International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Characterization of microorganisms associated with sweet potato spoilage

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International Journal of Science and Research Archive, 2025, 16(01), 1746-1755

Publication history: Received on 11 June 2025; revised on 20 July 2025; accepted on 22 July 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.16.1.2040

Abstract

Sweet potatoes are staple food crops, especially in developing countries. However, spoilage of sweet potatoes due to microbial contamination is a major problem, causing significant economic losses. The most common microorganisms associated with spoilage of sweet potatoes are Bacteria and Fungi. This study was aimed at characterization of microorganisms associated with sweet potato spoilage in Awka, Anambra State. Six samples of decaying sweet potatoes were obtained at different locations at Eke Awka Market in Awka. Nigeria. The samples were serially diluted and inoculated on nutrient agar and Sabourauds Dextrose Agar to assess for bacterial and fungal growths respectively. Discrete colonies were obtained and identified using appropriate microbiological and biochemical methods. From the result, the total bacterial count ranged from 1.02 x 10* to 5.40 x 10* while the mean fungal count ranged from 1.36 x 10^6 to 1.71 x 10^6. The result showed that all the samples had both bacterial and fungal isolates which can be associated with the spoilage mechanism. The fungal population is much more than the bacterial population and this shows that bacteria are the predominant microorganisms responsible for the spoilage of sweet potato during storage. The bacterial isolates include *Staphylococcus* spp and *Streptococcus* spp while the fungal isolates are *Candida albicans* and *Saccharomyces cerevisae*. Since sweet potatoes have been described as having thin, delicate skin that is easily damaged by cuts and abrasion during harvesting, transportation or distribution. concerted efforts should therefore be directed towards minimizing or reducing this so as to avoid physical damage to the tuber and thereby reducing or preventing microbial attack. In order to mitigate the rate at which microorganisms cause deterioration of sweet potato, several control techniques such proper washing of the harvested tuber, cleaning of transit containers, proper handling of the tuber in order to avoid injuries, good hygienic practices by the handlers, provision of good and healthy storage facilities must be put in place.

Keywords: Sweet Potatoes; Food Spoilage; Deterioration; Plant pathogens

1. Introduction

Food scarcity is one of the most urgent issues facing developing countries. According to Kana *et al*. (2012), 10% of the almost one billion individuals who suffer from acute hunger in these countries are said to have died as a result of hunger-related problems. Cassava, yam, sweet potatoes, and Irish potatoes are among the most significant root crops in Nigeria. Following harvest, these tubers experience losses due to pathological, physiological, or physical reasons, or a combination of these (Opara and Agugo, 2014).

Typically farmed as an annual crop, sweet potatoes (Ipomoea batatas) are a perennial crop with tuberous roots. Originating in Central America, it is now farmed extensively as a staple food in most regions of the world (Ecocrop, 2010). Sweet potatoes come in more than 403 kinds, with meat that can be white, yellow, red, purple, pink, violet, orange, or brown, and skin that can be yellow, red, orange, or brown (Ecocrop, 2010). The crop has excellent nutritional and health benefits.

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The plant's leaves, roots, and vines are among its many edible aspects. The tender leaves are frequently consumed by humans, the roots are frequently utilized as a source of carbohydrates, and the leafy stems are fed to animals (Woolfer, 2012). In addition to these, the crop has been shown to offer unexpected health benefits, such as preventing heart disease, arteriosclerosis, depression, emphysema, arthritis, stroke, muscle cramps, and stomach ulcers; lowering inflammation and arthritis; and curing bronchitis and stomach ulcers (Alum *et al*., 2013).

According to Oyeyipo (2012), sweet potatoes have a great deal of potential as a cost-effective and efficient food energy source. According to Oladoye *et al*. (2013), it is a significant source of anthocyanidins and antioxidants. It can be combined with yam to form pounded yam and amala. Rots have a major impact on the yield of sweet potatoes, particularly vegetable potatoes. A survey conducted in Iran found that 10% of sweet potatoes had pre-harvest rots and 20% had post-harvest rots (Bidarigh *et al*., 2012). These rots are a significant obstacle to Nigeria's efforts to ensure food security.

Plant pathogens, which include bacteria, viruses, and fungus, are to blame for the rise in global economic losses. Pests and diseases severely limit sweet potato productivity, reducing output by as much as 98% (Kapinga *et al*., 2010). The sweet potato weevil is the most significant limitation among sweet potato pests, followed by viruses (Qaim, 2019). Due to the crop's vulnerability to virus infection, sweet potato viruses are a major issue and are resulting in financial losses. Different African nations have reported varying yield reductions. Half of the yield loss is attributed to Nigeria and Uganda. Viruses have been linked to yield decreases of more than 90% in East Africa (Gibson *et al*., 2018).

According to Adeyonu *et al*. (2016), a large portion of Nigeria's population depends on sweet potatoes as a crop for food security. At several phases, such as field, harvest and storage (if improperly picked and stored), and marketing, sweet potato roots are regrettably vulnerable to numerous microbial illnesses. One of the main obstacles to sweet potatoes' potential as a crop for food and health security is the kind of spoiling that is frequently linked to them (Echerenwa & Umechuruba, 2014).

This has a number of negative consequences, including as a decline in the qualities of food, a significant loss of storage roots, the inability to obtain food products during the off-season, and a waste of agricultural inputs and limited resources like water. Additionally, it depletes investments and human labor, and it has a negative impact on people's ability to afford agricultural products. Additionally, food (sweet potato) safety is compromised by microbial spoiling, which poses a major health risk (Esnakula *et al*., 2013; Georgiadou *et al*., 2014).

Control measures are required because of the detrimental economic impact of fungal infections. Hydro-warming, gamma irradiation, and fungicide treatment are a few postharvest pathogen control techniques. These techniques have some disadvantages, such as being unavailable to Nigerian farmers, being harmful to the environment, being phytotoxic to humans, and having a high likelihood of causing resistance in the targeted pathogens, even though they have been shown to have intermediate effects in reducing spoilage and extending the shelf life of sweet potato tubers (Ray and Ravi, 2015). The use of plant extracts as innovative fungicides for plant protection has gained attention recently due to the disadvantages of conventional fungal and rot control methods (Okigbo and Nmeka, 2015; Okigbo and Omodamiro, 2016).

There are several publications describing the use of plant extracts to manage plant diseases since many botanicals have been thoroughly studied and found to have antibacterial qualities. Chromolena odorata (Siam weed), Ocimum gratissimum (wild basil), Moringa oleifera (moringa), and Zingiber officinale (ginger) are a few plants that have been investigated for their antibacterial qualities (Okigbo and Nmeka, 2015; Okigbo *et al*., 2019a). Food has always been scarce in the world's poorer countries. In these countries, about 1 billion people suffer from severe hunger, and 10% of them pass away as a result of hunger-related complications. The rapid growth of the human population, which puts strain on all sources of food supply, exacerbates this issue even more (Urom, 2014).

One of the biggest issues facing the globe today is how to maintain long-term sustainable development while providing food security for a growing population. The Food and Agricultural Organization estimates that in order to feed the world's population, which is expected to reach 9.3 billion people by 2050, food production will need to increase by 70%. Even worse, despite the fact that the number of people experiencing food insecurity is still too high (FAO, 2010), enormous amounts of food, including sweet potatoes, are lost annually and globally as a result of spoiling and pest infestations from farms to people. Microbe-induced spoiling and insufficient agricultural storage are the causes of this issue (Kana *et al*., 2012).

Reducing sweet potato production losses at postharvest depends on studies that will produce baseline data on the prevalence of postharvest spoilage microorganisms of sweet potatoes in Awka and a strong, drawback-free method for

controlling the rot pathogens. Reducing postharvest losses of sweet potatoes due to microbial degradation has significant potential advantages. It is essential for lowering poverty and the strain on water, climate, and ecosystems. It is also a tactic for improving food security and bridging the gap between the amount of food that is currently available and what will be required in 2050 to sustainably feed the world's estimated 9.3 billion people. The purpose of the study was to characterize the microbes linked to sweet potato deterioration in Awka, Anambra State.

2. Materials and methods

2.1. Study Area

The study was conducted in Anambra State, South Eastern Nigeria with a total area of 4,844km^2, located on Latitude 6.2209^{o}N and Longitude 6.9370^{o}E. The state is populated primarily by Igbos with farming as predominant occupation. Anambra State is bounded to the North by Kogi State, the west by Delta and Edo States, to the south by Imo and Rivers States, the southeast by Abia state and to the East by Enugu State. There are two distinct seasons, the wet and the dry seasons. The former takes place between April and October, while the latter occurs from November to March (Uneke *et al*., 2009). The state has pseudo-bimodal rainfall pattern spread from April to November with annual rainfall range between 1700mm-2060mm. The maximum mean annual temperature range is 27-36oC all through the year. Humidity is high (60-94.3%) with lowest levels during the dry season in April before the wet season begins (Longinus, 2015). The state has 21 Local Government Areas (LGAs).

2.2. Sample Collection

Replicate samples of decaying sweet potatoes were obtained at different locations at Eke Awka Market in Awka, Nigeria. They were collected in a sterile polythene bags and were brought into the laboratory in Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for microbial analysis.

2.3. Preparation and Sterilization of Media

The preparation and sterilization of media, water, and glass ware for the experiment all preceded the collection of the sample. All the glass wares were sterilized first by washing and rinsing with 70% ethanol. The pipettes were wrapped with paper and sterilized in a hot air oven. Nutrient agar and potato dextrose agar were used.

A weighing balance was used to measure 5.6 grams of nutrient agar. This was then mixed with 200ml of distilled water in a conical flask. After mixture, the conical flask was put on a heating mantle to homogenize the medium. After homogenization, it was then put in an autoclave and sterilized at 121°C for 15 minutes.

Thirty-nine grams (7.8g) of Sabourauds dextrose agar was weighed on a weighing balance and was mixed with 200ml of distilled water in a conical flask. The conical flask was placed on a heating mantle for homogenization. After homogenization, it was then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, 4ml of streptomycin was added aseptically (after the medium has cooled) to the molten PDA to inhibit the growth of bacteria.

2.4. Preparation of Samples

Cotton wool moistened with 70% ethanol was used to sterilize the workbench, hands and knife. The sterile knife was then used to cut the sample open and the decaying inner part was cut into pieces. A sterile pestle and mortar were used for homogenization. A weighing balance was then used to weigh one gram of the homogenized solid.

2.5. Isolation of Organisms from Sample

Using the method of Harrigan and McCance (2015), ten (10) grams of the sample was poured into 100 ml of sterile distilled water and the tube was shaken gently to ensure thorough mixing. After mixing, a serial dilution was then carried out by transferring 1ml from the first test-tube into the second test-tube that contains 9ml of distilled water. The second tube was mixed gently and 1ml was taken from the second tube into the third test-tube, and so on till the fifth test-tube (10^{-5} dilution factor).

Isolation of organisms was done using the spread plate method (Harrigan and McCance, 2015). One microliter (0.1ml) each from the third test-tube was pipetted using a new sterile pipette into nutrient agar and sabourauds dextrose agar. Also, 1ml each was pipetted from the fifth test-tube and poured into two empty sterile Petri-dishes (for NA and SDA). After the samples have been poured into the Petri-dishes, the media (NA and SDA) were poured into their respective plates (about 15ml was poured into the plates). Nutrient agar was used for bacteria while potato dextrose agar was

used for fungi. After the plates had set, they were then incubated at 37°C for 24 hours (only NA plates). The SDA plates were incubated at 25°C for 3-5 days.

2.6. Characterization of Bacteria and Fungi using Cultural Methods

2.6.1. Identification and characterization of bacteria isolates

The bacterial isolates were identified based on their cultural, morphological and biochemical reactions as described by Cheesbrough (2010) and Erin, (2012).

The following biochemical tests were carried out to enhance the characterization of the isolates.

- Gram staining

Gram staining of the isolate was carried out according to Cheesbrough, (2010). A thin smear of the organism was made on a clean microscopic slide. It was air dried and then heat-fixed by passing briefly over flame. Two drops of crystal violet were added to the smear for 1 minute, and then rinsed with clean water. Lugol's Iodine was added for 1 minute and washed. It was then decolorized by flooding with acetone for 30 seconds. The film was rinsed with water and counter stained with Safranin for 1 minute; it was rinsed with water again and allowed to dry. Microscopic observation was made using oil immersion objective lens.

- Catalase test

Catalase test was carried out according to Cheesbrough, (2010). A small part of the test colony was collected using a sterile wire loop and immersed into a sterile test tube containing 2-3ml 30% Hydrogen peroxide solution and observed for the appearance of effervescence.

- Haemolysis test

This test is used to check for the ability of some microorganisms to lyse red blood cells. A sterile syringe was used to collect human blood. The blood was mixed with nutrient agar media in a Petri dish. The organisms were streaked on the blood agar using a sterile wire loop and incubated for 48 hours at 37^{o}C. The cell lysis is observed by clearance zone around the smears.

- Citrate utilization test

This test was carried out according to Cheesbrough, (2010) in test tube slants of Simmon's citrate agar. A 0.1 ml aliquot of each test organism was then inoculated by spreading on the Simmon's citrate agar plate and incubated at 37˚C for 48h. Colour change from green to blue indicated that the organism was able to utilize citrate.

- Motility test (Soft stabbing method)

This was carried out according to Ogbo, 2005.The microorganism was stabbed into a sterilized semi solid Nutrient agar contained in a sterile test tube, using a sterile inoculating needle. It was incubated for 18 - 48 h at 35 - 37^{o}C and observed for diffused lines of turbidity emerging from the original line of inoculation. The test was used to differentiate motile organisms from non-motile ones.

- Sugar fermentation

Sugar fermentation test was carried out according to Njoku, (2013). Sugars such as glucose, fructose, sucrose, lactose, mannitol and maltose were added in peptone water in 1% (w/v) and with two drops of Bromothymol blue indicator, and then 1.5 ml aliquot each was distributed in standard assay tubes, each containing an inverted Durham tube. The sugar solutions were sterilized by autoclaving at 115^{0}C for 15 minutes and 200 µl of the bacterial samples were inoculated in each tube, and then incubated at 37 ºC; colour change and gas production was observed after 48 h.

2.7. Macroscopic and Microscopic Characterization of Fungal Isolates

Fungi identification was based on morphological characteristic described by Mathur and Kongsdal (2003) and Scot (2009). Macroscopic features such as texture of mycelia, spore or conidia-producing structures, type of pigmentation

were observed from fungal tissues grown on PDA. Microscopic characteristics (spore and mycelium shape and colour) of the isolates were examined by Lactophenol Cotton Blue (LPCB) wet mount preparation widely used in staining and observing fungi. The preparation had three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures and cotton blue which stains the chitin in the fungal cells. A drop of Lactophenol cotton blue stain was placed on a clean slide and a small portion of each mycelial colony aseptically taken using a sterile inoculating needle, placed and teased in a drop of lactophenol cotton blue and covered with a cover slip. The slide was finally examined under the microscope at low and high power objectives for colony characteristic such as colour and texture of mycelia, type of pigmentation and microscopic characteristics of spores such as shape and colour This technique shows various microscopic characteristics of the fungal isolates (Onuorah *et al*., 2015).

Yeast was identified on the basis of colony, cell morphology and biochemical tests. The cream coloured, non-mucoid colonies resembling Candida-like colonies were sub-cultured on corn meal agar and examined using the germ-tube test as described by Esimone *et al*. (2010).

2.7.1. Germ tube test

The germ tube test is a rapid, simple, and very valuable test for the identification of the most important yeast pathogen, *C. albicans*. A light inoculum of the yeast colony is made in 0.5 to 1.0ml of serum (for example, human, sheep, rabbit, or horse serum) so as to make a barely cloudy suspension. The mixture is incubated at 37°C for 2 to 3 h. After incubation a drop of the suspension is examined microscopically for germ tubes. This is a filamentous outgrowth from a yeast cell with no constriction present at the base.

3. Results

Table 1 Total Bacterial and Fungal count

Sample	Total Bacterial Count (cfu/g)	Total Fungal Count (sfu/g)
P1	5.40 x 10^4	1.36 x 10^6
P2	1.02 x 10^4	1.71 x 10^6

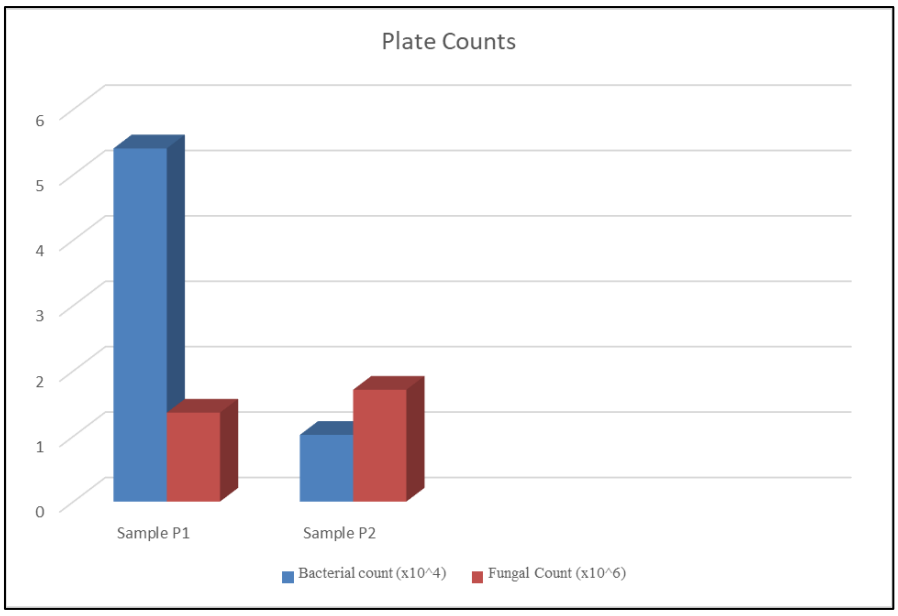

Figure 1 Total Bacterial and Fungal counts (x10^4)

Table 2 Identification of Bacterial Isolates based on Colony Morphology

Isolates	Shape	Colour	Opacity	Margin	Elevation	Surface
P1a	Circular	Cream	Transparent	Entire	Convex	Smooth
P1b	Circular	Light Yellow	Opaque	Entire	Convex	Smooth

Table 3 Identification of Bacterial Isolates based on Biochemical Reactions

Isolates	Gram Reaction	Shape	Motility	Catalase	Citrate Utilization	Haemolysis	Oxygen Demand
P1a	Positive	Cocci in clusters	Non motile	Positive	Positive	Negative	Facultative anaerobe
P1b	Positive	Cocci in chains	Non motile	Negative	Positive	Negative	Facultative anaerobe

Table 4 Identification of Bacterial Isolates based on Sugar Fermentation

Isolates	Sugar Fermentation							
	Glucose	Dextrose	Mannitol	Fructose	Galactose	Lactose	Sorbitol	Maltose
P1a	AG	AG	AG	AG	A	-	AG	-
P1b	AG	AG	AG	AG	AG	AG	AG	AG

KEY NOTES; A - Acid Produced with No Gas; Ag- Acid and Gas Produced ;- : No Acid Or Gas Produced

Table 5 Identification of Fungal Isolates

Isolates	Gram Reaction	Arrangement	Motility	Germ Tube Test
P2b	Positive	Long Oblong	Non motile	Positive
P2a	Positive	Medium Oblong	Non motile	Positive
P3b	Positive	Tiny Oblong	Non motile	Negative
P3a	Positive	Long Oblong	Non motile	Negative

Table 6 Identification of Fungal Isolates based on Sugar Fermentation

Isolates	Sugar Fermentation							
	Glucose	Dextrose	Mannitol	Fructose	Galactose	Lactose	Sorbitol	Maltose
P2a	A	A	A	AG	-	-	-	-
P2b	A	A	A	A	-	-	-	-
P3a	AG	AG	AG	A	AG	-	-	-
P3b	AG	AG	AG	A	AG	-	-	-

KEYNOTES; A - Acid Produced With No Gas; Ag- Acid And Gas Produced ;- : No Acid Or Gas Produced

Table 7 Probable bacterial and fungal isolates

Isolate	Probable Organism
P1a	*Staphylococcus* spp
P1b	*Streptococcus* spp
P2a	*Candida albicans*
P2b	*Candida albicans*
P3a	*Saccharomyces cerevisae*
P3b	*Saccharomyces cerevisae*

4. Discussion

Analysis was done on the microbiological deterioration of sweet potatoes in the city of Awka. The findings indicated that both bacterial and fungal isolates were present in every sample, which may be related to the mechanism of spoiling. While the fungal count ranged from 1.36×10^6 to 1.71×10^6, the total bacterial count ranged from 1.02×10^4 to 5.40×10^4. The fact that there are far more fungi than bacteria indicates that bacteria are the main microorganisms causing sweet potatoes to deteriorate while being stored. Chiejina and Ukeh's (2012) research, which also noted a greater fungus population, is consistent with this. Their great sensitivity to microbial assault is indicated by the high microbial numbers. This is because carbon dioxide and water are released into the atmosphere as gasses during the process of using the majority of the roots' carbohydrates for energy (Jenkins, 2012).

Streptococcus species and Staphylococcus aureus are the genera of bacteria that were isolated from the sweet potato sample. This work is comparable to that of Oladoye *et al*. (2013), who found that Staphylococcus was the most common bacterium among those he identified from a decomposing sweet potato. Numerous scientists have also reported the microorganisms that have been shown to be responsible for sweet potato deterioration during storage. According to Oladoye *et al*. (2013), bacteria that cause sweet potato spoiling include Staphylococcus, Bacillus, and Pseudomonas. These bacteria can create enzymes that can break down the tissues of sweet potatoes. *It was discovered that Aspergillus, Fusarium,* and *Geotrichum* were the causes of sweet potato spoiling (Khatoon *et al*., 2012; 2016). According to Enyiukwu *et al*. (2014), *Aspergillus fumigatus* is a pathogen that affects sweet potatoes.

In Awka, Anambra state, two fungal genera—*Candida* spp. and *Saccharomyces* spp.—were shown to be responsible for sweet potato rotting. Ten tubers from the Eke-Awka market in the Awka South Local Government Area of Anambra State were used in a related study by Agu *et al*. (2015) to investigate the fungi linked to sweet potato post-harvest loss. They detected three species of spoilage molds: *Rhizopus stolonifer, Aspergillus fumigatus,* and *Aspergillus niger*. These findings contradict with those of Gambari and Okinedo (2020), who found that postharvest rots of sweet potato tubers were caused by *Aspergillus niger, Mucor* species, *Fusarium* species, and *Penicillium* species.

In South West Nigeria, *Aspergillus flavus* is the most commonly isolated fungus from spoiled sweet potato tubers, according to Anaienyo and Ataga (2017). These discrepancies could be caused by a number of things, including sample size.

5. Conclusion

This study demonstrated that sweet potato deterioration during storage is caused by both bacteria and fungi. As a result, there are less sweet potatoes available on the market. A number of control measures must be implemented in order to slow down the rate at which microorganisms cause sweet potatoes to deteriorate. These include washing harvested tubers properly, cleaning transit containers, handling tubers carefully to prevent injuries, ensuring that handlers maintain good hygiene, and providing hygienic storage facilities.

Recommendations

In order to prevent physical damage to the tuber and, consequently, microbial attack, concerted efforts should be made to minimize or reduce the thin, delicate skin of sweet potatoes, which has been described as being easily damaged by cuts and abrasion during harvesting, transportation, or distribution. Furthermore, temperature and humidity have an

impact on the growth of spoilage organisms; lowering these factors can help slow the rate at which these microbes multiply and harm food while it is being stored.

Since temperature has a significant impact on microbial development, storing sweet potatoes at a lower temperature will reduce the pace of microbial growth. Storage with low humidity should be promoted as well since it slows the growth of bacteria and fungus linked to sweet potato spoiling. Therefore, reducing the behaviors that encourage microbial attack during storage can limit post-harvest deterioration or waste and increase food security and sustainability. Therefore, efforts ought to be focused on expanding the nation's food supply and preserving it.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest in any part of this work.

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