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Article

Hydrolytic Enzyme Production and Susceptibility to Antifungal Compounds of Opportunistic *Candida parapsilosis* Strains Isolated from *Cucurbitaceae* and *Rosaceae* Fruits

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Abstract: Endophytic yeast species were studied in the internal tissues of ripe cultivated vegetables and fruits. A total of 19 yeast species, 11 ascomycete species, and 8 basidiomycete species were observed in the internal tissues of all fruits examined. The opportunistic yeast *Candida parapsilosis* was present in all plants studied. Several virulence factors (production of hydrolytic enzymes and sensitivity to antifungal agents) were examined in all 107 isolates of *C. parapsilosis* from the internal tissues of fruits. The most virulent isolates were found in vegetables. *C. parapsilosis* is widespread in nature and is often isolated from a variety of non-human sources. It is frequently involved in invasive infections that seriously affect human health. This species poses a high risk to immunocompromised individuals, such as HIV patients and surgical patients or children whose immune systems are not sufficiently mature. Since virulent isolates of *Candida parapsilosis* have been found in vegetables and fruits; their raw consumption may not be safe. Finally, we emphasize the importance of ongoing phenotypic and genetic studies of endophytic isolates of *Candida parapsilosis* and their comparison with clinical isolates.

Keywords: *Candida parapsilosis*; endophytes; yeasts; resistance to antibiotics; virulence

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1. Introduction

Opportunistic *Candida* species are present in more than 50% of the human population. Infection caused by *Candida*, known as candidiasis, remains an important clinical problem, especially in immunocompromised patients [1]. In recent years, opportunistic mycoses caused not only by *Candida albicans* but also by *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* have become an urgent public health problem and are a common complication of many diseases. There are several reasons for the increasing incidence of fungal infections in the population. The deterioration of environmental conditions decreases the anti-infective resistance of humans, leading to an imbalance between the normal microbiome and the immune response of the body, to a strong activation of opportunistic fungi and, consequently, to an expansion of the spectrum of pathogens causing lesions of the skin and internal organs. The irrational use of antibiotics, cytostatics, hormonal drugs, radiation, and chemical therapies in the fight against the underlying disease also leads to a decrease in immunity and the development of drug-resistant strains of microorganisms [2,3].

Pathogenic and opportunistic species of the genus *Candida* are increasingly found not only in clinical settings but also in various natural substrates, especially in urban environments, such as urban soils in the area of household waste sites, urban plant substrates, etc. [4,5]. A recent large-scale analysis of endophytic yeasts from internal tissues of 54 types of vegetables, fruits, and nuts from 36 countries in agricultural crops revealed a high occurrence and abundance of the clinically important yeast *Candida parapsilosis* [6,7] in some internal tissues [8]. The endophytic community of opportunistic yeasts was represented

by the *C. parapsilosis* complex, comprising three distinct species: *Candida parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis* [9].

Endophytic yeasts are one of the most promising areas in the study of microbe–plant associations. They serve as an excellent model for the study of a number of current practical questions in agriculture, medicine, ecology, and systems. Endophytic yeasts are predominantly stored or developed in the internal tissues of fruits [10–16]. Unlike filamentous fungi, unicellular yeasts cannot spread by apical growth in the intercellular space. However, yeast cells can enter the plant through stomata, hydatodes, and mechanical micro-injuries of the cuticle and epidermis [17]. Some yeasts can produce exoglucanases that allow yeast cells to invade internal tissues from the surface by local destruction of the cuticle. [18]. In the internal tissues of fruits, endophytic yeasts are mainly represented by ascomycete species from the genera *Aureobasidium, Candida, Debaryomyces, Hanseniaspora, Metschnikowia, Meyerozyma, Pichia, Saccharomyces*, and *Wickerhamomyces* [10,13,14,16].

However, under anthropogenic impact, microbial complexes may change to include opportunistic species of the genus *Candida* in yeast communities. For clinically relevant yeast species, the change in biological properties of isolates toward virulence, i.e., their actual ability to cause disease, is well known [19–22]. The development of resistance to antifungal drugs can be considered an indicator of the change from "harmless" to "aggressive" behavior [22,23].

The aim of our work was to study the diversity of endophytic yeasts in ripe vegetables and fruits grown in fields near cities and to evaluate the virulence properties of isolated strains of opportunistic species.

2. Materials and Methods

2.1. Study Location and Sampling

Vegetables and fruits for the study of endophytic yeast diversity in internal tissues were collected in 2021 on the territory of farms in Moscow Region and Vladimir Region. A total of 65 vegetables and fruits were analyzed. Each sample of a ripe fruit, which had no visible damage, was treated separately (Table 1).

Table 1. Vegetables and fruits studied and their locations.

Names of Vegetables and Fruits	Samples	Location
Cucurbita pepo (variety "Zorka")	8	Vladimir Region
Cucurbita pepo (variety winter table pumpkin)	7	Vladimir Region
Cucurbita zucchini (variety "Black Beauty")	9	Vladimir Region
Malus domestica (variety "Cinnamon Stripe")	12	Moscow Region
Malus domestica (variety "Bessemianka Michurinskaya")	12	Vladimir Region
Prunus domestica	17	Moscow Region

2.2. Microbiological Analyses and Species Identification

To study endophytic yeast communities, vegetables and fruits were treated according to the following scheme: 70% ethanol, 30 min; 2% sodium hypochlorite, 30 min; 70% ethanol, 30 s; and washing in sterile distilled water for 10 min [11,24]. After the exocarp was removed with a sterile scalpel, the inner tissue was excised, homogenized, and poured with sterile water to obtain a 1:10 dilution. The suspensions were vortexed on a Multi Reax Vortexer (Heidolph Instruments, Germany) for 15 min at 2000 rpm. Three suspensions were prepared for each vegetable and fruit. The prepared suspensions were plated in four replicates each on glucose–peptone–yeast extract (GPY) agar (20 g/L glucose, 10 g/L peptone, 5 g/L yeast extract, 20 g/L agar) supplemented with chloramphenicol (500 mg/L) to prevent bacterial growth. A total of 780 plates were incubated at 22 °C for 5–7 days. The grown yeast colonies were classified into morphological types using a dissecting microscope (MSP-2 (LOMO, Russia)) and the number of isolates of each type was counted. From 5 to

7 isolates of each morphotype were taken in a pure culture. Purified yeast isolates were cryopreserved in 10% (*v*/*v*) glycerol in water solution at −80 °C in the yeast collection of the Soil Biology Department at Lomonosov Moscow State University (WDCM 1173).

Identification of yeast species was based on the ITS rDNA nucleotide sequence. DNA isolation and PCR were performed according to the procedure described previously [8,15]. DNA sequencing was performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) with subsequent analysis of the reaction products on an Applied Biosystems 3130xl Genetic Analyzer at the facilities of Evrogen (Moscow). For sequencing, the ITS5 primer (5′-GGA AGT AAA AGT CGT AAC AAG G) was used [8]. For species identification, nucleotide sequences were compared with those in public databases, using the BLAST NCBI (www.ncbi.nlm.nih.gov (accessed on 27 November 2022)) and the MycoID (www.mycobank.org (accessed on 29 November 2022)) tools. The ITS regions of the strains studied were 99.5–100% similar to the type strains. Sequences obtained for yeast species in the present study were deposited in GenBank database (OQ238753-OQ238770 and OM038460). All *Candida parapsilosis* isolates were further identified by growth on chromagar (HiCrome Candida Differential Agar Base, HiMedia Laboratories) and some of the strains were sequenced (supplementary data).

2.3. Production of Hydrolytic Enzymes

Enzymatic activities were measured by plate method. Aliquots (10 µL) of 48 h old cultured fungal cells (10^7) were spotted onto the surface of each agar medium (see below) and then incubated for 7 days at 37 °C. Colony diameter (a) and colony plus precipitation zone diameter (b) were measured using a digital paquimeter, and enzymatic activities were expressed as Pz values (Pz = a/b) as described by Price et al. [25]. According to this definition, low Pz values indicate high enzyme production and, inversely, high Pz values indicate low enzyme production. The Pz value was divided into four categories:

Pz = 1, no enzymatic activity; 0.999 ≥ Pz ≥ 0.700, low enzymatic activity; 0.699 ≥ Pz ≥ 0.400, medium enzymatic activity; Pz ≤ 0.399, high enzymatic activity [17].

2.3.1. Phospholipase Activity

Phospholipase activity was measured using an egg yolk agar plate (1 M NaCl, 5 mM $CaCl_2$, and 8% sterile egg yolk emulsion, pH 7.0) according to Price et al. [25]. The hydrolysis of lipid substrates present in egg yolk resulted in the formation of a calcium complex with fatty acids released by the action of the secreted enzymes, resulting in a precipitation zone around the colony. For strains in which phospholipase activity was detected, a more precise diagnosis of phospholipase A2 activity was performed. Twenty-four h cultures of *Candida parapsilosis* strains were used. Phospholipase A2 activity was determined by a modified titrometric method. To two test tubes (experiment and control), 1 mL of the initial concentration of yeast was added and placed in the thermostat at 60 °C for 15 min. Then, a 0.3% trypsin solution was added to both test tubes and placed in the refrigerator at 4 °C for one hour. Then, the lecithin solution was added to the experimental test tube, 0.6% calcium chloride was added to both samples, and the sample was placed in the thermostat at 37 °C for two hours. After this time, lecithin was added to the control sample and 10% calcium chloride was added to both test tubes to stop the reaction. A 0.1% alcoholic solution of phenolphthalein was added to the contents of the test tubes and titrated with a freshly prepared solution of 0.002 M sodium hydroxide solution until it turned faintly pink staining. The volume of solution used for titration of the control and experimental samples was recorded. Enzyme activity was calculated using the following formula: A = Vo−Vk (Vo and Vk are the volumes of alkali solution used for titration of the experimental and control samples, respectively; A was the enzyme activity expressed in mmol/L). The studies were performed over the course of a day with an interval of 8 h.

2.3.2. Protease Activity

Determination of protease production was assayed using the albumin agar plate (1.17% yeast carbon base (YCB) medium supplemented with 0.2% BSA, pH 4.0) as previously described by Rьchel et al. [26]. The protease activity resulted in a clear zone around the colony, which corresponded to the hydrolysis of the BSA present in the medium.

2.3.3. Hemolytic Activity

The determination of hemolysis was performed using the blood agar plate. The medium was prepared by adding 7 mL of fresh sheep blood to 100 mL of Sabouraud dextrose agar containing 3% glucose, as previously described by Luo et al. [27]. The presence of a distinct translucent halo around the inoculum site in transmitted light indicated positive hemolytic activity.

2.4. Antimycotic Sensitivity of Endophytic Candida parapsilosis Strains

Antimycotic susceptibility of *C. parapsilosis* strains was tested using Mueller–Hinton agar (HiMedia Laboratories Pvt. Ltd., India), which is a standard medium for the disk diffusion method [28]. Disks with two widely applicated antifungal drugs (HiMedia Laboratories Pvt. Ltd., India): Amphotericin B 100 (μg/disk) (AMO) and Fluconazole 25 (μg/disk), were used. As a control, the reference strain of *C. parapsilosis* ATCC 22019 was taken (CLSI QC strain for Disk Diffusion and MIC determination). A total of 107 endophytic isolates of *C. parapsilosis* were tested. Each of the 107 strains was tested in six plate replicates for both antimicrobial drugs.

2.5. Data Analyses

Statistical data processing and graphical presentation of the obtained results were carried out using Excel 2010 (Microsoft, Albuquerque, NM, USA) and Statistica 8.0 (StatSoft, USA) programs. The analysis of variance (ANOVA) was carried out for group comparisons. Statistical significance was judged at the level of $p < 0.05$. The number of yeast colonies was used to calculate the abundance of yeast cells (CFU) in each type of sample per dry weight. The relative abundances of the species were calculated as the proportion (%) of colonies that appeared on the plates.

3. Results

3.1. Abundance and Diversity of Endophytic Yeast Complexes in Vegetables and Fruits

The abundance of endophytic yeasts in the internal tissues of ripe fruits ranged from a minimum of 1.78 lg (CFU/g) in *Cucurbita pepo* (variety "Zorka") to a maximum of 5.89 lg (CFU/g) in *Malus domestica* (variety "Bessemianka Michurinskaya") (Table 2). A total of 19 yeast species, 11 ascomycete species, and 8 basidiomycete species were detected in the internal tissues of all examined fruits (Table 2). In most of the ripe fruits studied, the diversity of the ascomycete yeast species was higher than that of the basidiomycete species. The ratio of ascomycete yeasts to basidiomycete was particularly high in *Prunus domestica*, being 10 to 3.

Typical eurytopes (*Aureobasidium pullulans, Rhodotorula mucilaginosa*) were found in the internal tissues of all plants examined, as was the ascomycete yeast *Metschnikowia pulcherrima. M. pulcherrima* is regularly found in nutrient-rich plant material that serves as a feeding and breeding habitat for a variety of insects. These habitats include tree sap fluxes, flowers, and fruits [29]. Strains of *M. pulcherrima* are known to exhibit strong antagonistic activity against postharvest pathogens and are used as biocontrol agents [30,31]. The yeast species *Debaryomyces hansenii* was detected in all fruits tested except *Cucurbita pepo* (variety winter table pumpkin). Yeasts of the genus *Debaryomyces* include species isolated from various natural habitats, man-made environments, and clinical material [32,33]. The ascomycete species *Hanseniaspora uvarum* was observed in apples, plums, and a zucchini. *H. uvarum* is a widely distributed yeast species most commonly isolated from soil, insects, various ripe fruits, and fermenting musts [29]. It has also been collected from marine and

freshwater ecosystems [34] and from medical samples [35]. Among the basidiomycete yeasts, the typical epiphytic species *Papiliotrema flavescens* and *Filobasidium magnum* [36–39] and the cold-adapted yeast species *Cystofilobasidium infirmominiatum* [40,41] were present in the endophytic communities.

Table 2. Diversity of endophytic yeasts obtained from ripe vegetables and fruits (relative abundance with standard errors).

Yeast Species	*Cucurbita pepo* (Variety "Zorka")	*Cucurbita pepo* (Variety Winter Table Pumpkin)	*Cucurbita zucchini*	*Malus domestica* (Variety "Cinnamon Stripe")	*Malus domestica* (Variety "Bessemianka Michurinskaya")	*Prunus domestica*
Ascomycetes						
Aureobasidium pullulans	24.72 ± 0.21	21.44 ± 0.51	17.26 ± 0.21	11.23 ± 1.01	10.98 ± 0.12	10.81 ± 0.2
Barnettozyma californica	6.22 ± 0.23	5.11 ± 012	0.13 ± 0.04	–	–	5.87 ± 0.71
Candida parapsilosis	6.01 ± 0,67	8.41 ± 0.09	6.12 ± 0.23	2.09 ± 0.9	4.26 ± 0.02	1.91 ± 0,22
Candida saitoana	– *	–	–	2.17 ± 0.02	–	2.85 ± 0.21
Candida sake	–	–	2.41 ± 0.11	3.02 ± 0.04	1.45 ± 0.3	1.14 ± 076
Candida santamariae	–	–	–	9.01 ± 0.17	–	–
Debaryomyces hansenii	3.19 ± 0.56	–	1.17 ± 0.02	6.54 ± 0.11	11.28 ± 0.23	7.19 ± 1.1
Hanseniaspora uvarum	–	–	5.04 ± 0.11	11.67 ± 0.45	16.44 ± 0.36	14.84 ± 0.1
Metschnikowia pulcherrima	9.16 ± 0.34	12.44 ± 0.07	7.67 ± 0.12	19.41 ± 0.16	21.42 ± 0.77	24.08 ± 0.8
Pichia membranifaciens	–	–	–	–	–	2.11 ± 0.92
Starmerella sp.	–	–	–	–	–	0.31 ± 0.02
Basidiomycetes						
Cystofilobasidium infirmominiatum	21.55 ± 0.55	17.64 ± 0.21	9.24 ± 0.2	–	–	–
Filobasidium magnum	2.11 ± 0.31	–	–	17.44 ± 0.45	15.12 ± 0.02	0.61 ± 0.01
Leucosporidium egoroviorum	–	–	23.47 ± 0.16	–	–	–
Papiliotrema flavescens	11.23 ± 0.12	18.06 ± 0.2	0.24 ± 0.01	2.11 ± 0.17	2.97 ± 0.16	–
Pseudohyphozyma pustula	–	–	–	4.02 ± 0.13	–	–
Rhodotorula babjevae	–	0.77 ± 0.01	1.21 ± 0.5	0.91 ± 0.07	1.12 ± 0.11	–
Rhodotorula mucilaginosa	15.81 ± 0.81	16.13 ± 1.01	24.83 ± 0,34	10.38 ± 0.31	14.96 ± 0.62	27.53 ± 0.8
Vishniacozyma victoriae	–	–	1.21 ± 0.02	–	–	0.75 ± 0.01
Average abundance, lg (CFU/g)	1.78	2.01	3.12	5.62	5.89	3.54
Total species number	9	8	13	13	10	13
Ascomycete yeast species/ Basidiomycete yeast species	5/4	4/4	7/6	8/5	6/4	10/3

* "–" not found.

The opportunistic yeast species *Candida parapsilosis* was detected in the internal tissues of all vegetables and fruits studied. This species, along with *C. albicans*, *C. glabrata*, and *C. tropicalis*, is one of the most common species involved in invasive fungal infections [6,42]. Previously, we observed this opportunistic yeast species in plant substrates in urban environments with high anthropogenic impact (along highways): on pollen of wind-pollinated plants [5], in the internal tissues of *M. domestica* and *p. communis* fruits [15] (Table 2).

3.2. Production of Hydrolytic Enzymes and Antimycotic Susceptibility of Candida parapsilosis Endophytic Strains

During the study, a total of 107 endophytic strains of *Candida parapsilosis* were isolated. All isolated endophytic *Candida parapsilosis* strains were evaluated for some virulence properties: hydrolytic activity (Tables 3 and 5) and sensitivity to antifungal drugs (Tables 4 and 5).

Phospholipase activity was observed only in the two endophytic strains tested. Both strains were isolated from vegetable products (Table 3). High proteolytic activity was observed in all isolated strains (Table 3). Low hemolytic activity was observed in all strains isolated from vegetables. No hemolytic activity was detected in 4 of 22 strains isolated from apples, and low hemolytic activity was detected in the other strains. Two of the eighteen strains isolated from plums also showed no hemolytic activity, and the others showed low activity (Table 3). Most strains showed sensitivity to antifungal drugs (Table 4).

Fluconazole-resistant strains were isolated from all tested products. Only some strains from vegetables showed resistance to amphotericin B. Strains resistant to both antibiotics were isolated only from vegetable products (Figure 1).

Table 3. Hydrolytic enzyme production by endophytic strains belonging to *Candida parapsilosis* and isolated from ripe vegetables and fruits (mean values with standard errors).

Code	Substrate	Phospholipase †	Phospholipase A2 (mmol/L)	Protease †	Hemolysis †
ATCC 22019	control	1		0.35 ± 0.02	0.84 ± 0.05
CPE-01	*Cucurbita pepo*	1		0.32 ± 0.05	0.71 ± 0.01
CPE-02	*Cucurbita pepo*	1		0.28 ± 0.05	0.83 ± 0.01
CPE-03	*Cucurbita pepo*	1		0.28 ± 0.08	0.80 ± 0.05
CPE-04	*Cucurbita pepo*	1		0.35 ± 0.11	0.84 ± 0.01
CPE-05	*Cucurbita pepo*	0.51 ± 0.02	13.81 ± 0.03	0.34 ± 0.01	0.84 ± 0.05
CPE-06	*Cucurbita pepo*	1		0.22 ± 0.09	0.86 ± 0.02
CPE-07	*Cucurbita pepo*	1		0.15 ± 0.02	0.74 ± 0.05
CPE-08	*Cucurbita pepo*	1		0.30 ± 0.02	0.91 ± 0.10
CPE-09	*Cucurbita pepo*	1		0.32 ± 0.04	0.72 ± 0.03
CPE-10	*Cucurbita pepo*	1		0.36 ± 0.05	0.88 ± 0.05
CPE-11	*Cucurbita pepo*	1		0.31 ± 0.05	0.84 ± 0.11
CPE-12	*Cucurbita pepo*	1		0.26 ± 0.10	0.93 ± 0.01
CPE-13	*Cucurbita pepo*	1		0.33 ± 0.03	0.74 ± 0.05
CPE-14	*Cucurbita pepo*	1		0.36 ± 0.06	0.96 ± 0.06
CPE-15	*Cucurbita pepo*	1		0.34 ± 0.04	0.91 ± 0.11
CPE-16	*Cucurbita pepo*	1		0.30 ± 0.02	0.84 ± 0.03
CPE-17	*Cucurbita pepo*	1		0.31 ± 0.02	0.80 ± 0.12
CPE-18	*Cucurbita pepo*	1		0.34 ± 0.06	0.72 ± 0.21
CPE-19	*Cucurbita pepo*	1		0.41 ± 0.11	0.89 ± 0.01
CPE-20	*Cucurbita pepo*	1		0.35 ± 0.09	0.84 ± 0.04
CPE-21	*Cucurbita pepo*	1		0.32 ± 0.02	0.90 ± 0.04
CPE-22	*Cucurbita pepo*	1		0.31 ± 0.02	0.97 ± 0.05
CPE-23	*Cucurbita pepo*	1		0.45 ± 0.11	0.86 ± 0.02
CPE-24	*Cucurbita pepo*	1		0.30 ± 0.07	0.87 ± 0.01
CPE-25	*Cucurbita pepo*	1		0.32 ± 0.04	0.90 ± 0.01
CPE-26	*Cucurbita pepo*	1		0.32 ± 0.04	0.94 ± 0.03
CPE-27	*Cucurbita pepo*	1		0.38 ± 0.02	0.84 ± 0.05
CPE-28	*Cucurbita pepo*	1		0.34 ± 0.03	0.84 ± 0.16
CPE-29	*Cucurbita pepo*	1		0.25 ± 0.02	0.88 ± 0.01
CPE-30	*Cucurbita pepo*	1		0.29 ± 0.02	0.81 ± 0.01
CPE-31	*Cucurbita pepo*	1		0.35 ± 0.01	0.92 ± 0.09
CPE-32	*Cucurbita pepo*	1		0.34 ± 0.06	0.84 ± 0.10
CPE-33	*Cucurbita pepo*	1		0.34 ± 0.16	0.98 ± 0.02
CPE-34	*Cucurbita pepo*	1		0.31 ± 0.02	0.81 ± 0.02
CPE-35	*Cucurbita pepo*	1		0.30 ± 0.01	0.82 ± 0.02
CPE-36	*Cucurbita pepo*	1		0.30 ± 0.09	0.80 ± 0.11
CPE-37	*Cucurbita pepo*	1		0.37 ± 0.11	0.98 ± 0.06
CPE-38	*Cucurbita pepo*	1		0.35 ± 0.11	0.84 ± 0.02
CPE-39	*Cucurbita pepo*	1		0.38 ± 0.02	0.82 ± 0.02
CPE-40	*Cucurbita zucchini*	1		0.31 ± 0.02	0.81 ± 0.11
CPE-41	*Cucurbita zucchini*	1		0.38 ± 0.01	0.74 ± 0.23
CPE-42	*Cucurbita zucchini*	1		0.40 ± 0.02	0.80 ± 0.05
CPE-43	*Cucurbita zucchini*	1		0.65 ± 0.05	0.80 ± 0.02
CPE-44	*Cucurbita zucchini*	1		0.21 ± 0.05	0.94 ± 0.04
CPE-45	*Cucurbita zucchini*	1		0.33 ± 0.01	0.87 ± 0.04
CPE-46	*Cucurbita zucchini*	1		0.32 ± 0.08	0.82 ± 0.05
CPE-47	*Cucurbita zucchini*	1		0.32 ± 0.11	0.80 ± 0.01
CPE-48	*Cucurbita zucchini*	1		0.35 ± 0.05	0.73 ± 0.02
CPE-49	*Cucurbita zucchini*	1		0.33 ± 0.04	0.88 ± 0.02
CPE-50	*Cucurbita zucchini*	1		0.34 ± 0.04	0.90 ± 0.01
CPE-51	*Cucurbita zucchini*	1		0.34 ± 0.01	0.90 ± 0.01

Table 3. *Cont.*

Code	Substrate	Phospholipase †	Phospholipase A2 (mmol/L)	Protease †	Hemolysis †
CPE-52	*Cucurbita zucchini*	1		0.15 ± 0.02	0.91 ± 0.16
CPE-53	*Cucurbita zucchini*	1		0.30 ± 0.02	0.94 ± 0.41
CPE-54	*Cucurbita zucchini*	1		0.39 ± 0.06	0.82 ± 0.04
CPE-55	*Cucurbita zucchini*	1		0.26 ± 0.12	0.82 ± 0.04
CPE-56	*Cucurbita zucchini*	1		0.21 ± 0.07	0.81 ± 0.01
CPE-57	*Cucurbita zucchini*	1		0.30 ± 0.16	0.82 ± 0.01
CPE-58	*Cucurbita zucchini*	1		0.34 ± 0.01	0.84 ± 0.02
CPE-59	*Cucurbita zucchini*	1		0.34 ± 0.01	0.86 ± 0.02
CPE-60	*Cucurbita zucchini*	1		0.30 ± 0.05	0.88 ± 0.22
CPE-61	*Cucurbita zucchini*	1		0.31 ± 0.01	0.88 ± 0.13
CPE-62	*Cucurbita zucchini*	1		0.38 ± 0.04	0.81 ± 0.05
CPE-63	*Cucurbita zucchini*	0.54 ± 0.10	16.11 ± 0.05	0.11 ± 0.02	0.94 ± 0.01
CPE-64	*Cucurbita zucchini*	1		0.30 ± 0.11	0.74 ± 0.12
CPE-65	*Cucurbita zucchini*	1		0.30 ± 0.09	0.84 ± 0.01
CPE-66	*Cucurbita zucchini*	1		0.31 ± 0.05	0.74 ± 0.04
CPE-67	*Cucurbita zucchini*	1		0.29 ± 0.06	0.84 ± 0.04
CPE-68	*Malus domestica*	1		0.32 ± 0.12	0.94 ± 0.02
CPE-69	*Malus domestica*	1		0.38 ± 0.01	0.94 ± 0.01
CPE-70	*Malus domestica*	1		0.38 ± 0.02	0.94 ± 0.12
CPE-71	*Malus domestica*	1		0.36 ± 0.05	0.90 ± 0.01
CPE-72	*Malus domestica*	1		0.41 ± 0.06	1
CPE-73	*Malus domestica*	1		0.45 ± 0.05	0.84 ± 0.44
CPE-74	*Malus domestica*	1		0.32 ± 0.01	0.84 ± 0.06
CPE-75	*Malus domestica*	1		0.37 ± 0.07	0.84 ± 0.05
CPE-76	*Malus domestica*	1		0.36 ± 0.01	1
CPE-77	*Malus domestica*	1		0.30 ± 0.16	1
CPE-78	*Malus domestica*	1		0.39 ± 0.06	0.84 ± 0.05
CPE-79	*Malus domestica*	1		0.44 ± 0.06	0.82 ± 0.02
CPE-80	*Malus domestica*	1		0.33 ± 0.05	0.90 ± 0.02
CPE-81	*Malus domestica*	1		0.34 ± 0.01	0.90 ± 0.01
CPE-82	*Malus domestica*	1		0.35 ± 0.02	0.91 ± 0.02
CPE-83	*Malus domestica*	1		0.36 ± 0.03	0.86 ± 0.11
CPE-84	*Malus domestica*	1		0.37 ± 0.09	0.84 ± 0.07
CPE-85	*Malus domestica*	1		0.30 ± 0.11	0.82 ± 0.21
CPE-86	*Malus domestica*	1		0.42 ± 0.07	0.84 ± 0.01
CPE-87	*Malus domestica*	1		0.31 ± 0.01	0.89 ± 0.05
CPE-88	*Malus domestica*	1		0.30 ± 0.01	0.82 ± 0.05
CPE-89	*Malus domestica*	1		0.35 ± 0.02	1
CPE90	*Prunus domestica*	1		0.36 ± 0.04	0.89 ± 0.01
CPE91	*Prunus domestica*	1		0.35 ± 0.02	0.81 ± 0.03
CPE92	*Prunus domestica*	1		0.45 ± 0.22	0.82 ± 0.05
CPE93	*Prunus domestica*	1		0.44 ± 0.08	0.92 ± 0.04
CPE94	*Prunus domestica*	1		0.38 ± 0.01	0.84 ± 0.12
CPE95	*Prunus domestica*	1		0.31 ± 0.02	0.86 ± 0.18
CPE96	*Prunus domestica*	1		0.30 ± 0.02	0.86 ± 0.05
CPE97	*Prunus domestica*	1		0.39 ± 0.04	0.80 ± 0.01
CPE98	*Prunus domestica*	1		0.32 ± 0.01	0.81 ± 0.05
CPE99	*Prunus domestica*	1		0.34 ± 0.01	0.83 ± 0.05
CPE100	*Prunus domestica*	1		0.37 ± 0.01	1
CPE101	*Prunus domestica*	1		0.36 ± 0.04	0.82 ± 0.10
CPE102	*Prunus domestica*	1		0.33 ± 0.06	0.80 ± 0.06
CPE103	*Prunus domestica*	1		0.33 ± 0.13	1
CPE104	*Prunus domestica*	1		0.28 ± 0.01	0.96 ± 0.01
CPE105	*Prunus domestica*	1		0.38 ± 0.01	0.94 ± 0.04
CPE106	*Prunus domestica*	1		0.47 ± 0.02	0.81 ± 0.04
CPE107	*Prunus domestica*	1		0.32 ± 0.01	0.94 ± 0.01

† The protease, phospholipase, and hemolysis activities were measured by the formation of a clear halo around the colony and expressed as Pz value. Pz value was scored into four categories: Pz = 1, no enzymatic activity; $0.999 \geq Pz \geq 0.700$, low enzymatic activity; $0.699 \geq Pz \geq 0.400$, medium enzymatic activity; $Pz \leq 0.399$, high enzymatic activity.

Table 4. Endophytic strains of *Candida parapsilosis* isolated from ripe vegetables and fruits with antimycotic susceptibility (retarded growth with standard errors, mm).

		Amphotericin B	Fluconazole
Code	**Substrate**	**13–17**	**27–33**
ATCC 22019	control	17.9 ± 0.01	27.6 ± 0.02
CPE-01	*Cucurbita pepo*	17.1 ± 0.01	27.1 ± 0.05
CPE-02	*Cucurbita pepo*	17.1 ± 0.06	27.1 ± 0.11
CPE-03	*Cucurbita pepo*	16.2 ± 0.05	27.0 ± 0.04
CPE-04 †	*Cucurbita pepo*	19.2 ± 0.05	26.6 ± 0.01
CPE-05	*Cucurbita pepo*	12.5 ± 0.03	26.1 ± 0.01
CPE-06	*Cucurbita pepo*	18.6 ± 0.4	27.2 ± 0.11
CPE-07	*Cucurbita pepo*	17.6 ± 0.16	27.1 ± 0.03
CPE-08	*Cucurbita pepo*	14.2 ± 0.07	27.2 ± 0.11
CPE-09	*Cucurbita pepo*	17.9 ± 0.01	27.6 ± 0.11
CPE-10	*Cucurbita pepo*	17.2 ± 0.05	27.2 ± 0.10
CPE-11	*Cucurbita pepo*	16.9 ± 0.12	28.1 ± 0.05
CPE-12	*Cucurbita pepo*	17.2 ± 0.11	21.2 ± 0.05
CPE-13	*Cucurbita pepo*	18.2 ± 0.05	27.2 ± 0.05
CPE-14	*Cucurbita pepo*	15.7 ± 0.11	27.1 ± 0.02
CPE-15	*Cucurbita pepo*	17.3 ± 0.01	27.0 ± 0.33
CPE-16	*Cucurbita pepo*	17.0 ± 0.42	27.0 ± 0.01
CPE-17	*Cucurbita pepo*	17.1 ± 0.03	27.4 ± 0.04
CPE-18	*Cucurbita pepo*	16.8 ± 0.11	27.4 ± 0.02
CPE-19	*Cucurbita pepo*	17.5 ± 0.10	27.0 ± 0.02
CPE-20	*Cucurbita pepo*	16.1 ± 0.05	27.1 ± 0.10
CPE-21	*Cucurbita pepo*	14.5 ± 0.12	27.1 ± 0.12
CPE-22	*Cucurbita pepo*	17.2 ± 0.07	27.2 ± 0.06
CPE-23	*Cucurbita pepo*	11.9 ± 0.5	27.0 ± 0.05
CPE-24	*Cucurbita pepo*	17.9 ± 0.07	27.6 ± 0.23
CPE-25	*Cucurbita pepo*	17.9 ± 0.12	27.1 ± 0.01
CPE-26	*Cucurbita pepo*	16.9 ± 0.12	28.6 ± 0.02
CPE-27	*Cucurbita pepo*	17.1 ± 0.03	28.2 ± 0.02
CPE-28	*Cucurbita pepo*	17.0 ± 0.05	27.1 ± 0.13
CPE-29	*Cucurbita pepo*	17.2 ± 0.05	27.1 ± 0.04
CPE-30	*Cucurbita pepo*	17.2 ± 0.37	27.5 ± 0.02
CPE-31	*Cucurbita pepo*	17.4 ± 0.05	26.9 ± 0.01
CPE-32	*Cucurbita pepo*	16.9 ± 0.10	27.1 ± 0.54
CPE-33	*Cucurbita pepo*	17,2 ± 0.08	27.0 ± 0.11
CPE-34	*Cucurbita pepo*	17.1 ± 0.01	27.1 ± 0.02
CPE-35	*Cucurbita pepo*	16.9 ± 0.01	27.2 ± 0.55
CPE-36	*Cucurbita pepo*	15.4 ± 0.05	27.0 ± 0.2
CPE-37	*Cucurbita pepo*	16. 9 ± 0.21	27.0 ± 0.03
CPE-38	*Cucurbita pepo*	17.0 ± 0.16	27.2 ± 0.01
CPE-39	*Cucurbita pepo*	15.9 ± 0.05	27.1 ± 0.16
CPE-40	*Cucurbita zucchini*	14.9 ± 0.11	27.0 ± 0.12
CPE-41	*Cucurbita zucchini*	15.1 ± 0.12	27.4 ± 0.11
CPE-42	*Cucurbita zucchini*	17.0 ± 0.09	26.6 ± 0.11
CPE-43	*Cucurbita zucchini*	17.0 ± 0.40	27.6 ± 0.11
CPE-44	*Cucurbita zucchini*	13.6 ± 0.05	27.1 ± 0.09
CPE-45	*Cucurbita zucchini*	13.1 ± 0.01	27.0 ± 0.14
CPE-46	*Cucurbita zucchini*	14.9 ± 0.05	29.2 ± 0.11
CPE-47	*Cucurbita zucchini*	13.9 ± 0.11	27.0 ± 0.07
CPE-48	*Cucurbita zucchini*	12.9 ± 0.04	26.0 ± 0.05
CPE-49	*Cucurbita zucchini*	12.9 ± 0.11	26.2 ± 0.02
CPE-50	*Cucurbita zucchini*	17.1 ± 0.05	27.2 ± 0.15
CPE-51	*Cucurbita zucchini*	15.1 ± 0.01	27.2 ± 0.02
CPE-52	*Cucurbita zucchini*	14.9 ± 0.17	27.0 ± 0.06
CPE-53	*Cucurbita zucchini*	16.9 ± 0.11	27.0 ± 0.12

Table 4. *Cont.*

		Amphotericin B	Fluconazole
Code	**Substrate**	**13–17**	**27–33**
CPE-54	*Cucurbita zucchini*	16.9 ± 0.04	28.2 ± 0.20
CPE-55	*Cucurbita zucchini*	17.1 ± 0.01	29.0 ± 0.42
CPE-56	*Cucurbita zucchini*	13.8 ± 0.13	27.8 ± 0.04
CPE-57	*Cucurbita zucchini*	13.9 ± 0.30	27.0 ± 0.12
CPE-58	*Cucurbita zucchini*	14.9 ± 0.05	27.0 ± 0.05
CPE-59	*Cucurbita zucchini*	15.9 ± 0.44	27.0 ± 0.05
CPE-60	*Cucurbita zucchini*	17.1 ± 0.05	27.1 ± 0.11
CPE-61	*Cucurbita zucchini*	13.9 ± 0.01	27.9 ± 0.01
CPE-62	*Cucurbita zucchini*	13.5 ± 0.02	27.1 ± 0.05
CPE-63	*Cucurbita zucchini*	12.1 ± 0.04	26.9 ± 0.01
CPE-64	*Cucurbita zucchini*	15.9 ± 0.12	27.2 ± 0.44
CPE-65	*Cucurbita zucchini*	15.5 ± 0.01	27.6 ± 0.02
CPE-66	*Cucurbita zucchini*	17.2 ± 0.05	27.8 ± 0.01
CPE-67	*Cucurbita zucchini*	15.9 ± 0.18	27.5 ± 0.12
CPE-68	*Malus domestica*	17.9 ± 0.03	27.0 ± 0.01
CPE-69	*Malus domestica*	18.2 ± 0.10	27.0 ± 0.72
CPE-70	*Malus domestica*	18.6 ± 0.05	27.1 ± 0.12
CPE-71	*Malus domestica*	17.1 ± 0.37	27.9 ± 0.05
CPE-72	*Malus domestica*	17.5 ± 0.05	27.1 ± 0.11
CPE-73	*Malus domestica*	18.1 ± 0.07	28.2 ± 0.02
CPE-74	*Malus domestica*	17.1 ± 0.12	27.0 ± 0.23
CPE-75	*Malus domestica*	17.1 ± 0.05	27.0 ± 0.09
CPE-76	*Malus domestica*	19,9 ± 0.06	27.0 ± 0.05
CPE-77	*Malus domestica*	18.1 ± 0.01	27.8 ± 0.01
CPE-78	*Malus domestica*	16.9 ± 0.11	27.0 ± 0.11
CPE-79	*Malus domestica*	17.1 ± 0.02	26.6 ± 0.13
CPE-80	*Malus domestica*	17.1 ± 0.05	27.6 ± 0.04
CPE-81	*Malus domestica*	18,2 ± 0.12	26.8 ± 0.09
CPE-82	*Malus domestica*	16.8 ± 0.03	27.5 ± 0.11
CPE-83	*Malus domestica*	17.1 ± 0.40	27.0 ± 0.09
CPE-84	*Malus domestica*	17.0 ± 0.22	27.0 ± 0.23
CPE-85	*Malus domestica*	17.2 ± 0.01	27.0 ± 0.04
CPE-86	*Malus domestica*	17.2 ± 0.09	27.1 ± 0.05
CPE-87	*Malus domestica*	17.0 ± 0.11	27.9 ± 0.16
CPE-88	*Malus domestica*	17,6 ± 0.05	27.2 ± 0.07
CPE-89	*Malus domestica*	19.1 ± 0.21	27.0 ± 0.02
CPE-90	*Prunus domestica*	17.2 ± 0.03	27.0 ± 0.10
CPE-91	*Prunus domestica*	17.1 ± 0.44	27.1 ± 0.03
CPE-92	*Prunus domestica*	16.5 ± 0.13	27.1 ± 0.01
CPE-93	*Prunus domestica*	17.2 ± 0.02	25.6 ± 0.30
CPE-94	*Prunus domestica*	17.0 ± 0.02	27.0 ± 0.22
CPE-95	*Prunus domestica*	17.0 ± 0.04	27.0 ± 0.01
CPE-96	*Prunus domestica*	17.2 ± 0.18	27.1 ± 0.05
CPE-97	*Prunus domestica*	16.9 ± 0.05	27.0 ± 0.02
CPE-98	*Prunus domestica*	17.1 ± 0.01	27.5 ± 0.02
CPE-99	*Prunus domestica*	17.1 ± 0.11	27.2 ± 0.02
CPE-100	*Prunus domestica*	17.2 ± 0.03	27.6 ± 0.05
CPE-101	*Prunus domestica*	15.8 ± 0.55	27.0 ± 0.11
CPE-102	*Prunus domestica*	17.2 ± 0.16	27.0 ± 0.45
CPE-103	*Prunus domestica*	16.9 ± 0.44	27.3 ± 0.13
CPE-104	*Prunus domestica*	17.0 ± 0.05	27.1 ± 0.03
CPE-105	*Prunus domestica*	17.0 ± 0.01	27.0 ± 0.01
CPE-106	*Prunus domestica*	17.4 ± 0.12	27.0 ± 0.17
CPE-107	*Prunus domestica*	17.2 ± 0.02	27.2 ± 0.02

†—Values below the reference values are highlighted in gray.

Table 5. Mean Pz value of hydrolytic enzymes and growth retardation zone (mm) for endophytic strains of *Candida parapsilosis* isolated from ripe vegetables and fruits.

	Phospholipase	Protease	Hemolysis	Amphotericin B	Fluconazole
Cucurbita pepo	0.99 ± 0.0005	0.32 ± 0.05	0.85 ± 0.07	16.74 ± 0.11	27.06 ± 0.09
Cucurbita zucchini	0.98 ± 0.004	0.32 ± 0.05	0.84 ± 0.07	15.10 ± 0.10	27.31 ± 0.1
Malus domestica	– †	0.36 ± 0.05	0.89 ± 0.06	17.63 ± 0.14	27.22 ± 0.12
Prunus domestica	–	0.36 ± 0.04	0.76 ± 0.05	16.83 ± 0.14	27.04 ± 0.09

†—not observed.

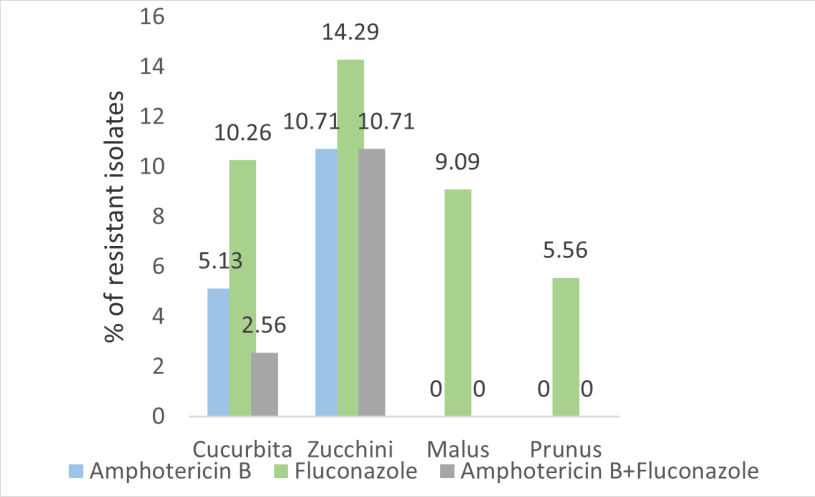

Figure 1. Share of endophytic strains of *Candida parapsilosis* isolated from ripe vegetables and fruits resistant to Amphotericin B, Fluconazole, and both antifungal drugs.

4. Discussion

Of course, not all yeasts found in fruits in this study can be classified as true or obligate endophytes that develop asymptomatically and only in internal tissues. We should consider the groups of yeasts that can live endophytically and consider the contaminated species separately. Yeasts with an endophytic lifestyle probably include typical epiphytic and eurytopic yeasts, for which the transition to an endophytic lifestyle in internal tissues is a strategy to avoid unfavorable environmental factors (solar radiation, desiccation, etc.).

Another, more complicated situation arises when considering the contaminating species [43]. Most likely, it is the opportunistic yeast *Candida parapsilosis*, which was found in the internal tissues of all samples examined.

Candida parapsilosis can be observed in domestic animals, insects, soil, marine environment, etc. [7,44]. In addition, *C. parapsilosis* is a commensal yeast of humans that frequently colonizes the skin. However, it can become pathogenic when host defense mechanisms change [6,20,22]. Due to its ability to adapt to different host niches, it can cause systemic infections in immunocompromised patients [45,46].

Recently, it has been shown that environmentally occurring strains of *Candida parapsilosis* isolated from urban topsoil can be resistant to common antifungal drugs [23,47,48].

Our study of endophytic strains isolated from vegetable and fruit products has shown that among endophytic strains of opportunistic *Candida parapsilosis,* there are those that possess virulence characteristics (produce active hydrolytic enzymes), such as clinical isolates [22], and are resistant to antifungals. A large proportion of these strains has been isolated from ripe vegetables. Consumption of such raw produce may not be safe, especially for immunocompromised individuals who are genetically predisposed to the development of fungal diseases and mycogenic allergies.

C. albicans remains one of the most important fungal pathogens; although, there is an increasing shift to non-albicans *Candida* spp. (NACS), particularly *C. parapsilosis* and *C. glabrata* [49]. They can cause a variety of health disorders in humans, ranging

from allergic syndromes and mucosal infections to life-threatening invasive diseases. The extensive use of antifungal drugs, both prophylactic and therapeutic, is considered to be one of the main causes of *Candida parapsilosis* resistance worldwide, and its occurrence poses a major challenge for treatment [50–53]. Thus, opportunistic microflora in the internal tissues of fruits is a serious problem. Therefore, further research on the extent and nature of the dissemination of resistant isolates of opportunistic *Candida* in food and the mode and mechanism of contamination of plant tissues is extremely important.

5. Conclusions

This study has shown that fruits of agricultural crops may not meet microbiological safety criteria. We have detected virulent isolates of *C. parapsilosis* resistant to fluconazole and amphotericin B in the internal tissues of ripe fruits. There is no doubt that further extensive research efforts addressing the distribution of virulent isolates of *Candida parapsilosis* in different fruits, pathogenic properties, antimicrobial susceptibility profile, genetic resistance mechanisms, and mechanisms of entry into plant tissues will help prevent infections.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol3010014/s1, Supplementary data.

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