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Identification of Novel Endophytic Yeast Strains from Tangerine Peel

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Abstract

Seven endophytic yeast strains were isolated from tangerine peel (Citrus reticulata Blanco) and genotyped through clustering with D1/D2 and ITS1-5.8S-ITS2 sequences from GenBank. Phenotypic characteristics were obtained through commercial kits and through assisted species identification. Indole-3-acetic acid (IAA) production by the yeast strains was assessed using Salkowski reagent and High-Performance Liquid chromatography (HPLC). The growth-promoting effects of the yeast were evaluated using the 'ragdoll' method. CRYb1, CRYb2 and CRYb7 isolates were identified as the closest species *Hanseniaspora opuntiae*. CRYb3 was identified as *Pichia kluyveri*. CRYb4, CRYb5 and CRYb6 were identified as *Meyerozyma guilliermondii*. CRYb1, CRYb5, CRYb6 and CRYb7 were found to be capable of IAA production. The most promising yeast strains now require further evaluation for their ability to promote plant growth in vitro and in vivo. These data increase our knowledge of the distribution and biological properties of endophytic yeast. This is important information that will be required to fully harness the growth-promoting properties of yeast strains.

Introduction

Endophytes are microorganisms that can be isolated from surface-sterilized plant tissue that do not damage the host plant [1]. In 2003, Azevedo et al. defined endophytes as microorganisms that inhabit the inner tissues of plants, causing no damage to the host. This definition excludes microorganisms such as mycorrhizal fungi and plant-nodulating bacteria. Endophytic microorganisms can colonize an ecological niche similar to that of phytopathogens, suggesting they have potential as biocontrol agents through their ability to inhibit plant pathogens [2, 3]. Endophytic microorganisms also produce metabolites similar to those produced by the host, and benefit the host through their ability to produce natural compounds that are lacking in host plants.

Comparatively, studies on the isolation, localization, and diversity of endophytic yeasts are limited. Isolation methods that are less biased towards filamentous fungi or bacteria may be necessary to accurately assess these endophytic yeast populations [4]. The majority of studies using endophytic yeasts involve trees and plants of the forests. *Cryptococcus* sp., *Debaryomyces* sp., *Sporobolomyces* sp., and *Rhodotorula* sp. are the most commonly isolated endophytic yeast strains [5]. Endophytic yeasts are present in a range of crop plants including apples, carrot, and sweet orange [6–8].

To-date, research on endophytic yeasts have focused on ecological studies; however, endophytic yeasts have an array of potential uses in biological control and enhanced plant growth. IAA is an auxin that has been widely studied in endophytes. The role of microbe-IAA in plant–microbe interactions has received increasing attention in recent years. IAA producing yeasts, such as *S. roseus*, *Candida valida*, *R. glutinis* and *Trichosporon asahii*, *Lindera* (*Williopsis*) *saturnus* and *R. mucilaginosa* can promote plant growth [9, 10]. Eight of the *Williopsis saturnus* endophytic yeast strains identified in maize roots produce the auxins IAA and IPYA (indole-3-pyruvic acid). The ability of *W. saturnus* to enhance plant growth was most pronounced in

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the presence of L-TRP [8]. In previous studies, three endophytic yeast strains found in poplar, *Rhodotorula graminis*, and two strains of *R. mucilaginosa* have also been shown to produce IAA [11]. Khan et al. found that bacterial and yeast endophytes from poplar and willow permit successful colonization, growth enhancement, and increased fruit yields of specific crops following the addition of poplar and willow endophytes under greenhouse conditions [12]. The genome of strain WP1 was the first endophytic yeast to be sequenced, and analysis of its genome may permit the discovery of genes that promote plant–microbe interactions [13].

The addition of yeast to crop plants to improved growth may be more publically acceptable than the addition of bacteria. A larger number of endophytic yeast resources are necessary, that can provide new knowledge of endophyte–plant associations and enhance their agricultural applications. The fungal endophytes associated with leaves, stems, trunks, and roots of *C. reticulata* were isolated and taxonomically identified [6]. However, the role of yeast in the biology of tangerine plants is poorly understood. The aims of this study were to: (i) isolate endophytic yeast from tangerine peels; (ii) identify specific isolates and focus on their phylogenetic associations using D1/D2 domains and ITS1-5.8S-ITS2; (iii) to examine the capacity of the yeast strains to produce IAA; (iv) to assess the ability of the isolated yeast strains to promote wheat growth.

Materials and Methods

Isolation of Endophytic Yeast from Tangerine Skin Peel

The tangerine (Citrus reticulata Blanco) which is native to Guangzhou in China were purchased from in April 2017. We collected three samples from each of five healthy tangerine. Tangerine peel (3 g) was surface sterilized with 70% ethanol for 1 min, 5% sodium hypochlorite for 5 min, and rinsed five times in sterile deionized water (dH_2O). The effectiveness of surface sterilization was verified by spreading the final rinse water onto YPD (1% Yeast extract, 2% Peptone, and 2% Dextrose). Sterilized tangerine peels were homogenized, and 100 µL of the homogenate added to YPD plates supplemented with tetracycline (100 µg/mL) [14]. A total of 15 stripe fragments per sample were used for yeast isolation. Plates were incubated at 27 °C until visible yeast colonies appeared. Colonies of varying morphologies were selected and purified by cross-streaking onto YPD plates. Purified yeast strains were suspended in YPD broth supplemented with 10% (v/v) glycerol and maintained at −80 °C.

Phenotypic Characterization

Yeast strains were characterized by their morphology according to standard methods [15]. Colony formation was investigated by cultivation on potato dextrose agar (PDA, contains: 20%, w/v, potato infusion; 2%, w/v, glucose; 1.5%, w/v, agar) and corn meal agar (2%, w/v, corn meal infusion; 1.5%, w/v, agar) in slide culture plates at 25 °C for up to 14 days. Three replicate plates were ecv.

Carbon-Utilization Characterization

A total of 14 organic compounds were used to characterize the levels of carbon-utilization by the yeast isolates. A commonly used method to distinguish yeast species is the comparison of their ability to utilize organic compounds as their sole carbon source [16].

Extraction of Yeast Genomic DNA

Yeast genomic DNA was prepared according to established protocols with modifications [17]. Briefly, yeast cultures were grown overnight in 10 mL of YPD broth at 28 °C and cells were collected by centrifuging at 12000×*g* for 1 min followed by washing in dH_2O. Cells were lysed by vortexing and the addition of glass beads. Lysates were clarified and cDNA was extracted using the phenol/chloroform/isoamyl alcohol method. Extracted DNA was precipitated in an equal volume of isopropanol at room temperature. DNA was resuspended in TE buffer and stored at −20 °C.

PCR Amplification

The D1/D2 region of the large ribosomal ribonucleic acid subunit (LSU rRNA) gene region was PCR amplified using NL1 (5′-TGCTGGAGCCATGGATC-3′) and NL4 (5′-TACTTG TTCGCTATCG GTCT-3′) primers [18]. The ITS1-5.8S-ITS2 rDNA sequence of the nuclear ribosomal internal transcribed spacer regions were PCR amplified using ITS1 (5′-TCCGTA GGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTAT TGATATGC-3′) [19]. PCR reactions contained 5 ng of DNA template, 0.2 mM upstream and downstream primers, 0.4 U Taq polymerase and 3.7 mM $MgCl_2$ (Takara) in PCR buffer (total volume 50 µl). The PCR reaction conditions were as follows: 5 min denaturation at 94 °C, 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final 72 °C extension for 7 min. PCR products were verified on 1% agarose gels.

Cloning and Sequencing

PCR products were gel purified using the HI Yield Gel/PCR DNA Fragments Extraction kit (RBC Bioscience) according to the manufacturer's protocol. Purified products were sequenced with NL1 and NL4 primers in the D1/D2 region, ITS1 and TIS4 primers in the ITS1-5.8S-ITS2 region of the LSU rRNA gene. Sequences were compared by BLAST searches [10] and aligned to related sequences retrieved from GenBank using the multiple alignment program. Phylogenetic trees were reconstructed from evolutionary distance data on Kimura's two-parameter correction [20] using the neighbor-joining method [21] and MEGA software version 7.0. Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) [22]. Nucleotide sequences obtained in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and assigned the accession numbers.

IAA Production Assay

Endophytic yeast isolates were assayed for their ability to produce IAA in YPD in the presence or absence of L-Trp. For these assays, yeast isolates were prepared from 5-day GPB cultures of approximately 1×10^8 Colony-Forming Units (CFU) mL^{-1}. YPD was supplemented with 0.5% filter-sterilized L-Trp (Millipore membranes, pore size 0.22 µm) and inoculated with 2 ml of each yeast strain. Cultures were incubated on a shaker at 250 rpm at 25 °C in darkness for 7 days. Non-inoculated flasks served as controls. On days 1, 2, 5, and 7, cells were isolated by centrifugation at 10,000 rpm for 5 min and 1 mL of supernatant was mixed with 2 mL of Salkowski reagent (2 mL 0.5 M $FeCl_3$ + 98 mL 35% $HClO_4$) [23]. Samples were incubated for 30 min and read on an Onliab EU-2600 spectrophotometer (Onliab, Shanghai) at a wavelength 530 nm. IAA solutions of known concentration were used to establish a standard curve. To further improve experimental accuracy, IAA production was also determined by HPLC. The 5 mL culture supernatants were extracted with an equal volume of ethyl acetate. After repeat extractions, the organic phase was evaporated in a vacuo and the resulting powder was re-dissolved in 1 mL MeOH. HPLC analysis was performed on the Ultimate 3000 system. Crude methanolic extracts of 20 µL IAA were injected onto a reverse phase XBridge C18 column (3.5 µm, 100 mm × 4.6 mm) equilibrated with 95:5 methanol/water. Extracts were resolved isocratically at a flow rate of 0.5 mL min^{-1}. The retention time of IAA was identified by comparison to standard samples [24]. IAA standard curves were obtained by calculating the IAA peak areas of different standard concentrations.

Assessment of Growth Promotion

The growth-promoting effects were evaluated using the 'ragdoll' method described by [25]. Briefly, seeds of wheat (*Triticum aestivum*) were surface sterilized in 5% sodium hypochlorite for 5 min and washed thoroughly in dH_2O. Sterilized seeds were soaked in yeast isolates (10^8 CFU ml^{-1}) for 1 h, placed in a gauze with 20 ml dH_2O, folded and rolled into a moderately tight tube, and placed in a plant culture bottle. Tubes were maintained at 28 ± 2 °C for 5 days. At the end of incubation period, germination rates (%), and root and shoot lengths were measured.

Results

Yeast Isolation from the Tangerine Peel

Seven yeast strains termed CRYb1, CRYb2, CRYb3, CRYb4, CRYb5, CRYb6 and CRYb7 were isolated from the tangerine peel. The endophytic yeast communities isolated included *Hanseniaspora opuntiae*, *Pichia kluyveri* and *Meyerozyma guilliermondii.*

Morphological Characteristics

The isolated yeasts were assessed for their morphological characteristics after culturing on YPD agar. Three of the isolates (CRYb3, CRYb5 and CRYb6) were pale-yellow, whilst four isolates (CRYb1, CRYb2, CRYb4 and CRYb7) were pink. All were round and smooth (Table 1).

Molecular Identification

CRYb1, CRYb2, CRYb3, CRYb4, CRYb5, CRYb6 and CRYb7 were genotyped through clustering with D1/D2 and ITS1-5.8S-ITS2 sequences from GenBank. The resulting dendrograms (Figs. 1 and 2) revealed high bootstrap values for specific strains. The two analyzed methods do not show different results. Upon analysis of the D1/D2 and ITS1-5.8S-ITS2 regions, CRYb1, CRYb2 and CRYb7 were most closely

Table 1 The morphological characteristics of the yeast isolates

Isolate number	CRYb1	CRYb2	CRYb3	CRYb4	CRYb5	CRYb6	CRYb7
Colony color	Pink	Pink	Pale-yellow	Pink	Pale-yellow	Pale-yellow	Pink
Colony shape	Round, smooth	Round, smooth	Round, smooth	Round, smooth	Round, smooth	Round, smooth	Round, smooth

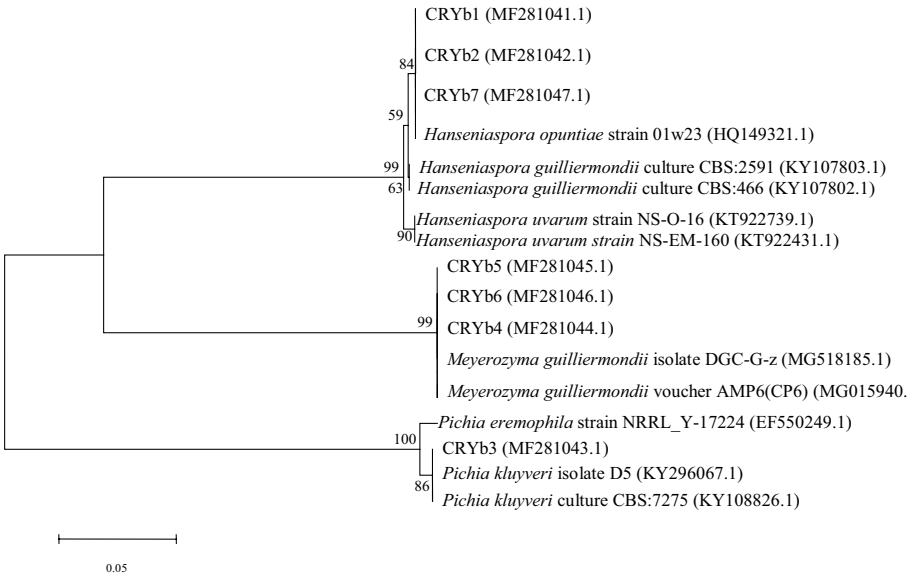

Fig. 1 Phylogenetic tree showing relatedness among large-subunit gene D1/D2 region sequences of yeast strains. The tree was constructed with neighbor-joining distance matrix. Bootstrap values (1000 tree interactions) are indicated at the nodes

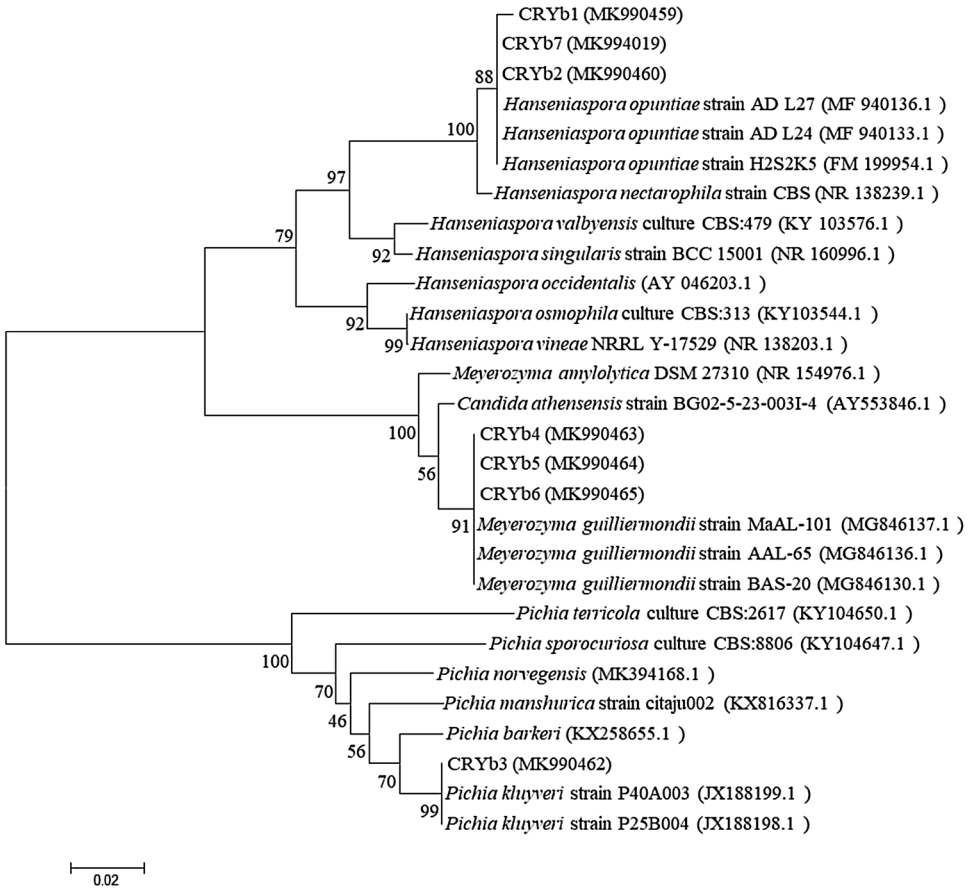

Fig. 2 Phylogenetic reconstruction based on ITS1–5.8S–ITS2 sequences of yeast strains. The tree was constructed using a neighbor-joining distance matrix. Bootstrap values (1000 tree interactions) are indicated at the nodes

related to *Hanseniaspora opuntiae*, whilst CRYb3 was more closely related to *Pichia kluyveri*. CRYb4, CRYb5 and CRYb6 were most closely related to *Meyerozyma guilliermondii.*

Carbon-Utilization Assays

Table 2 summarizes the utilization of 14 organic compounds by CRYb1, CRYb2, CRYb3, CRYb4, CRYb5, CRYb6 and CRYb7. The carbon source utilization data require further

Table 2 Summary of utilization of 14 carbon sources by yeast isolates

Chemicals	CRYb1	CRYb2	CRYb3	CRYb4	CRYb5	CRYb6	CRYb7
D-glucose	+	+	+	+	+	+	+
Glycerol	−	+	+	+	+	+	+
L-arabinose	−	−	+	+	−	+	+
Xylitol	+	−	+	−	+	−	−
D-galactose	+	+	+	+	+	+	+
Inositol	−	−	+	−	−	−	−
D-sorbitol	+	+	+	−	−	−	+
D-cellobiose	+	−	+	+	+	−	+
D-lactose	−	−	+	−	−	−	−
D-maltose	+	−	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
D-trehalose	+	−	+	+	+	+	+
D-melezitose	+	+	+	+	+	−	+
D-raffinose	+	+	+	+	+	+	+

+ Positive reaction, – negative reaction

confirmation that the CRYb1, CRYb2 and CRYb7 strains belong to *Hanseniaspora* sp., CRYb3 belongs to *Pichia* sp., CRYb4, CRYb5 and CRYb6 belong to *Meyerozyma* sp.

IAA Production

High-performance liquid chromatography was used to measure the IAA content due to its higher accuracy in comparison to traditional spectrophotometry. The spectrophotometric results show that CRYb1, CRYb5, CRYb6 and CRYb7 strains had higher levels of IAA production. In reference to this result, we measured the IAA yield of these strains at different culture times by high-performance liquid chromatography. The IAA in both standards and samples displayed consistent retention times (Supplementary Fig. 1). When incubated with 0.5% L-tryptophan, CRYb1, CRYb5, CRYb6 and CRYb7 displayed a time dependent increase in IAA production (Fig. 3). CRYb1 strains showed the highest levels of IAA production, whilst CRYb5 produced the lowest.

Influence of Yeast on the Agronomic Performance of Wheat

The effects of the yeast on the growth of wheat were demonstrated using the "ragdoll" method in which each isolate exhibited increased root lengths when compared to control samples. Of the yeasts tested, CRYb1 significantly enhanced both root and shoot lengths and increased the fresh wheat weight. CRYb7 significantly enhanced the root length, fresh weight and dry weight compared to controls (Table 3). Strains CRYb5, CRYb6 and CRYb7 also significantly enhanced shoot and root lengths.

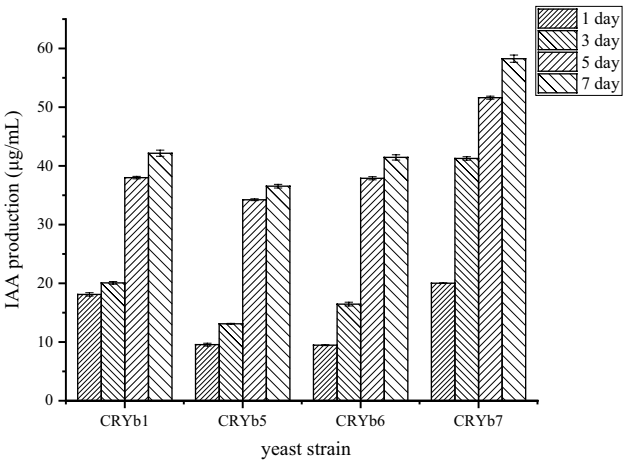

Fig. 3 IAA production by yeast strains incubated with 0.5% L-tryptophan

Discussion

In this study, seven yeast strains were isolated from the stems of tangerine peel and grown on YPD medium under aerobic conditions. Phylogenetic analysis of the rRNA sequences supported the identification of the yeast strains. Since ITS1-5.8S-ITS2 sequences are highly conserved in fungi [26], they were used to identify yeast, bind to 26srDNA, and enhance study accuracy. The two analyzed methods do not show different results. CRYb1, CRYb2 and CRYb7 were *H. opuntiae*, CRYb3 was *P. kluyveri*, CRYb4, CRYb5 and CRYb6 were *M. guilliermondii*. The *Hanseniaspora opuntiae* and *Meyerozyma guilliermondii* have been isolated from fruits and show good biological control efficacy [27–31]. In previous studies, *Pichia kluyveri* has

Table 3 Effect of the four yeasts on agronomic performance of wheat

Isolate number	Shoot length (cm)	Root length (cm)	Shoot/root	Fresh weight (g)	Dry weight (g)
CK	8.85 ± 1.01	9.01 ± 1.70	0.98	0.1858 ± 0.0190	0.0375 ± 0.0047
CRYb1	9.25 ± 1.83	11.21 ± 1.66	0.82	0.1921 ± 0.0292	0.0349 ± 0.0072
CRYb5	7.60 ± 1.12	6.16 ± 1.35	1.23	0.1740 ± 0.0187	0.0385 ± 0.0055
CRYb6	8.54 ± 1.20	6.29 ± 1.35	1.36	0.1776 ± 0.0301	0.0349 ± 0.0061
CRYb7	9.76 ± 1.77	8.25 ± 2.14	1.18	0.2015 ± 0.0219	0.0386 ± 0.0057

been widely used in industrial production and biological control [32, 33]. These endogenous yeasts are distributed and have many potential applications. Compared to other parts of the orange, the endogenous yeasts of orange peel are richer [6, 34]. This also depends on the environment in which the oranges grow. Unlike other tissues of sweet orange, the growth cycle of fruits and leaves is short, so colonization frequency and species richness of the yeasts can differ.

Four of the yeast strains produced high levels IAA in the presence of L-Trp. IAA is a phytochrome and one of the group of auxins known to improve plant growth through their ability to stimulate cell elongation, root initiation, seed germination and seedling growth [35]. As one of the most expensive energy-expensive amino acids [36] L-Trp is not synthesized by all yeast strains. Microorganisms incapable of synthesizing L-Trp rely on their plant hosts or the surrounding microbial sources [37]. Due to the availability of L-Trp in plant tissue, endophytic yeasts generally do not expend high levels of energy on their synthesis. Simultaneously, the ability of endophytes to convert L-Trp to IAA is beneficial to the host and represents a mutually advantageous plant–microbe system.

In this study, *H. opuntiae* CRYb1 induced the largest increase in root and shoot length, assessed using the "ragdoll" method. Similarly, CRYb7 led to varying degrees of wheat stem growth. These effects are related to enhanced IAA production. Surprisingly, CRYb5 and CRYb7 caused a strong inhibition of wheat root growth. Neither strain was colonized in wheat seeds. Colonization of yeast can impact the growth and development of the host [38]. Their absorption of nutrients and the production of metabolites may also be important reasons for the inhibition of wheat growth observed. These specific mechanism(s) require further in depth analysis.

Yeast strains are present on plant surfaces. The number of yeast cells in storage tissues are, on average, considerably lower than those on the plant surface [39]. Our data challenge the established notion of the distribution patterns of yeast strains in natural habitats and suggest that the peel of plants can be considered a typical habitat of yeast species. Plant tissues similar to tangerine peel, therefore, represents a promising source of new taxa, and a future model for studies of the coevolution of plant–microbial associations. In addition, knowledge of the distributional patterns and biological properties of endophytic yeasts is important from the perspective of fruit storage, potential allergic responses, the promotion of plant growth, and phytopathogen biocontrol.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Petrini O (1991) Fungal endophytes of tree leaves. In: Andrews I, Hirano SS (eds) Microbial ecology of leaves. Springer, New York, pp 179–197. https://doi.org/10.1007/978-1-4612-3168-4_9
2. Hallmann J, Quadt-Hallmann A, RodríGuez-Kábana R, Kloepper JW (1998) Interactions between meloidogyne incognita and endophytic bacteria in cotton and cucumber. Soil Biol Biochem 30(7):925–937. https://doi.org/10.1016/S0038-0717(97)00183-1
3. Lacava PT, Li W, Araújo WL, Azevedo JL, Hartung JS (2007) The endophyte Curtobacterium flaccumfaciens reduces symptoms caused by Xylella fastidiosa in Catharanthus roseus. J Microbiol 45(5):388–393. https://doi.org/10.1016/j.mimet.2007.08.002
4. Pirttilä AM, Pospiech H, Laukkanen H, Myllylä R, Hohtola A (2003) Two endophytic fungi in different tissues of scots pine buds (Pinus sylvestris L.). Microb Ecol 45(1):53–62. https://doi.org/10.1007/s00248-002-1038-8
5. Doty SL (2013) Endophytic yeasts: biology and applications. In: Aroca R (ed) Symbiotic Endophytes. Springer, Berlin, pp 335–343. https://doi.org/10.1007/978-3-642-39317-4_17

6. Sadeghi F, Samsampour D, Seyahooei MA, Bagheri A, Soltani J (2019) Diversity and spatiotemporal distribution of fungal endophytes associated with citrus reticulata cv. siyahoo. Curr Microbiol 76(3):279–289. https://doi.org/10.1007/s00284-019-01632-9
7. Camatti-Sartori V, da Silva-Ribeiro RT, Valdebenito-Sanhueza RM, Pagnocca FC, Echeverrigaray S, Azevedo JL (2005) Endophytic yeasts and filamentous fungi associated with southern Brazilian apple (Malus domestica) orchards subjected to conventional, integrated or organic cultivation. J Basic Microbiol 45(5):397–402. https://doi.org/10.1002/jobm.200410547
8. Nassar AH, El-Tarabily KA, Sivasithamparam K (2005) Promotion of plant growth by an auxin-producing isolate of the yeastwilliopsis saturnusendophytic in maize (zea maysl) roots. Biol Fertil Soils 42(2):97–108. https://doi.org/10.1007/s00374-005-0008-y
9. Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73(4):331–371. https://doi.org/10.1023/A:1001761008817
10. Schloss PD, Westcott SL (2011) Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. Appl Environ Microbiol 77(10):3219. https://doi.org/10.1128/AEM.02810-10
11. Xin G, Glawe D, Doty SL (2009) Characterization of three endophytic, indole-3-acetic acid-producing yeasts occurring in populus trees. Mycol Res 113(9):973–980. https://doi.org/10.1016/j.mycres.2009.06.001
12. Khan Z, Guelich G, Phan H, Redman R, Doty S (2012) Bacterial and yeast endophytes from poplar and willow promote growth in crop plants and grasses. ISRN Agronomy. 2012. https://doi.org/10.5402/2012/890280
13. Andrea F, Robert O, Asaf S, Jeremy S, Zareen K, Redman RS, Fleck ND, Erika L, Grigoriev IV, Doty SL (2015) Genome sequence of the plant growth promoting endophytic yeast Rhodotorula graminis WP1. Front Microbiol 6:978. https://doi.org/10.3389/fmicb.2015.00978
14. Pontecorvo G, Roper JA, Hemmons LM, Macdonald KD, Bufton AW (1953) The genetics of Aspergillus nidulans. Adv Genet 5(2):141–238. https://doi.org/10.1016/S0065-2660(08)60408-3
15. Kurtzman CP, Fell JW, Boekhout T, Robert V (2011) Chapter 7–methods for isolation, phenotypic characterization and maintenance of yeasts. Yeasts 1(14):87–110
16. Young TW (1999). The biochemistry and physiology of yeast growth. In Brewing microbiology. Springer, Boston, pp. 13–42. https://doi.org/10.1007/978-1-4684-0038-0_2
17. Ausubel FM, Baatout S (1996) Short protocols in molecular biology (3rd edn). Trends Genet 12(8):325–325. https://doi.org/10.1002/0471142727
18. Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73(4):331–371. https://doi.org/10.1023/A:1001761008817
19. White TJ, Bruns T, Lee SJWT, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols 18(1):315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
20. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2):111–120. https://doi.org/10.1007/bf01731581
21. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406. https://doi.org/10.1093/oxfordjournals.molbev.a040454
22. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
23. Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. Plant Physiol 26:192–195. https://doi.org/10.1104/pp.26.1.192
24. Sarwar A, Latif Z, Zhang S, Zhu J, Zechel DL, Bechthold A (2018) Biological control of potato common scab with rare Isatropolone C compound produced by plant growth promoting Streptomyces A1RT. Front Microbiol 9:1126. https://doi.org/10.3389/fmicb.2018.01126
25. Chamblee DS, Green JT (1995) Production and utilization of pastures and forages in North Carolina. Technical Bulletin, USA
26. Sugita T, Nishikawa A (2003) Fungal identification method based on DNA sequence analysis: reassessment of the methods of the Pharmaceutical Society of Japan and the Japanese Pharmacopoeia. J Health Sci 49(6):531–533. https://doi.org/10.1248/jhs.49.531
27. Ruiz-Moyano S, Martín A, Villalobos MC, Calle A, Serradilla MJ, Córdoba M, Hernández A (2016) Yeasts isolated from figs (Ficus carica L.) as biocontrol agents of postharvest fruit diseases. Food Microbiol 57:45–53. https://doi.org/10.1016/j.fm.2016.01.003
28. Hyun SH, Lee JG, Park WJ, Kim HK, Lee JS (2014) Isolation and diversity of yeasts from fruits and flowers of orchard in Sinammyeon of Yesan-gun, Chungcheongnam-do Korea. Korean J Mycol 42(1):21–27. https://doi.org/10.4489/KJM.2014.42.1.21
29. Liu R, Zhang Q, Chen F, Zhang X (2015) Analysis of culturable yeast diversity in spontaneously fermented orange wine, orange peel and orangery soil of a Ponkan plantation in China. Ann Microbiol 65(4):2387–2391. https://doi.org/10.1007/s13213-015-1081-6
30. Ren F, Dong W, Sun H, Yan DH (2019) Endophytic mycobiota of jingbai pear trees in north China. Forests 10(3):260. https://doi.org/10.3390/f10030260
31. Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, Singh K, Saxena, AK (2019). Endophytic fungi: biodiversity, ecological significance, and potential industrial applications. In Recent Advancement in White Biotechnology Through Fungi. Springer, Cham, pp. 1–62. https://doi.org/10.1007/978-3-030-10480-1_1
32. de Souza Varize C, Christofoleti-Furlan RM, Muynarsk EDSM, de Melo Pereira GV, Lopes LD, Basso LC (2019). Biotechnological applications of nonconventional yeasts. In Yeasts in Biotechnology. IntechOpen. https://doi.org/10.5772/intechopen.83035
33. Mannazzu I, Domizio P, Carboni G, Zara S, Zara G, Comitini F, Budroni M, Ciani M (2019) Yeast killer toxins: from ecological significance to application. Crit Rev Biotechnol 39:1–15. https://doi.org/10.1080/07388551.2019.1601679
34. Gai CS, Lacava PT, Maccheroni W Jr, Glienke C, Araújo WL, Miller TA, Azevedo JL (2009) Diversity of endophytic yeasts from sweet orange and their localization by scanning electron microscopy. J Basic Microbiol 49(5):441–451. https://doi.org/10.1002/jobm.200800328
35. Paul LK, Khurana JP (2008) Phytochrome-mediated light signaling in plants: emerging trends. Physiol Mol Biol Plants 14(1–2):9–22. https://doi.org/10.1007/s12298-008-0002-6
36. Hrazdina G, Jensen RA (1992) Spatial organization of enzymes in plant metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 43(1):241–267
37. Radwanski ER, Last RL (1995) Tryptophan biosynthesis and molecular genetics biochemical and molecular genetics. Plant Cell 7(7):921–934. https://doi.org/10.2307/3870047
38. Mehmood A, Khan N, Irshad M, Hamayun M (2018) IAA producing endopytic fungus fusariun oxysporum wlw colonize maize roots and promoted maize growth under hydroponic condition. Eur Exp Biol 8(4):24. https://doi.org/10.21767/2248-9215.100065
39. Isaeva OV, Glushakova AM, Garbuz SA, Kachalkin AV, Iiu C (2010) Endophytic yeast fungi in plant storage tissues. Biol Bull 37(1):26–34. https://doi.org/10.1134/S1062359010010048