

## Morphological Characterization and Determination of Mycotoxigenic and Non-mycotoxigenic Producing *Penicillium* SP from Bakery Food Products by UV Light and Ammonia Vapor Test

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### Abstract:

Products from bakeries are vital for a healthy diet. Both macronutrients and micronutrients, which are essential for preserving human health, are abundant in it and of great quality. The most popular goods are buns, bread, cupcakes, cookies, and toast. Due to inappropriate handling and poor sanitation, a variety of filamentous fungi are implicated in the spoiling of bakery food products. Mycotoxins are secondary metabolites made by filamentous fungi and found in food and feed. The purpose of this study was to identify the mycotoxigenic and non-mycotoxigenic isolates of *Penicillium* sp. using morphological characterization. Eighteen (18) isolates of *Penicillium* sp were obtained from bakery foods that were gathered from bakeries in rural locations, such as Mayakonda, Davangere, Anaberu, Alur, Attigere, Echagatta, Bathi, Kodaganur, Bada, sasalu around Davangere city. They were cultured on different media. Colony colour and texture were seen to assess macromorphological qualities, while spore colour, size, structure, conidiophore structure, conidia, and vesical form were inspected under a microscope to determine micromorphological characteristics. On coconut cream agar (CCA) medium plates, UV fluorescence of isolates was used to measure mycotoxin generation. *Penicillium* sp isolates from 16 (88.88%) of the isolates were positive for mycotoxin, while 2 (11.11%) of the isolates tested negative. The prevalence of *Penicillium* sp., a mycotoxin-producing organism, emphasises the need for action to eradicate their presence in food. The highest incidence of *Penicillium* sp. was recorded in samples collected from Anaberu, followed by Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Davangere, Mayakonda, and Sasalu.

**Key Words:** Bakery products, fungal spoilage, *Penicillium* sp, Coconut cream agar, Ammonia vapor test.

### 1. Introduction

The majority of the nation and its cultures consider pastries to be vital staple foods. The most widely consumed products include breads, buns, cupcakes. Cereals account for over half of our protein demands and the majority of our dietary calories, making bakery items an important source of nutrients for our diet. Carbohydrates, proteins, lipids, vitamins, calcium, iron, minerals, starch, and energy are among the typical nutrients present in baked foods. It works well to add more fibre to bread products. Wheat flour, water, sugar, salt, yeast, baking soda, baking powder, corn starch, milk, butter or margarine, honey, yoghurt, essence, cocoa powder, chocolate slabs, fruit jams and sweetener are all materials used in baking. (Bartkiene et al., 2008). In fresh bread and other baked products, the baking process often destroys mould spores. Therefore, a contaminated source might be the air, a bakery surface, a component of equipment, a food handler, raw materials, or even after baking, during the chilling, slicing, or packaging processes. This implies that any issues with mold-related deterioration must arise following baking. Due to airborne contamination brought on by the warmer temperature and more humid storage conditions, the fungal spore counts are higher in the summer than they are in the

winter. Unwanted odours were produced by microbial decomposition, which is frequently visible on a product's surface (Baily *et al.*, 1993).

Due to the presence of several filamentous fungi including *Rhizopus* sp., *Mucor* sp., *Aspergillus* sp., and *Fusarium* sp. in the rotting of bakery goods brought on by inappropriate handling and sanitation, losses of bakery goods attributable to these fungi range from 1.5% to 2.5% depending on the time of year, the kind of goods, and the processing techniques used. Because more bread is being manufactured without preservatives and frequently raw ingredients like bran and seeds are included, it is likely that the incidence of wheat bread spoiling brought on by these fungi has grown over the past several years. These bakery items might be spoiled, which might be a health risk or result in a minor food disease. These goods have been linked to food-borne illnesses in previous decades. In nature, *Penicillium* sp. is extensively dispersed and mostly discovered at cereal and grains. Both before and after harvest. Temperature and relative humidity are two environmental factors that have an impact on a plant's growth. Developing qualitative cultural methods for identifying mycotoxigenic *Penicillium* species cultivated on suitable conditions using the blue fluorescence technique (Jarvis *et al.*). These techniques either use a liquid medium or a solid medium, like coconut agar medium (CAM) or potato dextrose agar (PDA). Under long-wave UV light, *Penicillium* sp., a mycotoxin generator, was discovered. Mycotoxigenic isolates are quickly distinguished from non-mycotoxigenic isolates by the emergence of blue to blue-green fluorescent, while non-mycotoxigenic is non-produce fluorescent. The quick and accurate approach of ammonium hydroxide vapor-induced colour shift is frequently used to identify toxic and non-toxic strains of *Penicillium*. Ammonium hydroxide vapor-induced colour shift is a rapid and reliable method for differentiating between hazardous and non-toxic strains of *Penicillium*. A single colony formed using this method in the centre of the Petri dish. While non-mycotoxigenic *Penicillium* strains do not produce colour, the reverse of colonies of mycotoxigenic *Penicillium* strains turned pink when their medium was exposed to ammonia vapour by dropping ammonia hydroxide on it. This study's main focus is on the rapid detection of *Penicillium* sp. mycotoxigenic generating strains that were isolated from bakery items by UV light test and ammonia vapour test, as well as its preventative measures. (Malkkiet *al.*, 2005).

## 2. Materials and Methods

### 2.1. Collection of samples

There are 55 various varieties of bakery items, including bread, toast, cupcakes, buns, cookies, etc. were collected from rural side (Echagatta, Bada, Bathi, Mayakonda, Avargere, Sasalu, Alur, Attigere, Anaberu and Kodaganur) different bakery shops around Davangere city from which were decorated samples in open stall during July to December 2022. The collected samples were brought in sterile polythene bags to the laboratory for analysis and maintained at temperature 25-27°C for further processes (Daou *et al.*, 2021).

### 2.2. Isolation and identification of *Penicillium* sp

Using three different isolation techniques, including the spread plate approach, serial dilution method, and direct plate method, four different types of dextrose agar media, including Potato dextrose agar (PDA), Rose Bengal agar (RBA), Czapek (dox) agar (CZA), and Sabouraud's dextrose agar (SDA), were used to isolate *Penicillium* sp. (Al-kahtani, 2014). Chloramphenicol was used to produce the growth medium, which was then autoclaved at 121°C for 15 minutes. The sterilised medium was placed into sterile Petri plates under aseptic conditions after cooling for 15-20 minutes. After solidification, plates were maintained at 40°C until inoculation. For the direct plate approach, 1 gm of the bakery sample was immediately poured or sprinkled over the PDA+RBA+CZA+SDA solidified agar media and incubated for 3 to 7 days at 30-37°C. In the spread plate method, 1 g of sample was mixed with 9 ml of distilled water to create a homogenate, which was then evenly scattered across the surface of the media (PDA+RBA+CZA+SDA) using a sterile L-shaped spreader. Following that, the plates were maintained in an incubator for 3-7 days at 30-37°C. Fungi were found and identified after incubation utilising microscopic analysis and morphological traits (Patil & Kukade, 2020). As part of the serial dilution method, 1 gm of sample was thoroughly mixed with 9 ml of sterile water to create a 10<sup>-1</sup> dilution, which was then serially diluted up to 10<sup>-7</sup>. Spread plate inoculation was performed using 1 ml of the solution from each dilution on PDA+RBA+CZA+SDA solidified agar medium. After that, the plates were held at 30-37°C for 3-7 days. After incubation, fungi were studied and identified using microscopic findings and morphological characteristics (Patil & Kukade, 2020). Lactophenol cotton blue stain was used to stain the cotton, as demonstrated by Domsch *et al.* in 1980, Subramanian,

1983, Ellis & Ellis, 1997, Gilman, 2001, and Nagamani *et al.* in 2006; after staining; *Penicillium* sp were recognised based on morphological and microscopic observation.

**2.3. Mycotoxigenic Potential Determination of *Penicillium* sp**

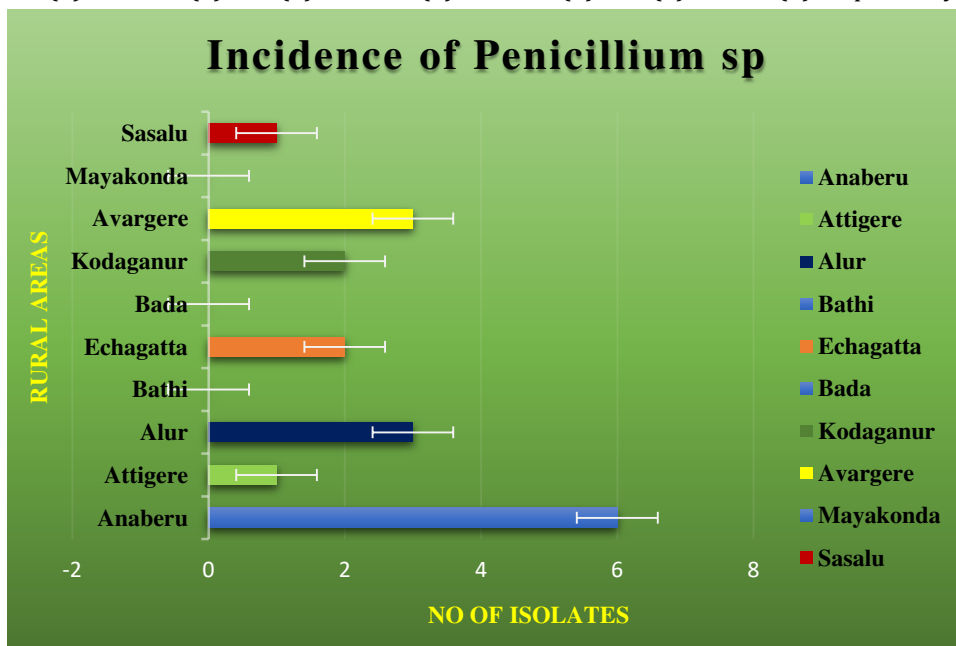
Coconut cream agar medium (CCAM) was used to test *Penicillium* species capacity to release toxins, revealing both toxigenic and non-toxigenic isolates. Fente et al. and Davis et al. state that coconut cream agar medium was made and sterilised in Petri plates using antibiotics. A well forms in the centre of the CCAM-containing plate after solidification. Spores from a *Penicillium* species culture that had been growing for 7 days were suspended in distilled water that contained 0.025% Tween 80. The Petri plate was then aseptically loaded with a 10 microliter suspension of spores. At 28°C, the infected plates underwent a 7-day incubation period. The inoculation plates were tested for toxin fluorescence after incubation by having them checked under UV light, and the findings were recorded.

After inoculation as single colonies on CAM using a cork borer with a 5 mm diameter, the fungal isolates were grown at 28 °C in the dark for 7 days. The Petri dish was then turned upside down, and the inside of the lid was coated with one or two drops of a concentrated ammonium hydroxide solution. The Petri dish was then inverted over the control plate and lid holding the ammonium hydroxide. (Alkhersanet *al.*,2016).

**3. Results**

**3.1. Detection of *Penicillium* sp**

In bakery food items, 18 isolates of *Penicillium* sp were discovered (Figure 1). By comparing the morphological characteristics of the isolates of *Penicillium* sp to the main descriptions given by Domschetal. 1980, Subramanian, 1983, Ellis & Ellis, 1997, Gilman, 2001, and Nagamani et al. 2006, the isolates were identified. The % detection of *Penicillium* sp in Anaberu, Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Avargere, Mayakonda, Sasalu. was 33.33% (6), 5.55% (1), 16.66% (3), 0% (0), 11.11% (2), 0% (0), 11.11% (2), 16.66% (3), 0% (0), 5.55% (1) respectively.



**Figure.1: Prevalence of *Penicillium* isolates in Anaberu, Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Avargere, Mayakonda, Sasalu**

### 3.2. Macroscopic properties of *Penicillium* sp on PDA and RBA

Figure 2 displays the colony morphology of *Penicillium* sp on PDA and RBA medium plates. After two days of incubation, *Penicillium* sp.'s mycelial colour was white, and after five days of incubation, the colonies on both plates took on a blue green hue. After 14<sup>th</sup> days of incubation colonies were 80-82mm in diameter, dominated by very light green centre with white surroundings.

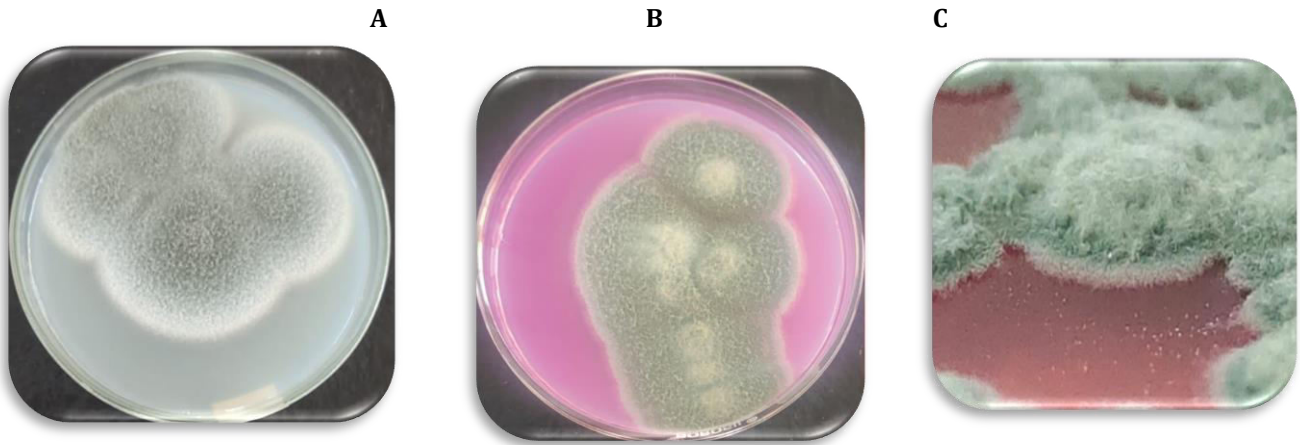


Figure 2. Colony morphology of *Penicillium* sp. on PDA and RBA medium plates. PDA plates, RBA plates, and 1.9x magnification

### 3.3. Microscopic characteristic of *Penicillium* sp

The microscopic features of isolated *Penicillium* are depicted in Figure 3. Conidiophores and septate hyphae are branching. Brush like clusters were observed at the end of the conidiophores. Conidia looks like round to ovoid.

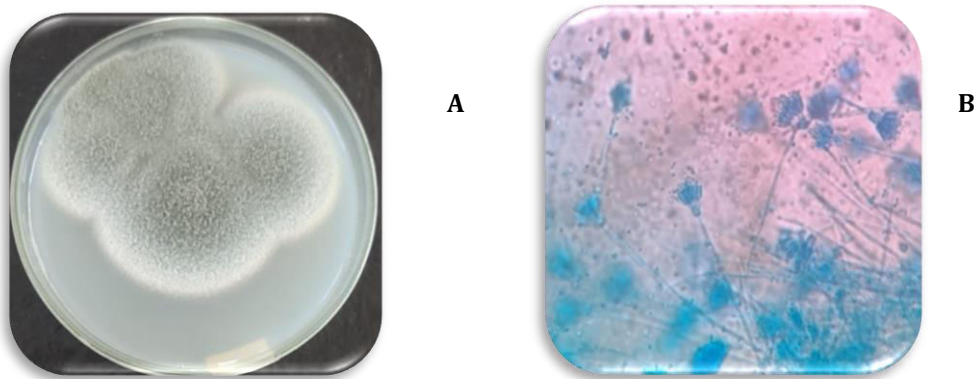


Figure 3. A. *Penicillium* sp. macroscopic features on PDA. B. *Penicillium* sp microscopic features with a 40X objective.

### 3.4. Screening of Mycotoxin Production

Figure 4 shows the outcomes of the *Penicillium* species UV light screening as well as fluorescent and non-fluorescent isolates of the *Penicillium* species on CCA. When exposed to UV light at 365 nm, 16 (88.88%) of the 18 isolates fluoresced blue on CCA medium plates. Table 1 reveals that of the 18 isolates, 88.88% (16) are capable of producing mycotoxin, while the remaining isolates (11.11% (2) do not exhibit blue fluorescence when exposed to UV light, indicating that they are not engaged in mycotoxin production. Figure 5 shows the distribution of *Penicillium* isolates

that are mycotoxigenic and those that are not in ten (10) different places. Three of the six isolates from the Anaberu bakery produced mycotoxin during testing, however the other three isolates did not. One isolate from the Attigere bakery samples had mycotoxin production found, and the other isolate also had mycotoxin production. Similar to this, two of the three isolates found in the Alur bakery samples produced mycotoxin, whereas the third strain did not. Out of the two isolates found in the Echagatta bakery samples, one produces mycotoxin. Two isolates from Kodaganur bakery samples proved positive for the formation of mycotoxin, and both two isolates did so. Two of the isolates from the Avargere bakery samples proved negative for mycotoxin generation, but one strain did. Last but not least, a single isolate from a sample from a bakery in Sasalu produced mycotoxin.

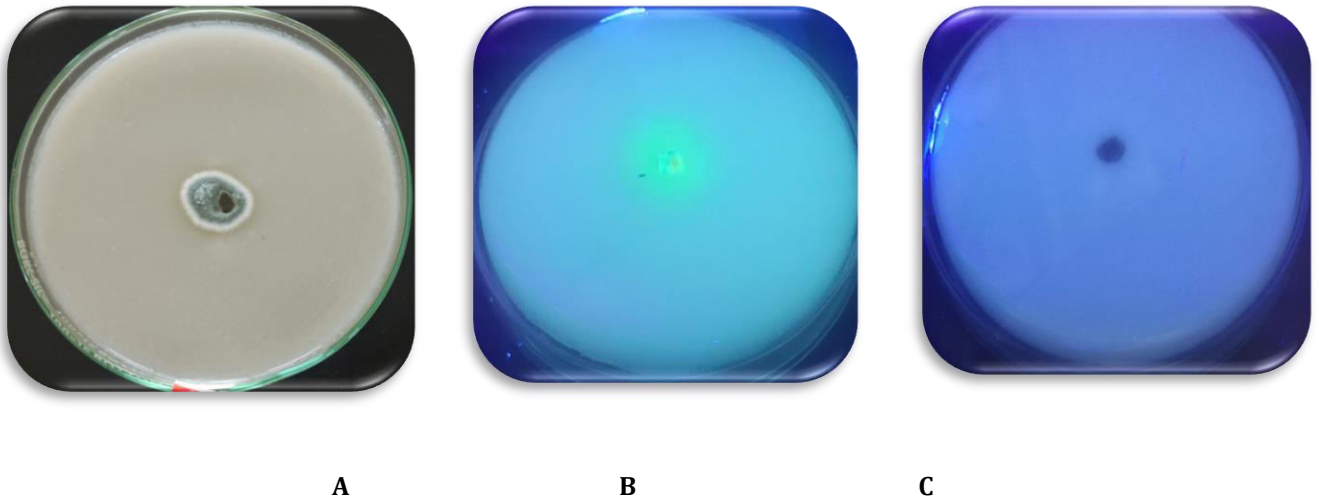


Figure.4. Mycotoxigenic and non-mycotoxigenic isolates of *Penicillium* observed under UV light. A. Growth of *Penicillium* sp on CCA medium plate. B. Mycotoxigenic production. C. Non-mycotoxigenic production.

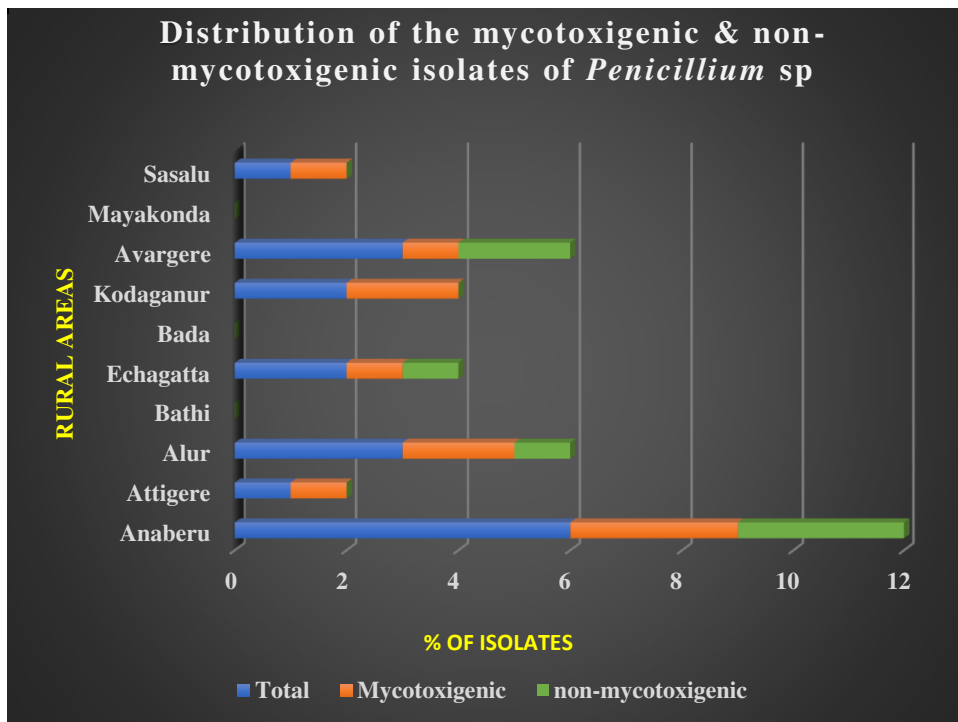


Figure.5. Distribution of *Penicillium* sp. mycotoxigenic & non-mycotoxigenic isolates in 10 bakery food samples gathered from rural locations

**Table 1. Identification of production of Mycotoxin through screening by UV fluorescence**

Sl.No	Isolates	Results
1	SR1	Positive
2	SR2	Positive
3	SR3	Positive
4	SR4	Negative
5	SR5	Positive
6	SR6	Positive
7	SR7	Positive
8	SR8	Positive
9	SR9	Positive
10	SR10	Positive
11	SR11	Negative
12	SR12	Positive
13	SR13	Positive
14	SR14	Positive
15	SR15	Positive
16	SR16	Positive
17	SR17	Positive
18	SR18	Positive

## Discussion

Anaberu bakery samples had the highest occurrence of *Penicillium* sp (33.33%), whereas Sasalu and Attigere bakery samples had the lowest prevalence (5.55%). Environmental variations may be to blame for the variations in *Penicillium* sp. prevalence in the various bakeries. A common technique for identifying mushrooms is to observe the key macro and micromorphological characteristics of cultures of diverse fungi on different media. The morphological Characterization was conducted in this work to emphasise the necessity of such fundamental identifying procedures for the fast screening of isolates in the majority of undeveloped nations, where access to uncommon instruments is a significant challenge. *Penicillium* isolates could be consistently identified by looking at specific physical traits. The colony shape of the isolates in this investigation matched *Penicillium* sp, as seen in Figures 2 and 3, according to Klich's interpretation of taxonomic descriptions. The microscopic and macroscopic characteristics shown in Figures 2 and 3 are similar to those of *A. flavus* and *Penicillium* species that Rodrigues et al. and Diba et al. have documented.

In this work, appropriate growth and sporulation were enabled by the use of Potato Dextrose Agar (PDA), Rose Bengal Agar (RBA), Czapek (dox) agar (CZA), and Sabouraud's dextrose agar (SDA) medium. This enabled a successful analysis of the macroscopic and microscopic properties of *Penicillium* sp. On the intentionally infected materials, *P.chrysogenum* developed quickly and left patches of discoloration that ranged from grey to bright green, according to Nielsen et al. In this study, the quick distinction between mycotoxigenic *Penicillium* sp. and non-mycotoxigenic *Penicillium* sp. was made possible by the use of CCA media. When 16 (88.88%) of the 18 isolates were subjected to 365 nm UV light, they fluoresced blue on CCA medium plates, indicating that they had the ability to produce mycotoxins. Culture-based approaches may be helpful for quick screening of isolates in the majority of underdeveloped nations where mycotoxin determination may be delayed due to a lack of sophisticated detection tools. Culture-based approaches are generally straightforward and affordable for mycotoxin detection. However, chromatographic techniques are generally employed in conjunction with these techniques.

## Conclusion

*Penicillium* sp. strains, both mycotoxigenic and non-mycotoxigenic, were discovered in bakery food products from various countries. Culture media and morphology are practical tools for *Penicillium* species in this study.

A rapid and reliable way for separating mycotoxigenic from non-mycotoxigenic isolates is to characterise and screen *Penicillium* isolates for mycotoxin detection under UV light (365nm).

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