*Journal homepage:* http://fens.usv.ro/index.php/FENS
*Journal of Faculty of Food Engineering,*
*Ştefan cel Mare University of Suceava, Romania*
*Volume XX, Issue 4 - 2021,* pag. 343 - 351

# SURVEY AND FOURIER TRANSFORM INFRARED MICROSCOPY (FTIR) ANALYSIS OF YEASTS ISOLATED FROM DAIRY PRODUCTS IN OSOGBO METROPOLIS

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Received 1st September 2021, accepted 28th December 2021

**Abstract:** *The purpose of this paper is to investigate the different yeast species in dairy products that can be harnessed industrially for biotechnological functions. Eighteen (18) different yeast species were obtained from thirty (30) different dairy products namely: yogurt, milk and cheese. pH, temperature and ethanol tolerance were conducted using standard methods while fungi isolation was done using culture-dependent methods. The antibacterial activity and FTIR analysis the yeast species were also done using standard methods. Result obtained showed that S. cerevisiae was the predominant fungi isolated (19.11%) while Chrysosporiumspp and C. fimetiwere the least with 1.47% occurrence each. All isolates showed moderate antibacterial activity on test pathogens. S. cerevisiae, C. krusei, C.glabrataand K. fragilis were analzyed for functional groups with the corresponding peaks 3439.19, 3437.26, 3439.19, 3431.48, 3417.98 and 3448.84($cm^{-1}$) respectively. All possess hydroxyl (O-H) and methylene functional group (C-H) while only D. hansenii(C-C) had an additional alkenyl group. In conclusion, representative isolate analyzed by FTIR can be employed industrially for fermentation processes while D. hanseniiis a promising organism for plastics degradation/ bioplastic production.*

**Keywords**: *Milk, yoghurt, cheese, antibacterial activity, ethanol tolerance, S. cerevisiae, D. hansenii*

## 1. Introduction

Dairy products such as milk, yoghurt and cheese (wara) are protein rich substrates in which probiotic yeasts can be easily isolated. Literature has reported that milk is one of the most satisfactory single food that can be prepared and consumed by humans. It contains proteins, vitamins, fats, carbohydrate and inorganic salts [1]. The proteineous nature of milk makes it a suitable growth medium for different types of organisms [2]. Cheese is one of the numerous food products that can be obtained by processing of milk and [3] has documented that about one-third of the total volume of milk produced in African is been used for cheese production. According to [4], there are more than 1000 varieties of cheese produced globally, each having a unique flavour, taste and form. There are four major ingredients involved in the production of cheese which are: milk, rennet, microorganisms and salt. Soft cheese can be used as a drug for certain infections and possesses a strong inhibitory activity against diarrhea causing infections [5], [6]. However, if milk is not properly pasteurized, it can become a vehicle for pathogenic microorganisms when processed into cheese and other delicacies. Yeast species have the ability to ferment lactose sugar and assimilate various acids such as citric, succinic and lactic acids [7]. The various impacts of probiotic yeast species in food processing involves: production of cheese, bread, and milk products, biopreservation which prevents the spoilage of food products as well as biofortification of foods [8], [9], [10]. Yeasts can also be used in the production of biofuel [11]. Fermented milk can be consumed as food beverages whose market and storage values are better improved than the raw milk. In addition, the major locally produced traditionally fermented milk products in Nigeria are wara and nunu which are made by using fresh milk collected from cow, sheep or goat. The African soft cheese (wara) is a good source of nutrients

*DOI: https://doi.org/10.4316/fens.2021.037*

such as protein, fat, calcium, iron, phosphorus, vitamins and essential amino acids [12]. Wara (local cheese) has higher moisture content than the hard cheese which makes it to have a lower shelf life due to spoilage by microorganisms. It can also be deep fried in groundnut oil for preservation over a long period of time. It can also be eaten in various forms in different African regions either as a raw cheese, flavoured snack, fried cake, meat substitute in dishes as well as filling in sandwiches [13].

The concentration of lactic acid bacteria in yogurt is higher than that of yeasts [14]. Yeasts have the ability to boost the immune system, induce anti-tumor effects, anti-cholestrolemic effect and also help to stabilize the growth of lactic acid bacteria. Yeasts are also leavening agents which can either be chemical or biological in nature. Microorganisms such as *Saccharomyces cerevisiae* can produce carbon dioxide from sugar utilization [15]. They can be used during fermentation process for baking and brewing. However, in brewing, alcohol is released by microorganisms which is of utmost need in the maturation and development of fermentation flavor [16].

## 2. Materials and methods

**Sample collection**

A total sample of 30 dairy products (10 soft cheeses (*wara*); 10 evaporated liquid milk and 10 yogurt samples) were purchased from the following local markets: Oja oba, Igbona, Orisunmibare and a supermarket (Ace), Osogbo, Osun State, Nigeria. The cheeses were collected into a sterile low density polythene bag and transported immediately to the Microbiology laboratory of Osun State University for further studies.

**Preparation of samples and pH measurement**

A 5.0 ml each sample was aseptically withdrawn using a clean pipette and transferred into a test tube containing 5.0ml of 0.1 % sterile peptone water. Ten-fold serial dilutions of each samples was prepared and 0.2ml of the appropriate dilutions ($10^{-3}$ and $10^{-5}$) were spread plated onto Potato Dextrose Agar (PDA) containing 100µg of chloramphenicol/ml. Plates were incubated at 30°C for 5-7days and purified by sub-culturing to obtain pure isolates. The pure cultures were stored in the refrigerator as stock on potato dextrose agar slants.

**pH measurement**

The pH of the samples were taken using pH meter (Orion pH meter (model 310, Orion Research Inc., Beverly, MA) which was first standardized with buffer solutions of pH 4, 7 and 9. 5g of the samples were pipetted out aseptically and transferred into test tubes. 20 ml of sterile distilled water was added and the mixture was shaken for 30 minutes on the rotary orbital shaker. The pH of the suspension was then determined by inserting the electrode of the pH meter into the solution and the pH values were read when the reading was stable [17].

**Characterization and identification of isolates**

The cultural characteristics of the isolates on the potato dextrose agar (PDA) plates were observed by staining the cultures with lactophenol blue on a sterile grease-free glass slide and placed under the microscope to view. Aplexopoulos (fungi compendium) was used to study properties such as the elevation, surface and colour were viewed and recorded [18].

**Thermotolerance test**

The ability of yeast isolates to grow at higher temperatures was characterized by culturing on potato dextrose agar and incubated at varying degrees of temperature (30, 45 and $65^0$C) for 72 hours [19]

**Ethanol tolerance tests**

The isolated yeast species were grown on potato dextrose broth containing varying ethanol concentrations (5, 10 and 15%) in order to determine their ethanol concentration level.

***In-vitro* production of inhibitory metabolites**

*In-vitro* antimicrobial activity of the screened yeast isolates were tested against test pathogenic microorganisms (*Escherichia coli* ATCC 43816, *Staphylococcusaureus* NCTC 6571, *Klebsiella pneumoniae* ATCC 25922, *Proteus mirabilis* ATCC 7002and

*Corynebacterium diphtheriae* ATCC 13813) using the agar well diffusion technique [20]. 24 hr old cultures of test organisms and antimicrobial producing yeasts isolates were aseptically transferred into sterile nutrient broth and incubated at 37ºC for 24 hr. The turbidity was adjusted to MacFarland standard and the numbers of cells were confirmed using the spectrophotometer. The promising antimicrobial producing yeast isolates were seeded on the surface of sterile Mueller Hinton agar (MHA) plates using sterile swab sticks. Wells of 6 mm diameter were bored into the agar plates. The yeasts isolates were centrifuged for 20 minutes at 6,000 rpm and 70µL, 80µL, 90µL and 100 µL of the supernatants (which contains active metabolites) was pipetted into the wells and incubated at $30^0$C for 24-48 hr. The zones of inhibition around the test organisms were measured and recorded appropriately. Test was conducted in triplicates and the mean value was recorded using statical analysis.

## FTIR (Fourier transform infrared spectroscopy)

Representative isolates namely:*M. furfur, D. hansenii, S. cerevisiae, C. krusei, C.glabrata*and *K. fragilis* were further analysed using the Fourier transform infrared spectroscopy in order to know the functional groups that these yeasts belong to, thus giving an insight to the biotechnological exploitations of these yeasts. The method of [21] was used with slight modifications.

## 3. Results and discussion

The pH of yogurt, milk and cheese ranged from 3.21-4.47, 5.30-6.60 and 5.63-5.87 respectively. The lowest pH (3.21) was recorded from yogurt while the highest (6.60) was recorded from Hollandia milk. Similar findings were reported by [22], [23] and [24] who recorded pH value range of 4.16-2.20, 2.00-5.50 and 1.30-7.80 from yeast species isolated from: Egyptian Karish cheese, cereal based Nigerian traditional fermented beverages namely (burukutu, kunu-zaki and ogi) and whole grain millet sourdoughs respectively. The ability of the yeast species to grow at low acidic pH confers microbial stability on the food. According to [25], the ability of moulds and yeasts at pH range of 2-8 demonstrates the ability of the yeasts to eliminate spoilage microorganisms, thus creating a conducive environment for the growth of food grade microorganims.

The total count of fungi species from each of the dairy sample ( yogurt, milk and cheese) is presented in Table 1 above. *S. cerevisiae* was the predominant fungiisolated (19.11%), followed by *B. bassiana*and*D. hansenii*(8.82%); *M. furfur and B. hawaliensis*(7.35%); *A. kalrae, C. krusei*and*P. purpurogen*(5.88%);*C. bantiana, K. fragilis*and*M. ferrugineum*(4.4.1%); *P. funiculosum, B. ranarum, C. glabrata, F. oxysporum*and*N. dimidiatum*(2.94%) while*Chrysosporiumspp* and *C. fimeti*were the least isolated with 1.47% occurrence each. The predominance of the yeast *S. cerevisiae*in yogurt and cheese had been earlier reported in literature according to the works of [26], [27], [28] from *Amasi* of cow milk, *Sameel* made of cow, goat, camel or sheep milk, *Chal* made of camel milk and sourdough made of millet respectively. However, it is noteworthy to mention that some of the isolated yeast species are normal flora of the skin, the *Calotropis procera* leaf (used as coagulant for cheese production), utensils used, food handlers as well as the environment (air, water and soil) such as: *B. bassiana, B.hawaliensis, C. glabrata, Chrysosporiumspp, C. krusei, F. oxysporum, C. fimeti, C. bantiana, M. ferrugineum* and *P. funiculosum.*

**Table 1**

**Total count of isolated yeasts from each dairy sample**

| Presumptive organisms | TEM | EAM | TAC | TOTAL |
|---|---|---|---|---|
| *Arthrographiskalrae* | 2 | 0 | 2 | 4 |
| *Basidiobolusranarum* | 0 | 2 | 0 | 2 |
| *Beauveria bassiana* | 2 | 2 | 2 | 6 |
| *Bipolarishawaliensis* | 2 | 0 | 3 | 5 |
| *Candida glabrata* | 0 | 0 | 2 | 2 |
| *Candida krusei* | 0 | 0 | 4 | 4 |
| *Chaetomium fimeti* | 0 | 1 | 0 | 1 |
| *Chrysosporiumspp* | 0 | 1 | 0 | 1 |
| *Cladophialophorabantiana* | 0 | 3 | 0 | 3 |
| *Derbaryomyceshansenii* | 1 | 0 | 5 | 6 |
| *Fusarium oxysporum* | 0 | 2 | 0 | 2 |
| *Kluyveromyces fragilis* | 1 | 0 | 2 | 3 |
| *Malassezia furfur* | 1 | 0 | 4 | 5 |
| *Microsporumferrugineum* | 0 | 3 | 0 | 3 |
| *Neoscytalidiumdimidiatum* | 0 | 2 | 0 | 2 |
| *Pencilliumpurpurogen* | 4 | 0 | 0 | 4 |
| *Penicillium funiculosum* | 0 | 2 | 0 | 2 |
| *Saccharomyces cerevisiae* | 5 | 0 | 8 | 13 |
| **TOTAL** | 18 | 18 | 32 | 68 |

**Key**: TEM= Yogurt; EAM= Milk; TAC= Cheese

Table 2 gives the information on the ethanol and temperature tolerance of the isolated yeast species.

All the isolates were subjected to growth at 5, 10 and 15% respectively and the result showed that they all grew well at 5 and 10% while *Arthrographis kalrae, Basidiobolus ranarum, Candida glabrata, Candida krusei, kluyveromycesfragilis Fusarium oxysporum*and *Microsporumferrugineum* did not grow at 15% ethanol according to Table 2. This denotes that these yeast species cannot tolerate high ethanol concentration as the growth of organisms at high ethanol concentration show to be species dependent. Furthermore, ethanol tolerance of the yeast isolates shows that they can be used as starter cultures in alcohol production because the fermentation process will be stopped if the organisms cannot tolerate the ethanol produced which is an indication that the yeast cell walls can withstand osmotic stress according to the reports of [29],[30], [31] and [32].

Key: GT= Gentamycin(control)

**Table 2**

**Ethanol and temperature tolerance of isolated yeasts**

| Presumptive organisms | Ethanol concentration | | | Temperature range | | |
|---|---|---|---|---|---|---|
| | 5% | 10% | 15% | 45°C | 37°C | 30°C |
| *Arthrographiskalrae* | + | + | - | + | + | + |
| *Basidiobolusranarum* | + | + | _ | + | + | + |
| *Beauveria bassiana* | + | + | + | + | + | + |
| *Bipolarishawaliensis* | + | + | + | + | + | + |
| *Candida glabrata* | + | + | - | + | + | + |
| *Candida krusei* | + | + | - | + | + | + |
| *Chaetomium fimeti* | + | + | + | + | + | + |
| *Chrysosporiumspp* | + | + | + | + | + | + |
| *Cladophialophorabantiana* | + | + | + | + | + | + |
| *Derbaryomyceshansenii* | + | + | + | + | + | + |
| *Fusarium oxysporum* | + | + | _ | + | + | + |
| *Kluyveromyces fragilis* | + | + | - | + | + | + |
| *Malassezia furfur* | + | + | + | + | + | + |
| *Microsporumferrugineum* | + | + | - | + | + | + |
| *Neoscytalidiumdimidiatum* | + | + | + | + | + | + |
| *Pencilliumpurpurogen* | + | + | + | + | + | + |
| *Penicillium funiculosum* | + | + | + | + | + | + |
| *Saccharomyces cerevisiae* | + | + | + | + | + | + |

**Table 3**

**Antibcaterial activity of isolated yeast species against pathogenic test organisms by measuring zone of diameter (mm)**

| Yeast Isolates | *P. mirabilis* | *S. aureus* | *K. pneumoniae* | *E.coli* | *C. diphtheriae* | Gentamycin (30µg) |
|---|---|---|---|---|---|---|
| ***S. cerevisiae*** | 9.92±0.03 | 9.00±1.13 | 11.20±0.01 | 13.99±1.23 | 9.69±0.09 | 18.00±0.01 |
| ***C. krusei*** | 9.45±0.17 | 10.00±1.02 | 10.90±0.11 | 10.20±1.06 | 10.00±0.03 | 22.00±0.11 |
| ***C. glabrata*** | 9.64±0.03 | 8.67±1.03 | 13.01±0.33 | 9.33±1.09 | 9.54±0.03 | 21.00±0.21 |
| ***M. furfur*** | 9.88±0.09 | 12.34±1.32 | 9.67±0.11 | 9.61±1.32 | 9.70±0.03 | 21.00±0.33 |
| ***D. hansenii*** | 9.27±0.06 | 9.90±1.06 | 9.45±0.03 | 10.01±1.44 | 9.41±0.03 | 18.00±0.10 |
| ***k. fragilis*** | 9.11±0.04 | 9.62±0.02 | 9.21±0.22 | 9.23±0.05 | 9.22±0.03 | 16.00±0.30 |

*Values are means ± standard deviation of duplicates at $p < 0.05$.*

In addition, growth at 30, 37 and 45$^{o}$C was also carried out on all the isolates and the result showed that there was a 100% growth in all the three (3) subjected temperatures. [33] had earlier documented that the ability of yeast species to grow within a wide temperature range (30-45$^{o}$C) confirms a vast difference in their thermostability, which is a suitable quality to be considered for use in fermentation processes.

The antibacterial effect (Table 3) of the *S. cerevisaie, C.krusei, C. glabrata, M. furfur, D. hansenii* and *K fragilis* against*P.mirabilis, S. aureus, K. pneumoniae, E. coli* and *C. diphtheriae* showed these corresponding zone of inhibition (mm):9.92, 9.00, 11.20, 13.99 and 9.69; 9.45, 10.00, 10.90, 10.20 and 10.00; 9.64, 8.67, 13.01, 9.33 and 9.54; 9.88, 12.34, 9.67, 9.61 and 9.70; 9.27, 9.90, 9.45, 10.01 and 9.41; 9.11, 9.62, 9.21, 9.23 and 9.22 respectively. However, [33] had ealier documented high antibacterial activity of *C. intermedia, C. kefyr*and *C. lusitaniae* against*S. aureus, E.coli* while they documented moderate activity of *C. tropicalis, C. lusitaniae* and *S. cerevisiae* against *E. coli.* In addition, they also documented low activity of *C. intermedia, C. kefyr,C.lusitaniae, C. tropicalis* and *S. cerevisiae* against *Pseudomonas aeruginosa*. Furthermore, the work of [34] also support the claims of this study.

Six (6) representative fungi in this study were further analyzed using Fourier transform infrared spectroscopy (FTIR) to further identify the yeast fungi species (Figures 1-2). The six fungi are: *D. hansenii, M. furfur, S. cerevisiae, C. krusei, C.glabrata* and *K. fragilis* with the corresponding peaks: 3439.19, 3437.26, 3439.19, 3431.48, 3417.98 and 3448.84(cm$^{-1}$) respectively. They all have stretch type of vibration in their FTIR spectra reading and single intensity. All possess hydroxyl and methylene functional group (Table 4) while only *D. hansenii* has an additional alkenyl group. Hydroxyl group O-H consists of one atom of oxygen covalently bonded to one atom of hydrogen (alcohols and carboxylic acid) and methylene group comprises of two hydrogen atoms bounded to one carbon atom (methyl chloride, methyl alcohol/methanol) while alkenyl belongs to the vinyl group (vinyl chloride, a precursor to polyvinyl chloride) which is a functional group formed by removing a hydrogen atom

from an alkene. In this study, the result obtained shows that *D. hansenii, S. cerevisiae, C. krusei, C. glabrata* and *K. fragilis* can be employed on an industrial scale fermentation for the production of alcohols, methyl chloride methyl alcohol (which can be used as an anti-freeze) and methanol based on the FTIR analysis of their functional group.

**Table 4**

**FTIR spectra showing the different functional groups of the yeast species**

| S/N | Types of vibration | Absorption, $cm^{-1}$ ,Class of compounds and Chemical Formula | | | | | | Intensity |
|---|---|---|---|---|---|---|---|---|
| | | *Derbaryomyce shansenii* | *Malassezia furfur* | *Saccharomyces cerevisiae* | *Candida krusei* | *Kluveromyces fragilis* | *Candidaglabrata* | |
| 1. | Stretch | 3439.19 (Hydroxyl group) O-H | 3437.26 (Hydroxyl group) O-H | 3439.19 (Hydroxyl group) O-H | 3431.49 (Hydroxyl group) O-H | 3443.84 (Hydroxyl group) O-H | 3417.95 (Hydroxyl group) O-H | Single |
| 2. | Stretch | 2908.75 (Methylene group) C-H | 2929.97 (Methylene group) C-H | 2960.83 (Methylene group) C-H | 2366.74 (Methylene group) C-H | 2935.76 (Methylene group) C-H | 2943.47 (Methylene group) C-H | Single |
| 3. | Stretch | 2353.23 (Methylene group) C-H | 2675.36 (Methylene group) C-H | 2677.29 (Methylene group) C-H | 2000.25 (Methylene group) C-H | 2360.95 (Methylene group) C-H | 2677.29 (Methylene group) C-H | Single |
| 4. | Stretch | 1635.69 (Alkenyl Group) C-C | 2376.38 (Methylene group) C-H | 2000.25 (Methylene group) C-H | - | 2007.96 (Methylene group) C-H | 2362.88 (Methylene group) C-H | Single |

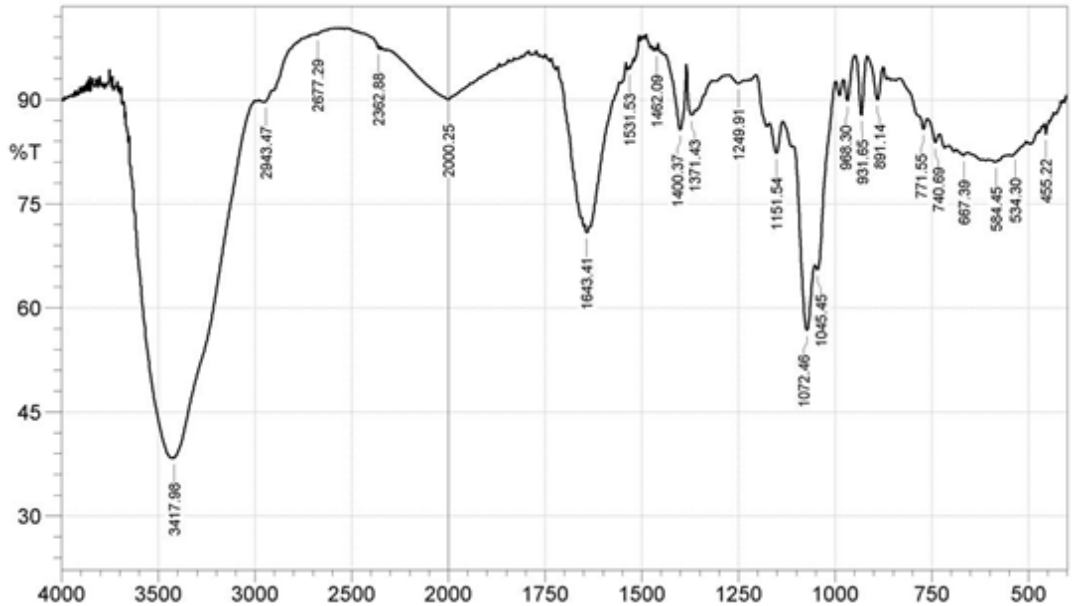

**Fig. 1: FTIR spectra generated from*S. cerevisiae***

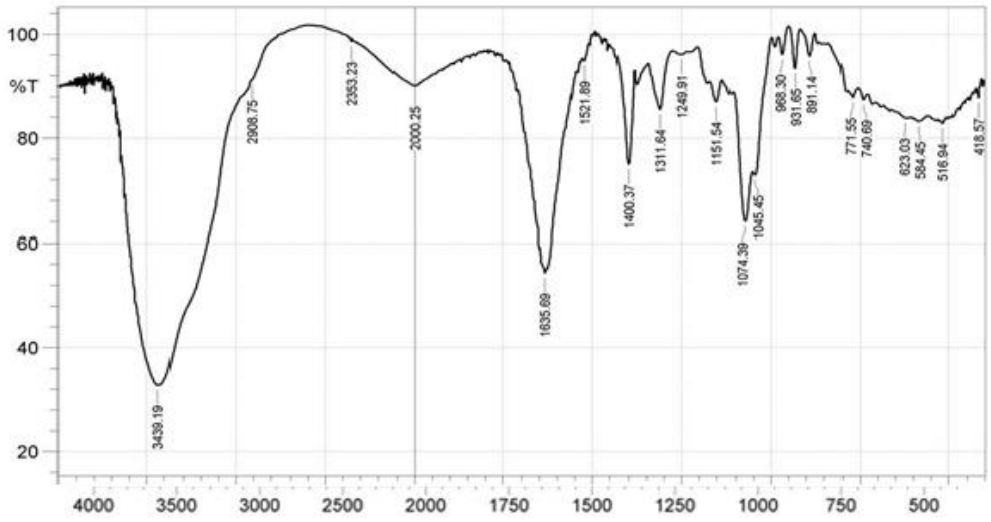


**Fig.2: FTIR spectra generated from *D. hansenii***

Furthermore, *D. hansenii* possesses an additional functional group (C-C) that makes a potential source for the production of polyvinyl chloride which is a bioplastic. Interestingly, there is limited information about it. [35], [36] also obtained similar results using FTIR to analyze the functional groups of *S. cerevisiae.*

## 4. Conclusions

In conclusion, the results obtained from this study shows that *D. hansenii* and *S. cerevisiae* are potential yeasts that can be used as starter cultures for industrial fermentation processes of foods and beverages. More interestingly, both yeast species can also for employed for bioplastic production as the FTIR analysis has confirmed this. There, it is recommended that further studies should be carried to optimize the bioplastic production processes.

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