Yeasts from Nanfeng mandarin plants: occurrence, diversity and capability to produce indole-3-acetic acid

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ABSTRACT

Plant growth promoters produced by microorganisms can play a significant role in the induction of some important physiological responses in the growth and development of plants. In this study, we provided a first insight into revealing the diversity of cultivable yeasts associated with Nanfeng mandarin (Citrus reticulata cv. Blanco) in China. Their capability to produce indole-3-acetic acid (IAA) was analyzed. A total of 796 yeast strains were obtained by the enrichment isolation technique from citrus soil, citrus leaves, citrus peel and citrus pulp of Nanfeng mandarin samples. On the basis of the 26S rDNA partial sequence analysis, the strains were identified as 14 yeast species in 9 genera belonging to Hanseniaspora sp., Pichia sp., Candida sp., Sporidiobolus sp., Meyerozyma sp., Symmetriospora sp., Rhodotorula sp., Starmerella sp. and Aureobasidium sp. The most abundant species in citrus soil were Starmerella meliponinorum and Meyerozyma caribbica. The species prevailing in citrus peel was Hanseniaspora opuntiae. Citrus pulp was rich in Meyerozyma guilliermondii. Additionally, Aureobasidium pullulans and H. opuntiae were the dominant species in citrus leaves. All yeast species obtained were accessed for the capability to produce IAA: 26 strains in seven species showed the capability of producing IAA. Rhodotorula paludigena produced the highest IAA concentrations of 76.22 mg/L. Our study confirms the phylogenetic diversity of yeast associated with Nanfeng mandarin and highlights that these yeast strains are promising resources of microbial fertilizer.

Introduction

Nanfeng mandarin (Citrus reticulata cv. Blanco) is one of the most economically important fruit tree crops in the world [1]. Nanfeng mandarin, with a cultivation history of 1300 years, is well known in China and is produced in the Nanfeng (NF) and Nancheng (NC) counties of Jiangxi Province [2]. Nanfeng mandarin requires a temperature of 23–29°C, annual precipitation of 1500–2000 mm and humidity of 80–85% for optimum growth [3]. Nanfeng mandarin is one of the most important exported fruits and it is globally popular for its nutritive value, sweet and sour flavour and thin, tender skin [4]. Current researches on Nanfeng mandarin and other citrus fruit have focused on improving the unique flavour and excellent traits of tangerine; the methods to grow, pick and preserve the citrus fruit; the analysis of its chemical composition [5, 6]; the biological activity of its chemical constituents, their effect on human health and some methods to enhance the beneficial constituents of the fruit by chemical or physical means [7–9]. However, there has been little research on the microbial diversity and specifically, yeast diversity, associated with Nanfeng mandarin.

Yeasts are widely distributed in natural environment including the leaf, flower, fruit and other organs of trees and the orchard soil. Some previous studies have focused on the yeast diversity of strawberry, grapes and so on [10, 11]. However, there are few studies on the yeast diversity of Nanfeng mandarin. Bryschherzberg and Seidel [12] revealed the yeast diversity on grapes in...
two German wine-growing regions. The metabolites of yeast have a great influence on the aroma components, colour and texture of wine, as suggested by Tofalo et al. [13]. Based on previous studies and some of the characteristics of yeast, we speculate that the flavour and quality of Nanfeng mandarin may be dependent on a certain relationship between yeasts and Nanfeng mandarin. At the same time, it is known that plant-associated microorganisms are extremely rich and have various biological functions: some of them can produce beneficial biologically active compounds [14, 15], reduce the availability of heavy metals [16], improve the resistance of host plants to abiotic stresses [17, 18], promote the agricultural disease resistance and have a growth-promoting action on plants [19–22]. It is believed that many of the plant-associated yeasts, like other microbes, have a definite biological function [23, 24].

Indole-3-acetic acid (IAA), an indole compound and a common source of endogenous growth hormone, is known to adjust various developmental and physiological processes, and stimulate the rapid and long-term response of plants [25]. It is a naturally occurring auxin with broad physiological effects. Plants and microorganisms including bacteria [26], yeasts [27, 28], actinomycetes [29] and filamentous fungi [30] can produce IAA; therefore, these microbes have been recognized as good sources of biofertilizer [31]. Applications of IAA-producing yeasts, such as Candida valida, Rhodotorula glutinis, Trichosporon asahii, Lindera saturnus and Rhodotorula mucilaginosa, to promote plant growth have been reported [32]. However, there is little information on IAA-producing yeasts of the Nanfeng mandarin.

To the best of our knowledge, this study is the first to report the diversity of cultivable yeasts associated with Nanfeng mandarin in China and their ability to produce IAA. It is important to isolate and identify the yeast populations of NF and NC areas where Nanfeng mandarin is mainly produced, and assess the capacity of these yeasts to produce IAA in vitro. This assessment will provide the foundation for the development of biofertilizers using these yeasts.

**Materials and methods**

**Sample collection**

The yeast isolates were obtained from different cultivars of citrus trees located in NF and NC County in October 2016 (Figure 1). NF (N 27°12’48” and E116°31’31”) and NC (N27°33’ and E113°40’12”) are located in Jiangxi Province, China. Seven samples each were obtained from the two places. Soil samples (about 10 g/sample) were taken from a depth of about 10 cm. Nanfeng mandarin samples, weighing around 500–1000 g each, were collected.
Undamaged leaves were collected from each selected individual tree. All the collected samples were stored in sterile plastic tubes and transported immediately to the laboratory and stored at 4 °C within 24 h of collection.

**Isolation and identification of Nanfeng mandarin-associated yeasts from Citrus reticulate cv. Blanco**

Large impurities visible in the soil were removed. The soil samples were dried and fed through a 2-mm sieve in the sterile operation room in order to obtain 10-g samples. The leaves and Nanfeng mandarin samples were washed with sterile distilled water, after which the pulp and peel were separated from each other in a sterile condition. The treated samples (10 g) were then cut into 1-cm³ pieces.

Yeasts were isolated by an enrichment technique carried out as described by Limtong et al. with certain modifications [33]. The treated samples were ground and placed in Erlenmeyer flasks containing 200 mL of YPD (1% yeast extract, 2% peptone and 2% glucose) and agitated on a rotary shaker at 150 rpm for 30 min. The solution was then diluted with sterile water and aliquots of 100 µL from these serial dilutions (1 × 10⁻⁵, 1 × 10⁻⁴ and 1 × 10⁻³) were plated on PDA culture medium (20% potato, 2% peptone, 2% glucose and 2% agar) supplemented with 100 mg/mL streptomycin or chloramphenicol. After incubation at 25 °C for 3 days, the colonies were counted and the means and standard deviations of three replicates were calculated. Representative colonies were selected by dereplication based on their phenotypic and morphological characteristics such as colony colour (milky white, rose red, creamy white, orange), size (diameter size), surface (smooth or rough), border type (circle, radial) and growth rate and picked for pure culture [34]. Purified yeast strains were suspended in PDA broth supplemented with 20% v/v glycerol and maintained at −80 °C for future use.

**DNA extraction, amplification of yeast D1/D2 26S rDNA fragment sequence and its phylogenetic analysis**

Yeast strain identification was performed by sequence analysis of the region D1/D2 of the rDNA large subunit (LSU) as described by Barnett et al. [34]. Amplification of the referred region in the 26S rDNA gene was done with the primers NL1 (5′-GCATATCAATAAGGGAGGAAAGG-3′) and NL4 (5′-GTCCGTTTCAAGACG-3′) as proposed by Kurtzman and Robnet [35]. DNA extraction and analysis was performed as described by Querol et al. [36]. Polymerase chain reaction (PCR) and amplifications were performed as described by Santo et al. [10].

The amplified products were sequenced by Sangon Biotech (Shanghai) Co. Ltd. The obtained sequences were compared for homology with the sequences of the described species available in the GenBank database at the National Center for Biotechnology Information (NCBI) using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The D1/D2 26S rDNA region sequence of the yeasts was initially aligned using the program package MEGA 6 [37]. Then, the sequence was used as a query to search for similar sequences from GenBank using the FASTA and BLAST programs for identification.

Afterwards, the resulting sequences were aligned with the ClustalX software with gaps treated as missing data [38]. The phylogenetic tree was constructed using the neighbour-joining method [39]. The bootstrap analysis was done for 1000 replicates to assess the reliable level of the nodes of the tree.

**Determination of indole-3-acetic acid production**

Production of IAA by the yeasts was quantitatively analyzed [32]. A yeast culture grown for 1–2 days on YM agar at 25 °C was inoculated in 5 mL of yeast extract-peptone-dextrose (YPD) broth (10 g/L yeast extract, 2 g/L peptone and 2 g/L dextrose) supplemented with 1 g/L L-tryptophan in a test tube and incubated for 7 days at 30 ± 2 °C under shaker conditions at 150 rpm. After 7 days, an aliquot of 1.5 mL of the culture broth was centrifuged at 6791 × g for 5 min and the supernatant was collected for determination of IAA concentration. Subsequently, 1 mL of supernatant was added to 1 mL of Salkowski reagent prepared in 12 g/L FeCl₃ and 7.9 mol/L H₂SO₄, and the intensity of pink colour developed in the mixture after 30 min was quantified with a spectrophotometer (UV-1800, Mapada) at a wave length of 530 nm against a calibration curve.

**Statistical analysis**

Yeasts were tabulated and summarized according to their isolation percentages. Graphics and tables were drawn by Origin. All measurements were performed in triplicates. One-way analysis of variance (ANOVA) was performed and differences were considered statistically significant when \( p < 0.05 \).

**Results and discussion**

**Isolation of yeasts**

The diversity of yeast species in the orange pulp, leaves, soil and peel was investigated in this study. A total of 796 strains were isolated: 510 strains were
recovered from the orchard soil, 72 strains were isolated from orange peel, 62 from orange pulp and 152 strains from orange leaves. If the sampling area is considered, 220 strains were isolated from NF and 576 strains were isolated from NC. A total of 796 yeast isolates were assigned to 24 morphotypes using dereplication. Among these, 14 isolates were representative, whereas 10 ones were different. Figure 2 and Table 1

**Figure 2.** Colonies and microscopic characteristics of represented yeasts.
show the phenotypic characters and morphological characteristics of the representative strains.

Identification of yeasts

On the basis of sequence analysis of the region D1/D2 of the 26S rDNA fragment, all 24 morphotypes were identified to belong to 14 species and they were grouped into 9 genera: Hanseniaspora (3 species), Pichia (1 species), Candida (2 species), Sporidiobolus (1 species), Meyerozyma (2 species), Symmetrospora (2 species), Rhodotorula (1 species) sp., Starmerella (1 species) and Aureobasidium (1 species) (Table 2). Most of the yeast isolates belonged to the Ascomycota clade, whereas some belonged to the phylum Basidiomycota. These strains were assigned numbers (N1-1, N1-2, N1-3, N2-1, N3-1, N3-2, N4-1, N5-1, N6-1, N6-2, N7-1, N7-2, N8-1, N8-2, N9-1, N9-2, N10-1, N11-1, N12-1, N13-1, N14-1, N14-2, N14-3 and N14-4) and a phylogenetic tree based on the D1/D2 26S rDNA domain sequence alignment was constructed (Figure 3).

Based on these experiments, it was found that Hanseniaspora uvarum, Hanseniaspora opuntiae, Pichia kluveri, Pichia terricola, Aureobasidium pullulans, Candida humilis, Candida tropicalis, Barnettzyma californica and Torulaspora delbrueckii are associated with tangerine. Among these, H. uvarum, H. opuntiae, P. kluveri and A. pullulans are the predominating species [40, 41]. As observed from Figure 3, close phylogenetic relationship exists between the 26S rDNA D1/D2 region sequences corresponding to N1-1, N1-2, N1-3, N2-1, N3-1 and N3-2, and these strains belong to the genus of the highest proportion (25%). The sequences of D1/D2 26S rDNA region of N5-1, N6-1 and N6-2 were evolutionarily close, belonged to the genus Candida, forming the second group with a proportion of 12.5%. The sequence of D1/D2 26S rDNA region corresponding to N4-1 was evolutionarily close to genus Pichia, forming the third group with a proportion of 4.2%. The sequence of 26S rDNA D1/D2 region of N7-1 and N7-2 belonged to genus Sporidiobolus, forming the fourth group with a proportion of 8.3%. The analyzed partial 26S rDNA gene sequence revealed that the evolutionary relationship of the strains N8-1, N8-2, N9-1 and N9-2 was close to the genus Meyerozyma, forming the fifth group with a proportion of 16.67%. N10-1 and N11-1 belonged to Symmetrospora, N12-1 belonged to Rhodotorula, N13-1 belonged to Starmerella, whereas N14-1, N14-2, N14-3 and N14-4 belonged to the genus Aureobasidium. They formed the sixth, the seventh, the eighth and the ninth group, respectively, each having a proportion of 8.3, 4.2, 4.2 and 16.67%, respectively. Previous studies have shown that H. opuntiae, H. uvarum, Hanseniaspora thailandica, Meyerozyma guilliermondii, A. pullulans and P. kluveri are the most abundant yeast species in citrus. H. uvarum and P. kluveri are also isolated from apples, grapes and table olives, whereas Meyerozyma caribbica, Rhodotorula paludigena and Sporidiobolus pararoseus are less frequent in citrus [24, 42, 43].

Further analysis of the sequenced genomes revealed that only one yeast strain (N12-1) showed a lower homology of 98% with R. paludigena (KU316709.1). Other strains were associated with the corresponding model strain with a higher homology. This confirms that the results of the sequence analysis of yeast 26S rDNA D1/D2 are credible.

Some of the isolated strains may have strong biochemical functions and application potential. Generally, the functional roles of yeast can be divided into three categories: biological agents, biological fertilizers and flavour enhancers. Many studies have explored the potential of yeast for biological control. For example, Hanseniaspora inhibits the grey mould decay and affects the postharvest quality parameters [44] as well as the postharvest diseases in strawberries [45]. Sporidiobolus is also known to be a biocontrol agent that is effective against postharvest diseases in table grapes [46, 47]. Meyerozyma has antifungal activity [48]. Aureobasidium and Rhodotorula have potential as biocontrol agents: they compete for nutrients and space to inhibit the growth of plant pathogens [49]. In this sense, most yeasts exhibit effects of biological control. Among the yeasts isolated by us, M. guilliermondii is useful in controlling plant soft rot, whereas R. paludigena and A. pullulans have the potential to reduce citrus green mould and thereby, to promote plant growth.

Different yeast species isolated from different samples and different sampling regions

The yeast abundance in the citrus samples was estimated. H. uvarum was isolated from citrus soil and H. opuntiae was the prevalent species in orange peel.
| Strain no. | Taxa       | GenBank accession no. | Most closely related strain (s) (accession no.) | Classification of matched species                                                                 | Cover (%) | Identi. (%) | Production of IAA(mg/L) |
|-----------|------------|-----------------------|-------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------|-------------|------------------------|
| N1-1      | Hanseniaspora | MF979188              | Hanseniaspora apuntiae (KJ794647.1)              | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 99        | 100         | 15.331                 |
| N1-2      | Hanseniaspora | MF979189              | Hanseniaspora apuntiae (KT226114.1)              | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 98        | 99          | 14.295                 |
| N1-3      | Hanseniaspora | MF979190              | Hanseniaspora apuntiae (KP975393.1)              | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 97        | 100         | 5.692                  |
| N2-1      | Hanseniaspora | MF979191              | Hanseniaspora thailandica (DQ404527.1)           | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 98        | 99          | 13.173                 |
| N3-1      | Hanseniaspora | MF979192              | Hanseniaspora uvarum (JQ678680.1)                | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 99        | 100         | 4.446                  |
| N3-2      | Hanseniaspora | MF979193              | Hanseniaspora uvarum (KU862643.1)                | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycodaceae; Hanseniaspora | 100       | 99          | 30.837                 |
| N4-1      | Pichia      | MF979194              | Pichia kluyveri (JQ771714.1)                     | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Pichiaceae; Pichia            | 100       | 99          | 7.646                  |
| N5-1      | Candida      | MF979195              | Candida cf. Azyma (EF601042.1)                   | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Trichomonascaceae; Wickerhamiella; Wickerhamiella/ Candida clade | 98        | 99          | 7.687                  |
| N6-1      | Candida      | MF979196              | Candida metapsilosis (KY106577.1)                | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Debaryomycetaceae; Candida/ Lodderomyces clade; Candida | 98        | 100         | 16.332                 |
| N6-2      | Candida      | MF979197              | Candida metapsilosis (FJ746062.1)                | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Debaryomycetaceae; Candida/ Lodderomyces clade; Candida | 99        | 99          | 8.061                  |
| N7-1      | Sporidiobolus | MF979198              | Sporidiobolus pararoseus (KC783407.1)            | Basidiomycota; Pucciniomycotina; Microbotryomycetaceae; Sporidiobolales; Sporidiobolaceae; Sporidiobolus | 99        | 99          | 19.532                 |
| N7-2      | Sporidiobolus | MF979199              | Sporidiobolus pararoseus (KU167710)              | Basidiomycota; Pucciniomycotina; Microbotryomycetaceae; Sporidiobolales; Sporidiobolaceae; Sporidiobolus | 98        | 99          | 16.332                 |
| N8-1      | Meyerozyma   | MF979200              | Meyerozyma guilliermondii (KJ794675.1)           | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Debaryomycetaceae; Meyerozyma | 99        | 99          | 7.355                  |
| N8-2      | Meyerozyma   | MF979201              | Meyerozyma guilliermondii (JX049423.1)           | Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Debaryomycetaceae; Meyerozyma | 98        | 100         | 5.318                  |

(continued)
Byrsonima crassifolia and A. pullulans were isolated from orange leaves [51]. The isolation data indicated that most species of yeasts were isolated from citrus leaves. This included 12 species of yeasts, four out of which, namely Candida metapsilosis, Symmetrospora spp., Candida cf. azyma and P. kluuyveri only, occurred in the leaves, and did not appear in the other samples. On the contrary, H. thailandica was obtained from the soil and peel, but it did not appear in the leaves and pulp. It is noteworthy that there were only three species of yeast isolated from peel, two of which were also obtained from the soil. More intriguingly, the number of yeast strains isolated from soil and peel were the same, but the compositions were very different, Starmerella meliponinorum being the only yeast strain common to both. It is generally recognized that different yeasts have different functions and therefore, the presence of different yeasts on different plant parts may produce different effects (Figure 4).

The samples were collected from two different counties (NF and NC counties) which are both Nanfeng mandarin production sites but the fruits from NF county taste more delicious and are more popular with the public. Different factors such as soil and climate may act alone or in combination to cause this phenomenon. Interestingly, comparison between the yeast samples from the two places (Figure 5) showed that the yeast species are more abundant in NC. Although M. caribbica is the only common yeast to be isolated from both the places, its proportion is much higher in samples from NC than in samples from NF.

Table 2. Continued.

| Strain no. | Taxa       | GenBank accession no. | Most closely related strain(s) (accession no.) | Classification of matched species | Cover (%) | Ident. (%) | Production of IAA(mg/L) |
|-----------|------------|-----------------------|-----------------------------------------------|----------------------------------|-----------|------------|------------------------|
| N9-1      | Meyerozyma | MF979202              | Meyerozyma caribbica (KY108517.1)              | Saccharomycetales; Debaromyces; Meyerozyma Ascomycota; Saccharomycota; Saccharomyces; Debaromyces; Meyerozyma Ascomycota; Saccharomycota; Saccharomyces | 97        | 100        | 10.235                |
| N9-2      | Meyerozyma | MF979230              | Meyerozyma caribbica (KCS44483.1)              | Saccharomycetes; Debaromyces; Meyerozyma Ascomycota; Saccharomyces; Debaromyces; Meyerozyma Ascomycota; Saccharomyces; Saccharomyces | 98        | 99         | 11.760                |
| Symmetrospora N10-1 | Symmetrospora MF979203 | Symmetrospora sp. DMKU-SE130(LC177043.1) | Basidiomycota; Pucciniomyces; Cystobasidium; Cystobasidium incertae sedis; Symmetrospora | Symmetrospora | 96        | 99         | 15.293                |
| N11-1     | Symmetrospora MF979204 | Symmetrospora sp. UFMC-A670(KMS27125.1) | Basidiomycota; Pucciniomyces; Cystobasidium; Cystobasidium incertae sedis; Symmetrospora | Symmetrospora | 98        | 99         | 12.799                |
| Rhodotorula N12-1 | Rhodotorula MF979205 | Rhodotorula paludigena (KU316709.1) | Basidiomycota; Pucciniomyces; Microbotryomycetes; Sporidiobolales; Sporidiobolaceae; Rhodotorula | Rhodotorula | 99        | 97         | 76.221                |
| Starmerella N13-1 | Starmerella MF979206 | Starmerella meliponinorum (KY109785.1) | Ascomycota; Saccharomycota; Saccharomycetes; Saccharomycetales; Saccharomycetales incertae sedis; Starmerella | Starmerella | 100       | 99         | 4.836                |
| Aureobasidium N14-1 | Aureobasidium MF979207 | Aureobasidium pullulans (KX893329.1) | Ascomycota; Pezizomyces; Dothideomycetes; Dothideomycetidae; Dothideales; Aureobasidiales; Aureobasidium | Aureobasidium | 97        | 99         | 30.671                |
| N14-2     | Aureobasidium MF979208 | Aureobasidium pullulans (KX893328.1) | Ascomycota; Pezizomyces; Dothideomycetes; Dothideomycetidae; Dothideales; Aureobasidiales; Aureobasidium | Aureobasidium | 97        | 99         | 15.293                |
| N14-3     | Aureobasidium MF979209 | Aureobasidium pullulans (JQ916048.1) | Ascomycota; Pezizomyces; Dothideomycetes; Dothideomycetidae; Dothideales; Aureobasidiales; Aureobasidium | Aureobasidium | 100       | 99         | 13.88                |
| N14-4     | Aureobasidium MF979210 | Aureobasidium pullulan (KX893329) | Ascomycota; Pezizomyces; Dothideomycetes; Dothideomycetidae; Dothideales; Aureobasidiales; Aureobasidium | Aureobasidium | 98        | 99         | 14.586                |

[50]. Byrsonima crassifolia and A. pullulans were isolated from orange leaves [51]. The isolation data indicated that most species of yeasts were isolated from citrus leaves. This included 12 species of yeasts, four out of which, namely Candida metapsilosis, Symmetrospora spp., Candida cf. azyma and P. kluuyveri only, occurred in the leaves, and did not appear in the other samples. On the contrary, H. thailandica was obtained from the soil and peel, but it did not appear in the leaves and pulp. It is noteworthy that there were only three species of yeast isolated from peel, two of which were also obtained from the soil. More intriguingly, the number of yeast strains isolated from soil and peel were the same, but the compositions were very different, Starmerella meliponinorum being the only yeast strain common to both. It is generally recognized that different yeasts have different functions and therefore, the presence of different yeasts on different plant parts may produce different effects (Figure 4).

The samples were collected from two different counties (NF and NC counties) which are both Nanfeng mandarin production sites but the fruits from NF county taste more delicious and are more popular with the public. Different factors such as soil and climate may act alone or in combination to cause this phenomenon. Interestingly, comparison between the yeast samples from the two places (Figure 5) showed that the yeast species are more abundant in NC. Although M. caribbica is the only common yeast to be isolated from both the places, its proportion is much higher in samples from NC than in samples from NF.
Therefore, the taste of Nanfeng mandarin may be related to the diversity of yeasts. These results are in agreement with a similar report that the yeast diversity of Xinjiang grape, especially non-Saccharomyces, can affect the quality and flavour of grape wine [52].

**Indole-3-acetic acid production**

Previous research has revealed that some yeast strains are not only potential biocontrol agents, but also potential biological fertilizers owing to their ability to produce beneficial substances. *Pichia* spp. can be used as an expression vector for the effective expression of useful substances [53, 54]. Production of bio-alcohols by *Candida* has also been carried out [55]. The yeast *C. maltosa* can produce IAA, thereby promoting the growth of plants [56]. Cloete et al. found evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* Pillans [57]. Each soil fraction has a distinct yeast assemblage depending on the soil nutrient availability and the physiological capacities of the yeast. Endophytic yeasts, like *Pichia fermentans* and *Candida raienensis*, play an important role in gall formation [58]. *Rhodotorula acheniorum*, *R. mucilaginosa* and *R. glutinis* produce some plant growth-promoting

**Figure 3.** Phylogenetic tree based on neighbour-joining analysis of the 26S rDNA D1/D2 domain sequences of the yeast isolates obtained from Nanfeng mandarin.
The yeasts that we obtained, such as *H. uvarum*, *R. paludigena* and *A. pullulans*, may also have a plant-growth promoting effect, although further experiments are needed to confirm this.

Among the 26 strains of yeast, 9 strains demonstrated the ability to produce IAA when cultivated in YPD broth supplemented with 0.1% L-tryptophan (Table 2). The other 17 strains grew in this medium, but no IAA was produced. *R. paludigena* produced relatively high concentrations (76.22 mg/L) of IAA. This result indicated that IAA production was strain-dependent. Some strains of some species were able to produce IAA, whereas others were not. Other strains such as *A. pullulans* produced IAA. However, the concentration of IAA produced was relatively low.

Simultaneously, yeasts also play an important role in food fermentation. Their unique characteristics impart a unique taste to the food. *C. tropicalis*, *Pichia membranifaciens*, *Candida boidinii* and *Saccharomyces cerevisiae* are involved in the natural fermentation process of table olives causing a change in their taste [43, 60]. *S. cerevisiae* can impact the fragrance of cherry wines [60]. Whether the yeasts obtained by us can be potentially applied in changing the flavour of tangerine-flavoured beverages still needs further study.

**Conclusion**

Our present study revealed the phylogenetic diversity of yeast associated with Nanfeng mandarin and highlights that the IAA-producing yeast strains could be used as promising resources of microbial fertilizers. The yeast species identified in this study have also been reported to be found in the soil, leaves and fruits of many other hosts from different biomes. Until date, the research on the use of yeasts as biofertilizer suggests that yeasts possess this effect. Moreover, further and more thorough studies of Nanfeng mandarin yeast are needed. The results of this study represent an important step in the phylogenetic analysis of yeast isolates collected from citrus trees of the Jiangxi Nanfeng. Moreover, they provide an insight into the eco-friendly use of yeast resources.

**Disclosure statement**

The authors declare there is no conflict of interest for their study.

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**References**

[1] Lin L, Chen JY. Study on fruit storability of 5 superior Nanfeng mandarin lines. South China Fruits. 2010;39:9–13.
[2] Li ZZ, Liu GR, Yuan FS, et al. Investigation on production environments of Nanfeng orange. Acta Agric Jiangxi. 2005;17:1–7.
[3] Xu H, Ye C, Yang L, et al. Effects of superabsorbent polymer on soil moisture content of Nanfeng tangerine-orchard. Chin Agric Sci Bull. 2010;26:255–258.
[4] Zhang FJ, Lian-Xiang DU, Wang M. Research on the fermentation technology of low-alcohol Nanfeng mandarin orange wine. Liquor-Making Sci Technol. 2011;2:93–95.
[5] Zheng H, Zhang Q, Quan J, et al. Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. Food Chem. 2016;205:112–121.
[6] Strano MC, Altieri G, Admane N, et al. Advance in citrus postharvest management: diseases, cold storage and quality evaluation. Citrus Pathol. 2017;7:139–159.
[7] Rampersaud GC, Valim MF. 100% citrus juice: nutritional contribution, dietary benefits, and association with anthropometric measures. Crit Rev Food Sci Nutr. 2017;57:129–140.
[8] Onakpoya I, O’Sullivan J, Heneghan C, et al. The effect of grapefruits (Citrus paradisi) on body weight and cardiovascular risk factors: a systematic review and meta-analysis of randomized clinical trials. Crit Rev Food Sci Nutr. 2017;57:602–612.
[9] Barreca D, Gattuso G, Bellocco E, et al. Flavanones: citrus phytochemicals with health-promoting properties. Biofactors. 2017;43:495–506.
[10] Santo DE, Galego L, Gonçalves T, et al. Yeast diversity in the Mediterranean strawberry tree (Arbutus unedo L.) fruits’ fermentations. Food Res Int. 2012;47(1):45–50.
[11] Di ME, Ercolini D, Coppola S. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. Int J Food Microbiol. 2007;117(2):201–210.
[12] Bryscherzberg M, Seidel M. Yeast diversity on grapes in two German wine growing regions. Int J Food Microbiol. 2015;214:137–144.
[13] Tofalo R, Schirone M, Telera GC, et al. Influence of organic viticulture on non-Saccharomyces, wine yeast populations. Ann Microbiol. 2011;61(1):57–66.
[14] Ye Y, Xiao Y, Ma L, et al. Flavipin in Chaetomium globosum CDW7, an endophytic fungus from Ginkgo biloba, contributes to antioxidant activity. Appl Microbiol Biotechnol. 2013;97:7131–7139.
[15] Mv T, Andersen B. MB1533 is a Defensin-like antimicrobial peptide from the intracellular meristem endophyte of scots pine Methylobacterium extorquens DSM13060. J Microbiol Biochem Technol. 2015;8:1–5.
[16] Kumar KV, Singh N, Behl HM, et al. Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in Brassica juncea grown in fly ash amended soil. Chemosphere. 2008;72:678–683.
[17] Esitken A, Yildiz HE, Ercisli S, et al. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci Hortic. 2010;124:62–66.
[18] Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci. 2004;166:525–530.
[19] Kloeper JW, Ran L, Zablutowicz RM. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 1989;7:39–44.
[20] Taghavi S, Barac T, Greenberg B, et al. Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. Appl Environ Microbiol. 2005;71:8500–8505.
[21] Wang Z, Li T, Wen X, et al. Fungal communities in rhizosphere soil under conservation tillage shift in response to plant growth. Front Microbiol. 2017;8:1301.
[22] Karthik M, Pushpakanth P, Krishnamoorthy R. Endophytic bacteria associated with banana cultivars and their inoculation effect on plant growth. J Hort Sci Biotechnol. 2017;92:568–576.
[23] Mestre MC, Rosa CA, Safar SV, et al. Yeast communities associated with the bulk-soil, rhizosphere and ectomycorrhizosphere of a Nothofagus pumilio forest in northwestern Patagonia, Argentina. FEMS Microbiol Ecol. 2011;78:531–541.
[24] Ruiz-Moyano S, Martin A, Villalobos MC, et al. Yeasts isolated from figs (Ficus carica L) as biocontrol agents of postharvest fruit diseases. Food Microbiol. 2016;57:45–53.
[25] Cleland RE. Auxin and cell elongation. The Netherlands: Springer; 1987. p. 204–220.
[26] Long X, Chen X, Chen Y, et al. Isolation and characterization endophytic bacteria from hyperaccumulator Sedum alfredi Hance and their potential to promote phytoextraction of zinc polluted soil. World J Microbiol Biotechnol. 2011;27:1197–1207.
[27] El-Tarabily KA. Suppression of Rhizoctonia solani diseases of sugar beet by antagonistic and plant growth-promoting yeasts. J Appl Microbiol. 2004;96:69–75.
[28] Nassar AH, Eltarabily KA, Sivasithamparam K. Promotion of plant growth by an auxin-producing isolate of the yeast Williopsis saturnus endophytic in maize (Zea mays L.) roots. Biol Fert Soils. 2005;42:97–108.
[29] Khamna S, Yokota A, Peberdy JF, et al. Indole-3-acetic acid production by Streptomyces sp. isolated from some Thai medicinal plant rhizosphere soils. Eur Asian J Biosci. 2010;4:23–32.
[30] Ruanpanun P, Tangchitsomkid N, Hyde KD, et al. Horizontal material inocula for enhancing crop productivity. Trends Biotechnol. 1987;61:576–580.
[31] Biotechnology & BIOTECHNOLOGICAL EQUIPMENT 1505

[32] Xin G, Glawe D, Doty SL. Characterization of three endophytic, indole-3-acetic acid producing yeasts occurring in Populus trees. Mycol Res. 2009;113:973–980.
[33] Limtong S, Yongmanitchai W, Tun MM, et al. Kazachstaniam siamensis sp. nov., an ascomycetous yeast species from forest soil in Thailand. Int J Syst Evol Microbiol. 2007;57:419–422.
[34] Barnett JA, Payne RW, Yarrow D. Yeasts: characteristics and identification. 3rd ed. Cambridge (UK): Cambridge University Press; 2000.

[35] Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek. 1998;73:331–371.

[36] Querol A, Barrio E, Ramón D. A comparative study of different methods of yeast strain characterization. Syst Appl Microbiol. 1992;15:439–446.

[37] Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–2739.

[38] Thompson JD, Gibson TJ, Plewniak F, et al. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25:4876–4882.

[39] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–425.

[40] Giorello FM, Berná L, Greif G, et al. Genome sequence of the native apiculate wine yeast Hanseniaspora vinaeae T02/19AF. Genome Announc. 2013;2:BR31–BR40.

[41] Lachance MA. The biodiversity, ecology, and biogeography of ascomycetous yeasts. In: Martin F, editor. The ecological genetics of fungi. Hoboken (NJ): Wiley Press; 2013.

[42] Pereira EL, Ramalhosa E, Borges A, et al. Yeast dynamics during the natural fermentation process of table olives (Nigrinhas de Freixo cv.). Food Microbiol. 2015;46:582–586.

[43] Camatti-Sartori V, da Silva-Ribeiro RT, Valdebenito-Sanhueza RM. Endophytic yeasts and filamentous fungi associated with southern Brazilian apple (Malus domestica) orchards subjected to conventional, integrated or organic cultivation. J Basic Microbiol. 2005;45:397–402.

[44] Liu HM, Guo JH, Cheng YJ, et al. Control of gray mold of grape by Hanseniaspora uvarum and its effects on postharvest quality parameters. Ann Microbiol. 2010;60:31–35.

[45] Cai Z, Yang R, Xiao H, et al. Effect of preharvest application of Hanseniaspora uvarum on postharvest diseases in strawberries. Postharves Biol Tec. 2015;100:52–58.

[46] Li Q, Li C, Li P, et al. The biocontrol effect of Sporidiobolus pararoseus Y16 against postharvest diseases in table grapes caused by Aspergillus niger and the possible mechanisms involved. Biol Control. 2017;113:18–25.

[47] Cadez N, Zupan J, Raspor P. The effect of fungicides on yeast communities associated with grape berries. FEMS Yeast Res. 2010;10:619–630.

[48] Coda R, Rizzello CG, Di Cagno R, et al. Antifungal activity of Meyerozyma guilliermondii: identification of active compounds synthesized during dough fermentation and their effect on long-term storage of wheat bread. Food Microbiol. 2013;33:243–251.

[49] Eugenio MS, Helson Mario MDV, Geisianunga augusta MM. Yeasts from native Brazilian Cerrado plants: occurrence, diversity and use in the biocontrol of citrus green mould. Fungal Biol. 2015;119(11):984–993.

[50] Liu R, Zhang Q, Chen F, et al. Analysis of culturable yeast diversity in spontaneously fermented orange wine, orange peel and orangery soil of a Ponkan plantation in China. Ann Microbiol. 2015;65:2387–2391.

[51] Sánchez-Torres P, Tuset JJ. Molecular insights into fungicide resistance in sensitive and resistant Penicillium digitatum strains infecting citrus. Postharvest Biotechnol. 2011;59:159–165.

[52] Xu YN, Li Q, Liu QP, et al. Research progress of non-Saccharomyces yeasts in Xinjiang. Food Indus. 2015;49(7):1378–1383.

[53] Weinacker D, Rabet C, Zepeeda AB, et al. Applications of recombinant Pichia pastoris in the healthcare industry. Braz J Microbiol. 2014;44:1043.

[54] Ahmad M, Hirz M, Pichler H, et al. Protein expression in Pichia pastoris: recent achievements and perspectives for heterologous protein production. Appl Microbiol Biotechnol. 2014;98:5301–5317.

[55] Winkelhausen E, Velickova E, Amartey SA, et al. Production of bioalcohols by yeasts from Saccharomyces and Candida genera. J Biotechnol. 2008;136:5454.

[56] Limtong S, Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. World J Microbiol Biotechnol. 2012;28:3323–3335.

[57] Cloete KJ, Valentine AJ, Standar MA, et al. Evidence of symbiosis between the soil yeast Cryptococcus laurentii and a sclerophyllous medicinal shrub, Agathosma betulina (Berg.) Pillans. Microb Ecol. 2009;57:624–632.

[58] Glushakova AM, Kachalkin MM. Yeasts from native Brazilian Cerrado plants: occurrence, diversity and use in the biocontrol of citrus green mould. Fungal Biol. 2015;119(11):984–993.

[59] Glushakova AM, Kachalkin MM. Yeasts from native Brazilian Cerrado plants: occurrence, diversity and use in the biocontrol of citrus green mould. Fungal Biol. 2015;119(11):984–993.

[60] Sun SY, Gong HS, Jiang XM, et al. Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with Saccharomyces cerevisiae on alcoholic fermentation behaviour and wine aroma of cherry wines. Food Microbiol. 2014;44:15–23.