https://bjm.ui.ac.ir/?lang=en

Journal of Microbial Biology
E-ISSN: 3060-7647
14rd Year, Vol. 14, No. 56, 2025 pp. 69- 75
Received: 19/10/2025 Accepted: 14/01/2026

(Research Paper)

Isolation and identification of the yeast *Saturnispora silvae* from rotting carrots from the market

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Abstract

Fruits and vegetables, particularly carrots (Daucus carota L.), play a vital role in human health by providing dietary fiber, essential micronutrients, carotenoids, and antioxidant compounds, which collectively reduce the risk of chronic diseases. However, postharvest microbial spoilage significantly threatens carrot quality and safety, leading to 20-40% losses in developing countries due to the presence of bacteria, molds, and yeasts. This study aimed to isolate and identify yeast species from spoiled carrot samples collected from markets. Spoiled carrots were collected from local markets in Karaj. Under sterile conditions, a portion of the infected carrot tissue was suspended in saline solution and cultured on potato dextrose agar (PDA) plates supplemented with 30 mg/L of chloramphenicol. Yeast isolates were identified based on colony morphology, microscopic characteristics, growth temperature range, biochemical tests, and sequence analysis of the amplified D1/D2 domain of the 26S rRNA gene. The isolated yeast strain produced ovoid to spheroidal vegetative cells measuring 2–5 µm in diameter. It can ferment glucose and assimilate citrate. The growth temperature range, biochemical characteristics, and phylogenetic tree analysis confirmed the identification of the isolated yeast as *Saturnispora silvae*. This study reports the first isolation of *Saturnispora silvae* from spoiled market carrots, demonstrating its ability to ferment glucose and colonize damaged tissues, potentially exacerbating post-harvest spoilage.

Keywords: Carrot, Yeast, Rotten, PCR.

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BEHGAR, M., Shawrang, P., Motamedi Sedeh, F., Roshan, R., Amiri, F. Isolation and Identification of the yeast Saturnispora silvae from rotting carrots from the Market. *Journal of Microbial Biology*, 2025; 14(56): 69-75. doi: 10.22108/bjm.2026.147095.1656

Introduction

Fruits and vegetables are essential components of a healthy diet, providing dietary fiber, various micronutrients (including polyphenols, pigments, and terpenes), and antioxidant compounds that collectively reduce the risk of chronic diseases such as cardiovascular diseases, obesity, diabetes, and cancer (1, 2). Among vegetables, carrots (Daucus carota L.) are widely consumed in the country, both fresh and processed (e.g., in salads and juices), owing to their high levels of carotenoids and antioxidants (3). However, ensuring the safety and quality of carrots requires minimizing post-harvest microbial contamination and loss.

Beyond nutritional value, controlling plant diseases in vegetables, such as carrots, is critical for both human health and economic reasons. Research has shown that spoilage and transmission of diseases through fruits and vegetables have been on the rise in recent years (4). Consequently, efforts to isolate and identify the pathogenic agents responsible for spoilage and disease outbreaks are recommended as control measures (5).

Moreover, in addition to the risk of disease transmission, postharvest spoilage due to microbial activity is a primary concern. For instance, significant microbial spoilage of carrots has been observed in local markets in Nigeria, where various microorganisms contribute to deterioration (6). Studies indicate that approximately 20–40% of harvested root vegetables, including carrots, may be lost in developing countries due to various post-harvest factors, with microbial spoilage playing a significant role (7, 8).

Furthermore, yeasts such as *Meyerozyma guilliermondii* and *Pichia* species have been implicated in texture modification, gas production, and overall spoilage of fresh carrot juice and shredded carrots (9). Microbial spoilage of carrots during post-harvest storage often involves a combination of bacteria, molds, and yeasts, with yeasts contributing to soft rot and quality loss under refrigerated conditions (10, 11).

Yeasts play a significant role in the microbial spoilage of vegetables, particularly minimally processed or stored root vegetables such as carrots. In ready-to-use grated carrots stored under modified atmosphere packaging, yeasts such as *Cryptococcus albidus* and *Candida lambica* have been identified as dominant spoilage organisms, contributing to exudation and the development of off-odors (12). Their significance stems from their tolerance to low oxygen, low pH, and preservatives, allowing them to dominate spoilage alongside lactic acid bacteria in ready-to-use products, thereby limiting shelf life and causing economic losses (11, 13). The present study aimed to isolate and identify yeast species from spoiled carrot samples collected from the market.

Materials and Methods

Sample Collection

Spoiled carrot samples were collected from a local market in Karaj, Alborz Province, during the winter of 2023. During sampling, carrots with slight spoilage and a relatively healthy appearance were selected and sent to the laboratory. The selected carrot samples exhibited minor symptoms of cavity spot disease.

Yeast Isolation

Yeast isolation was performed by aseptically excising a small portion of the deeper infected carrot tissue using a sterile scalpel under a laminar flow hood. The tissue sample was then transferred to sterile 0.9% (w/v) saline solution and homogenized. After vortexing, dilutions were prepared and cultured on Potato Dextrose Agar plates containing 30 mg/L chloramphenicol at room temperature. Initial cultures showed mixed microbial growth, including that of filamentous fungi. To reduce contamination, serial dilution and plating were repeated until pure yeast colonies were obtained. Colonies were examined after 2 days of incubation (14). The linear serial dilution method was used for the entire isolation process. Following purification by repeated streaking, the yeast isolate was cultured on PDA and incubated at 25 °C for 3 and 14 days to prepare slides for microscopic examination (15).

For phenotypic identification, the isolate was tested for growth at various temperatures (15-40 °C) and subjected to biochemical assimilation/fermentation tests using diagnostic disks. For the biochemical tests, the desired chemicals included dextrose, sucrose, maltose, xylose, galactose, raffinose, lactose, citrate, and urea in sterile test tubes containing 500 µL of saline solution, and a fresh colony of yeast was added to each tube using a loop and incubated for one day at 35 °C (16). Yeast staining was performed using a Gram staining kit (Siba Biotech).

Molecular Identification of Isolated Strains

DNA Extraction: Fresh yeast colonies were initially harvested from Potato Dextrose Agar cultures using a sterile loop under a laminar flow hood and transferred into 1.5 mL microcentrifuge tubes. The extraction process was carried out using Sina Clone (Sina PureTM DNA KIT) based on the

DNA extraction method for Gram-negative bacteria with slight modifications.

In summary, 150 µL of prelysis buffer and five microliters of Ribonuclease A added to the harvested yeast colonies. The mixture was vortexed and incubated for 15 min at 37 °C. Subsequently, 50 µL of Proteinase K was added to the microtube and incubated for 40 min at 55 °C. The extraction process was continued according to the manufacturer's instructions. The extracted DNA was stored at -20 °C for subsequent tests.

Table 1- Conditions of the PCR Reaction.

	Temperature (°C)	Time (min)	Cycles
Initial denaturation	94	3	1
Denaturation	94	1	30
Annealing	58	1	
Extension	72	1	
Final extension	72	3	1

Yeast Identification Using PCR: To identify the yeast species isolated in this study, the D1/D2 region of the large subunit (LSU) rDNA was amplified using primers NL1 and NL4 (5′-GCATATCAATAAGCGGAAAAG-3′ and 5′-GGTCCGTGTTTCAAGACGG-3′, respectively). For the PCR reaction, a pre-prepared master mix (AMPLICON, 2x Master Mix RED, Cat. No.: A190301) was used according to the manufacturer's recommendations in a volume of 25 µL (17). The PCR program is shown in Table 1.

Sequencing

To determine the sequence, the PCR product along with the corresponding primers was sent to Codon Company for sequencing. The obtained sequence was compared with sequences available in the GenBank database using BLAST, and closely related species sequences were aligned. A phylogenetic tree was constructed using the neighbor-joining (NJ) method based on evolutionary distances calculated using Kimura's two-parameter model in MEGA software version 10 (18). The confidence levels of the clades were estimated through bootstrap analysis with 1000 replications. The obtained nucleotide sequence was submitted to GenBank under accession number PP968850.1 MB 02.

Results

Culture Characteristics

On potato dextrose agar (PDA), the isolated yeast formed creamy-white, smooth, glistening colonies with regular, well-defined margins (Fig. 1).

Fig. 1- Morphology of an isolated yeast colony.

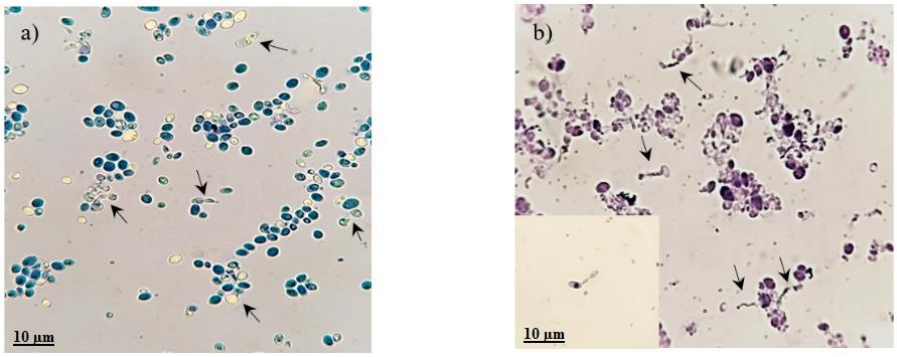

Fig. 2- Microscopic morphology of the isolated yeast. (a) Budding cells on PDA after 3 days at 25 °C. (b) Pseudohyphae formed on PDA after 14 days at 25 °C.

After 3 days of incubation on potato dextrose agar (PDA) at 25 °C, light microscopic observation showed ovoid to ellipsoidal vegetative cells (2–5 µm in diameter) that occurred singly or in pairs. Reproduction was vegetative through multilateral budding, and no sexual structures were observed. Prolonged incubation (14 days) in the same medium resulted in the formation of rudimentary or weak pseudohyphae (Fig. 2).

Physiological and Biochemical Characteristics

The results of the biochemical tests and growth at different temperatures are presented in Table 2. The yeast fermented glucose; however, it did not utilize other substrates, including xylose, maltose, sucrose, galactose, raffinose, lactose, and urea. The isolated yeast demonstrated the ability to grow on PDA medium at temperatures ranging from 15 to 30 °C, but it could not survive at 37 °C or higher.

Phylogenetic Analysis

Amplification of the D1/D2 region using the primers NL1 and NL4 resulted in the production of a fragment of approximately 600 bp on agarose gel electrophoresis (Fig. 3).

Table 2- Growth reactions and other characteristics.

Fermentation						Assimiation			Growth Temperature (ºC)					
Glucose	Lactose	Galactose	Raffinose	Xylose	Sucrose	Maltose	Citrate	Urease	15	19	25	30	37	40
+	-	-	-	-	-	-	+	-	+	+	+	+		-

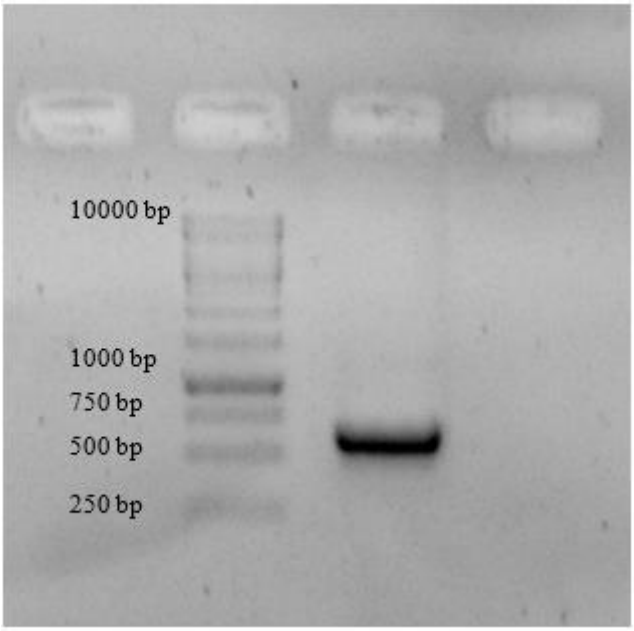

Fig. 3- The amplification of the D1/D2 region by PCR resulted in a fragment of approximately 600 bp.

Sequencing of the above fragment, which was 606 bp long, revealed a high bootstrap support (88%) for yeast *Saturnispora silvae* based on the BLAST results from the Global Gene Bank and phylogenetic analyses (Fig. 4).

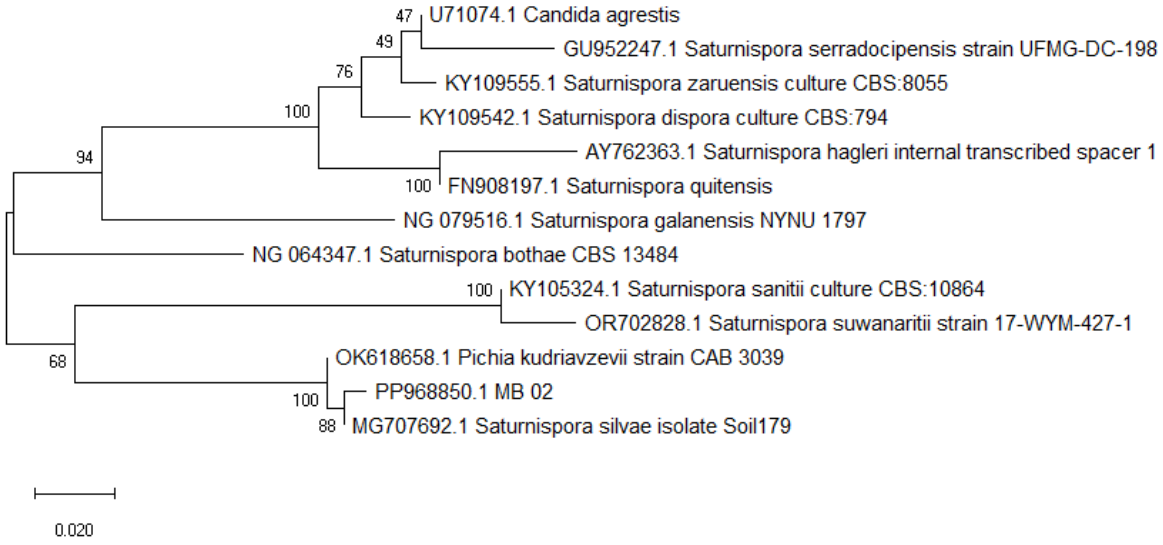

Fig. 4- Neighbour-joining phylogram showing the placement of isolated yeast based on the large subunit rRNA gene D1/D2 sequences. Numbers at nodes represent the percentages from 1000 replicate bootstrap resampling.

Subsequently, the corresponding fragment was registered in the gene database with accession number PP968850.1. No substitutions or gaps were observed between the above fragments in the isolated yeast and *Saturnispora silvae* in the database.

Discussion and Conclusion

The growth temperature range, biochemical characteristics, microscopic examination, and phylogenetic tree analysis confirmed the identification of the isolated yeast as *Saturnispora silvae* (previously known as *Candida silvae*) following recent taxonomic revision (19). This observation aligns with the taxonomic diversity of the genus, which encompasses 19 described species, some of which form ascospores, while others remain asexual (20, 21).

Microscopic examination revealed ovoid to ellipsoidal yeast cells measuring approximately 2–5 µm in diameter, occurring singly, in pairs, or in small clusters, with multilateral budding on a narrow base (22). This observed size range falls within the typical morphological variability of the *Saturnispora* clade, where vegetative cells are generally spheroidal to ovoidal and measure approximately 2–8 µm, with variations influenced by growth conditions and ploidy status (23, 24). These observations support the identification of the isolate as *S. silvae*, in agreement with the species' original isolation from clinical sources and its phylogenetic reassignment (23).

Species of *Saturnispora* have been isolated from various sources and habitats, including forest litter (20, 21), aquatic habitats (25), mangrove areas (26), decaying wood (19, 15), and other environments. To the best of our knowledge, this study is the first to isolate *Saturnispora silvae* from decaying carrots, thereby expanding the known ecological niches of this genus beyond previously reported plant decomposition substrates.

However, because carrots are root vegetables that grow underground, there is a high possibility of microorganisms transferring to their surface. Species of the genus *Saturnispora* have also previously been isolated from apples (27) and vetiver grass (28).

The biochemical profile of the isolated yeast revealed positive glucose fermentation and citrate assimilation, while it was negative for urease, xylose, maltose, sucrose, galactose, raffinose, and lactose. This pattern aligns closely with that of most *Saturnispora* species, which typically ferment glucose strongly but exhibit limited or negative assimilation of complex carbon sources, such as xylose and cellobiose (15, 21, 25). These results support the hypothesis that *Saturnispora* species persist in decaying plant material primarily by assimilating simple sugars (such as glucose) liberated through the hydrolytic activity of cohabiting microorganisms on cellulose and pectin, rather than by directly metabolizing complex polysaccharides (29).

Glucose fermentation has been reported in most species of this genus (*Saturnispora*) (16, 28, 29). Citrate utilization has been observed to be positive in some species, such as *S. kantuleensis*, and absent in others, including *S. sekii*, *S. bothae*, and *S. quitensis* (26, 21, 15). The high glucose content in carrots (16–22% of total sugars (29)) provides an ideal substrate for *S. silvae*, facilitating its colonization and potentially contributing to post-harvest spoilage.

The presence of observed cavity spot symptoms in the sampled carrots, which are often associated with pectinolytic pathogens such as *Pythium* spp. that produce cellulases and pectinases to macerate cell walls (30), indicates that *S. silvae* likely acts as a secondary opportunistic agent. This is consistent with the broader patterns of yeast spoilage observed in root vegetables and minimally processed carrots, where opportunistic yeasts (e.g., *Cryptococcus albidus*, *Candida lambica*, *Meyerozyma guillermondii*, and *Pichia* spp.) proliferate following initial tissue damage by primary bacterial or fungal pathogens, leading to sensory defects such as off-odors, exudation, and softening (11, 12, 13).

The present study has certain limitations that should be considered. The findings are from a limited number of spoiled carrot samples collected from local markets. This small sample size restricts generalizations about the prevalence, diversity, or consistent role of *Saturnispora silvae* in carrot spoilage across different regions, seasons, or market conditions. Larger-scale surveys involving more extensive sampling are needed to confirm the broader ecological and spoilage significance of this yeast.

Conclusion

This study reports the first isolation of *Saturnispora silvae* from spoiled market carrots, adding a new substrate to its known habitats. By fermenting glucose and colonizing damaged carrot tissues, this yeast can lead to increased spoilage under typical postharvest conditions. However, the findings are based on a single isolate from limited samples, restricting generalizations about its prevalence or diversity in carrot spoilage in market settings. Further large-scale studies across diverse market conditions are suggested to better elucidate this yeast's role in postharvest carrot spoilage.

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