of the information of the sample variance by summarizing the features of the original dataset (Ringnér, 2008; Corradi *et al.*, 2020). The PCA loading plots (Fig.4) displayed the relationship between the original variables and the principal components, and the orthogonal vectors in the loading plots indicated the degree of correlations among variables. Hence, PC1, PC2 and PC3 were used in this study as new activity indicators to individually evaluate the antibacterial activities of the broth extracts, the antioxidant activities of the broth extracts and the antioxidant activities of the mycelial extracts. The PC scores and *F* were successfully calculated to individually evaluate the corresponding bioactivities and the comprehensive bioactivity. The rankings of the fungi based on their activities were extremely clear according to the PC scores and *F* values, which allowed the comparison of multiple fungal

activities.

The activity evaluation indexes  $P_1$ ,  $P_2$ , and  $P_3$  were used to evaluate the antibacterial activities of broth extracts, antioxidant activities of both extracts and antioxidant activities of mycelial extracts. According to the PC1 score, *Aspergillus* spp. showed the highest antibacterial activity. Among the top 20 genera ranked in terms of antibacterial activity, 15 belonged to the *Aspergillus* genus, and the others were *Penicillium simplicissimum* (ranked 5th), *Fusarium fujikuroi* (ranked 8th), *Absidia corymbifera* (ranked 9th), *Penicillium camponotum* (ranked 10th) and *Xenomyrothecium tongaense* (ranked 17th). *Aspergillus clavatonanicus* showed the highest antibacterial activity against *S. paratyphi*, *E. coli*, *S. aureus* and *M. luteus*, with clear zone diameters of  $(27.0 \pm 0.6)$  mm,  $(26.6 \pm 0.7)$  mm,  $(28.5 \pm 0.2)$  mm and  $(32.9 \pm 0.4)$  mm, respectively. With respect to antioxidant activity, each phylum had a representative strain among the top 20 genera. The broth extract with the highest ranking was obtained from *Nectria haematococca*, which could completely scavenge DPPH under the experimental conditions. With respect to Cu<sup>2+</sup> reduction, the extract at a concentration of 1.00 mg mL<sup>-1</sup> was found to be equivalent to  $(2.83 \pm 0.03)$  mg mL<sup>-1</sup> Vc. The mycelial extract with the highest antioxidant activity was obtained from *Aspergillus niger*, and this extract exhibited strong abilities to scavenge DPPH ( $91\% \pm 1\%$ ) and hydroxyl groups ( $97\% \pm 6\%$ ) and a moderate ability to reduce Cu<sup>2+</sup>. The PC1, PC2 and PC3 scores were successfully used to evaluate the corresponding activities.

The comprehensive activity of a fungal strain was evaluated and represented by *F*. Among the 118 mangrove-derived fungi, *Aspergillus* spp. showed the best comprehensive activity. The analysis of the *F* values showed that 16 fungi among the top 20 strains in terms of comprehensive activity were *Aspergillus* spp., and the other four strains were *Nectria haematococca* (ranked 9th), *Penicillium simplicissimum* (ranked 12th), *Acremonium cellulyticus* (ranked 13th) and *Trichosporon faecale* (ranked 16th). *Aspergillus clavatonanicus* exhibited the highest comprehensive activity, followed by *Aspergillus flavipes* and *Aspergillus citrinoterreus*. The comprehensive bioactivity of fungi was expediently and accurately evaluated by *F* (Eq. (1)), which considers all activity indicators.

The fungi with high comprehensive bioactivity identified based on *F* values were worth further study. *A. clavatonanicus* has often been isolated and reported as an endophytic fungus with high antimicrobial activity, and Huang reported its activity against the mycelial growth of two phytopathogens, *Peronophythora litchi* and *Rhizoctonia solani*, with inhibition rates of 98.16% and 79.34%, respectively (Huang *et al.*, 2010). Mishra showed that *A. clavatonanicus* exhibits strong antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* with minimum inhibitory concentrations (MICs) of 0.078, 0.156 and 0.312 mg mL<sup>-1</sup>, respectively (Mishra *et al.*, 2017). Twenty-eight volatile compounds and known antibiotics, such as ampicillin, streptomycin, chloramphenicol, rifampicin, miconazole, ketokonazole

and fluconazole, have been detected in the secondary metabolite pool produced by *A. clavatonanicus* (Mishra *et al.*, 2017). Mishra concluded that *A. clavatonanicus* could be exploited not only in the development of biocontrol agents for crop disease management but also as a sustainable and alternative resource for the discovery of potent antimicrobial metabolites based on the detection of beta-ketosynthase domains in polyketide synthase gene clusters and adenylation domains in nonribosomal peptide synthase gene clusters. In this study, *A. clavatonanicus* not only showed strong antibacterial activity but also effectively scavenged DPPH and hydroxyl radicals with scavenging rates of  $95\% \pm 1\%$  and  $88\% \pm 3\%$ , respectively. In  $\text{Cu}^{2+}$  reduction,  $1.00 \text{ mg mL}^{-1}$  broth extract of *A. clavatonanicus* was equivalent to  $(1.77 \pm 0.09) \text{ mg mL}^{-1}$  Vc. The fungus *A. flavipes* have been well known for producing an abundance of metabolites, such as pectinase (Martínez-Trujillo *et al.*, 2011), butyrolactones (Nagia *et al.*, 2012), aspochalasin (Kwon *et al.*, 2012) and L-methioninase (El-Sayed, 2011), which endowed the fungus with antioxidant and antimicrobial activities (Guo and Wang, 2017; Si *et al.*, 2018). Guo and Wang employed a Plackett–Burman design and a Box–Behnken design to optimize the fermentation conditions of *A. flavipes* and reported the optimized production of substances with antibacterial activity against aquatic pathogenic *V. harveyi* from *A. flavipes* with potential for aquatic applications (Guo and Wang, 2017). Furthermore, Si *et al.* (2018) used the OSMAC strategy to isolate cytotoxic cytochalasins, which could be potential antitumor drug candidates, from *A. flavipes* and found that these exhibited significant cytotoxic activities against three human cancer cell lines (THP1, HL-60 and PC3) with  $\text{IC}_{50}$  values ranging from  $3.00$  to  $15.10 \mu\text{mol L}^{-1}$ . *A. citrinoterreus* without any reported bioactivity was first identified and named by Guinea *et al.* (2015). In our study, *A. citrinoterreus* showed strong antioxidant activity and moderate antibacterial activity. Its broth extract showed a high RSC for DPPH and hydroxyl radicals, with values of  $95 \pm 3\%$  and  $96 \pm 3\%$ , respectively, and at a concentration of  $1.00 \text{ mg mL}^{-1}$ , this extract was equivalent to  $(3.57 \pm 0.07) \text{ mg mL}^{-1}$  Vc. This broth extract also showed antibacterial activity against *Staphylococcus aureus* and *Micrococcus luteus*, as demonstrated by their formation of inhibition zones with diameters of  $(21.2 \pm 1) \text{ mm}$  and  $(12.0 \pm 1.3) \text{ mm}$ , respectively. The fungi identified based on *F*, which showed higher multibioactivity, showed great potential in the exploitation of natural products and the development of new drugs.

To extend the method, the PCs and *F*-values of the 118 fungi will be used as a database for comparing normalized activity data reported in the future as long as the same activity assays will be used in the analyses. Values of PCs and *F* greater than 0 indicate that the corresponding fungal activity is better than average level, and values greater than 1 indicate that the corresponding fungi exhibit high activity performance and are worth further research. Furthermore, the activity bioassays can be replaced by other analyses to establish a database for screening

fungi with desired activity. Hence, PCA is a time-saving, scientific method with a strong ability to evaluate and screen multiactive fungi.

## 5 Conclusions

Mangrove-derived fungi were diverse and bioactive. In the mangrove ecosystem of the Maowei Sea, Ascomycota was the most dominant phylum, and *Aspergillus* and *Penicillium* were the two major genera. For accurate evaluation of fungal bioactivity, PCA was successfully employed to evaluate and screen high-quality fungi with bioactivity. The results showed that PCA allowed the scientific comparison of multiactive fungi. Among the 118 tested mangrove-derived fungi, *Aspergillus* spp. showed the best comprehensive activity. Fungi such as *A. clavatonanicus*, *A. flavipes* and *A. citrinoterreus*, which exhibited high comprehensive bioactivity as demonstrated by PCA, showed great potential in the exploitation of natural products and the development of new drugs. Furthermore, a database that included information on the activities of 118 fungi was provided such that other researchers could conveniently compare fungal activities in the future work. The application of PCA provides a scientific basis for evaluating and screening fungi with multiple activities, and this method thus affects the discovery and development of new drugs. Mangrove-derived fungi recognized as prolific sources of natural products are worth sustained attention and further study.

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