

FRUITS AND BERRIES OF UZBEKISTAN ARE POTENTIAL SOURCES OF ENDOPHYTIC YEASTS

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Abstract. *The article presents studies on the isolation of endophytic yeasts from generative and storage parts of plants. 17 isolates of yeast cultures were isolated, 12 of which were identified by MALDI-TOF. The genera Pichia, Hansenia, Klyuveromyces, Nakazawae and Metschnikowia were discovered. The greatest diversity of endophytic yeasts is found in sweet cherry (Prunus avium). 50% of culture isolates were identified as Pichia species. 50% of the identified isolates were found to belong to Pichia type I. Also, a rare isolated culture - Nakazawae holstii - was found.*

Keywords: *Endophytic yeasts, Pichia, Hansenia, Klyuveromyces, Nakazawae, Metschnikowia*

Endophytic yeasts are a group of microorganisms that live in the internal tissues of plants without harming their hosts. They are increasingly recognized as sources of bioactive compounds for plant growth promotion, stress tolerance, and pharmaceutical and agricultural applications [1]. Unlike pathogenic fungi, endophytic yeasts establish mutualistic or commensal relationships with plants, contributing to their health and fitness under various conditions [2]. Endophytic yeasts belong to different taxonomic groups in the division of fungi, which mainly include Candida, Cryptococcus, Pseudozyma, Metschnikowia and Rhodotorula, among others. These yeasts have developed unique adaptations that allow them to colonize plant tissues, including the ability to metabolize complex carbon sources such as cellulose and lignin, which are abundant in plant cell walls [3].

2. Research methods

2.1. Collection of plant materials

In order to isolate endophytic yeast isolates, the flowers of the plants were taken from the trees in Yunusabad district of Tashkent, a few hours before the experiment in March and April. In May, the fruits of the plants were brought for experiment: the Victoria variety of strawberries - Hasanboy of Tashkent, the Karalevsky variety of strawberries from Kibray of Tashkent, apricots from Surkhandarya region, white and black mulberries from Yangiyol district of Tashkent, and cherries from Namangan region. Tangerines, kiwis, and beets were taken from the Yunusabad area of Tashkent city. The stored apples were cut from the tree one season before the experiment. Plants were identified according to their morphological characteristics.

2.2. Medias

Initially, malt, sabura, potato dextrose agar was taken as a nutrient medium. Potato dextrose broth nutrient composition (g/l): Potato - 200.0, glucose - 20.0, pH-3.5±1. Composition of Saburo nutrient medium (g/l): glucose - 40.0, peptone - 10.0, pH-6.5±1.

2.3. Isolation of endophytic yeasts

Endophytic yeasts were isolated from fresh fruits and generative parts of plants. [Abdel-Hafez et al., (2015) Fruits were surface sterilized in 70% alcohol and 3% sodium hypochlorite for 3-5 minutes. Fruit samples were cut into small pieces up to 5 mm and placed on the surface of potato dextrose agar medium. 200 mg/l of cefotaxime was added to the medium to inhibit the growth of the bacteria. In the work, the method of direct separation and deep inoculation of the liquid medium was used. using ethanol 70%, NaOCl 3%) and exposure time for surface sterilization. The ground samples were placed in petri dishes and inoculated with cefatxim (200µg/ml) as an antibiotic and potato dextrose broth, sabura, We used malt. Incubation was carried out for 5-14 days at a temperature of 28-30C and a shaker with a rotation speed of 180. After that, after the appearance of visual changes (turbidity, gas formation), the bar was taken into petri dishes by the method. Yeast-like colonies in Petri dishes were subjected to microscopic examination and transferred to cups with the same medium without antibiotics.

2.4. Identification of endophytic yeasts.

The obtained isolates were initially classified according to Kurtzman et al. (2000). Then it was identified by the MALTI-TOFF method. MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) is a widely used and widely used method for identifying pathogenic bacteria, especially microaerobes, anaerobes, mycobacteria and fungi in the microbiological clinical laboratory.

3. Results

There is increasing attention to the study of endophytic yeasts that live symbiotically with endophytic bacteria and fungi in various parts of plants. Currently, endophytic yeasts are poorly studied subjects, which is probably due to the difficulties of their isolation. But endophytic yeasts have been found to be of great importance for plant physiology, health and protection. It is known that yeasts prefer generative (flowers, seeds) and storage (fruits, roots, roots) parts of plants rich in carbohydrates. Storage parts are plant organs or tissues that serve to store nutrients, energy, and water that can be used by the plant to maintain metabolic activity under stress, nutrient deficiency, or adverse environmental conditions. By synthesizing biologically active compounds such as growth hormones and antibiotics, yeast help improve plant growth, development, and resistance to stressful conditions, as well as protect plants from pathogens. In this work, cherry (*Prunus avium*), peach (*Prunus persica*), apricot (*Prunus Armeniaca*), walnut (*Jungales regia*), currant (*Ribes aureum*), apple tree (*Malus domestica*), ripe fruits of tangerine (*Citrus reticulata*), kiwifruit (*Actinidia deliciosa*), beet roots (*Beta vulgaris*), royal and victorian varieties of strawberry (*Fragaria vesca*), black mulberry (*Morus nigra*), white mulberry (*Morus alba*) fruits, experiments were conducted to obtain endophytic yeasts.

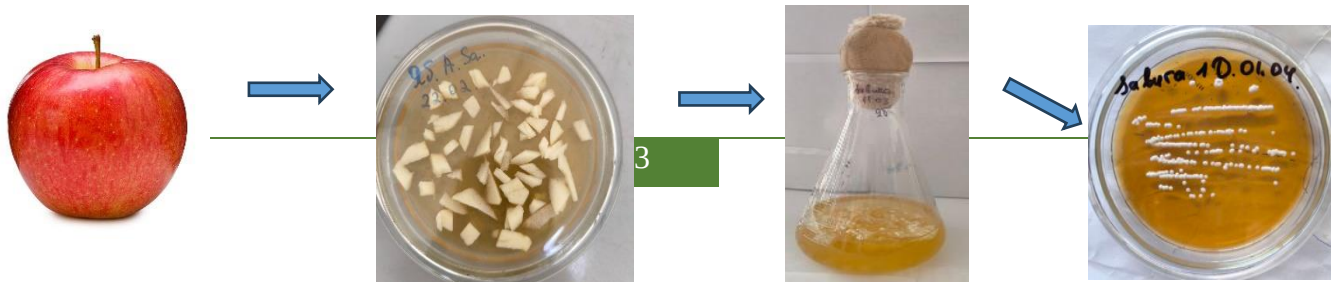
Table 1. Plant sources for obtaining endophytic yeasts

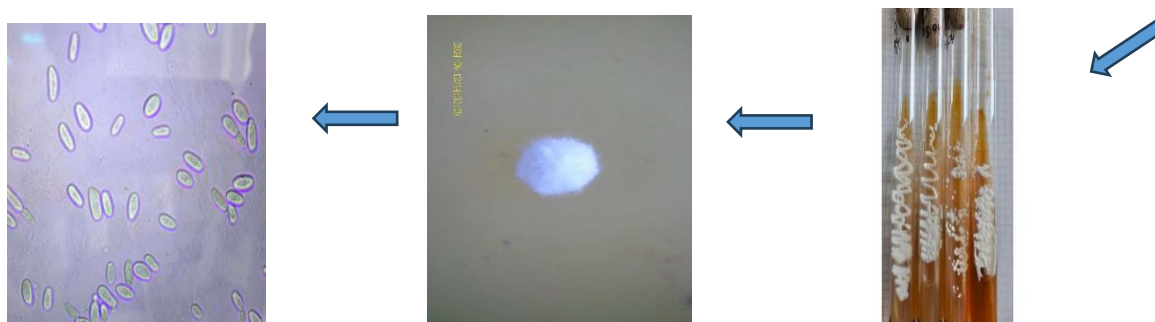
	Name of plants	Picture	Pl ant part	Endo phytic yeast isolates obtained	MALDI- TOF result

1	Kiwi <i>Actinidia deliciosa</i>		Fr uit	-	-
2	Tang erine <i>Citrus reticulata</i>		Fr uit	-	-
3	Beetr oot <i>Beta vulgaris</i>		Ro ot	-	-
1	Appl e <i>Malu s domestica</i>		Fr uit	1D, 2D	- -
2	Cherr y <i>Cerasus vulgaris</i>		Fl ower	3D	-
3	Kara kat <i>Ribes aureum</i>		Fl ower	4D	-
4	Peac h <i>Prun us persica</i>		Fl ower	5D	-

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5	Strawberry karalevsky <i>Fragaria vesca</i>		Fr uit	QK	<i>Pichia klyuveri</i>
6	Strawberry Victoria <i>Fragaria vesca</i>		Fr uit	QV1 QV2 QV3	<i>Pichia kudriavzevii</i> <i>Hansenia opuntiae</i> <i>Pichia kudriavzevii</i>
7	White mulberry <i>Morus alba</i>		Fr uit	OT	<i>Pichia kudriavzevii</i>
8	Black mulberry <i>Morus nigra</i>		Fr uit	QT	<i>Pichia kudriavzevii</i>
9	Cherry <i>Prunus avium</i>		Fr uit	G1 GP GOK	<i>Kluyveromyces marxianus</i> Nakazawa holstii <i>Pichia kudriavzevii</i>
10	Apricot <i>Prunus armeniaca</i>		Fr uit	O`OK O`M K O`KK	<i>Hansenia spora uvarum</i> <i>Metchnikowiakowia pulcherrima</i> <i>Metchnikowiakowia pulcherrima</i>





After the obtained isolates were collected, they were taken to MALDI TOF for identification. The advantages of matrix-assisted laser desorption ionization (MALDI) TOF MS have been evident for a quarter of a century. At first, it has several advantages, such as the fact that it requires little time, the amount of sample required is small, and it is cheap. The subsequent success of MALDI analysis was provided by its expanded base, and it can be said that it has achieved great progress in this regard. As can be seen from the table, 17 isolates of yeast cultures were initially isolated, 12 of which were identified by the MALDI-TOF method. The genera *Pichia*, *Hansenia*, *Kluyveromyces*, *Nakazawae* and *Metschnikowia* were discovered. The greatest diversity of endophytic yeasts is found in sweet cherry (*Prunus avium*). 50% of the identified endophytic yeast cultures are representatives of the genus *Pichia*. I would also like to emphasize the discovery of such rare culture as *Nakazawae holstii*.

Conclusion: As a result of the research, it was found that the fresh flowers of plants are better than their stored fruits as a source of endophytic yeasts. But apples are an exception. Endophytic yeasts were not found in kiwi and mandarin. Probably, the composition of the endophyte microflora of these fruits is affected by the increase of ascorbic acid. In general, fruits and berries of the republic are a rich and promising source of endophytic yeasts. The next stage of the work will be to study the different biological activities of the isolated endophytic cultures.

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