

## **Short Research Article**

### **Prevalence of *Candida* species in 'Thairu' a traditional fermented milk**

#### ABSTRACT

Aims: To study the prevalence of *Candida* species in 'Thairu', a traditional fermented milk prepared in the households of Kerala.

Comment [SdS1]: Separate the words

Study Design: Collection of household 'Thairu' samples followed by enumeration and identification of yeast using API 20CAUX Kit. Determination of pH, acidity of samples.

Comment [SdS2]: Check if this is correct

Place and Duration of Study: September 2021- December 2021.

Methodology: A total of 30 household 'Thairu' samples were collected in sterile bottles. The samples were serially diluted in normal saline and pour plated on to Yeast Extract Glucose Chloramphenicol Agar for isolation and enumeration. The pH was measured using pH meter by directly inserting the probe to the homogenized sample. Titratable acidity in per cent lactic acid was measured using N/10 NaOH. The isolates were identified based on the sugar fermentation pattern using API 20C AUX kit (Biomérieux, France).

Results: The yeast count in the samples ranged from 5.0 to 6.7 log CFU/g with an average of  $5.89 \pm 0.38$  log CFU/g. Average acidity and pH of the samples were  $1.92 \pm 0.34$  % LA and  $3.59 \pm 0.60$  respectively. A total of 23 yeast isolates were identified based on morphology and carbohydrate fermentation pattern using API 20C AUX kit (Biomérieux, France). 86.95 per cent of isolates were belonging to *Candida* species.

Conclusion: Observations of the study revealed the high prevalence of *Candida* species in traditional fermented milk 'Thairu'. Predominant *Candida* species were lactose fermenters, but the presence of few species with spoilage and pathogenicity potential were also detected. The safety assessment of *Candida* species is essential before applying them as starter cultures for food fermentations.

Keywords: *Candida*, fermented milk, yeast, starter culture, traditional food, safety

## 1. INTRODUCTION

'Thairu' is the traditional fermented milk product which serves as the key ingredient in the preparation of Kerala cuisines like 'pulissery', 'kalan' and 'pachadi'. The product is prepared in households by back slopping usually in cow milk. The microflora of this product varies from region to region within the state itself owing to the changes in culinary practices, incubation temperature and environmental conditions. Lactic acid bacteria, yeast and mold and enterococci play a significant role in fermentation of traditional milks (Mutukumira 1995). The presence of lactic acid bacteria and enterococci in traditional fermented milks of Kerala have been previously reported (Chandran *et al.*, 2022, Krishna *et al.*, 2021). The presence of yeast and molds are considered as indicators of poor hygiene and thus regarded as undesirable in fermented milks like dahi and yoghurt. Yeasts can tolerate extreme conditions like low pH, low water activity and high salt which helps them to proliferate in fermented foods and cause spoilage. But in traditional fermented foodlike kefir and kumiss the role of yeast in development of technological and sensory attributes is well studied (Gadaga *et al.*, 2000). The ability of yeast to grow in milk is mainly dependent on the assimilation of protein, fat, lactose and citrate present in milk. The effect of reduction in pH of milk during fermentation is very less for yeast when compared to lactic acid bacteria (Alvarez-Martin *et al.*, 2008).

Among yeast, *Candida* is a frequently isolated species from cocoa, cheese, meat, vegetables and spontaneously fermented milks. *Candida* is also used as co-culture along with *Saccharomyces* and *Lactobacillus* to produce fermented food products (Zohre and Erten, 2002). At the same time *Candida* genus, especially *Candida albicans* is widely known to cause infections and hence may represent a potential risk to public health (Rajkowska and Kunicka-Styczynsk, 2018). Lack of good manufacturing practices like proper pasteurization of milk and use of defined starter cultures can result in contamination of product with pathogens. The yeast communities in fermented milk products are generally linked to the production environment. Diversity in yeast strains isolated from different regions in this study can be attributed to this reason. The containers used for milk fermentation plays an important role in the presence of yeasts in fermented milks and yoghurts (Augustinet *et al.*, 2014). The acidophilic character of yeast along with low sensitivity to the antagonistic activity of lactic acid bacteria can support the growth of yeast in fermented

**Comment [SdS3]:** Only one space between words is better.

milks. Hence the study on the prevalence of *Candida* species in traditionally fermented milks are important from technological as well as safety point of view.

## 2. MATERIALS AND METHOD

### 2.1 Sample collection

Thirty samples of freshly prepared 'Thairu' were collected aseptically in sterile bottle from different households in Kerala. The samples were stored at 4°C during transportation and analysed within 12 h of sampling.

Comment [SdS4]: analyzed

### 2.2 Enumeration and isolation of yeast

The samples were serially diluted in normal saline and pour plated on to Yeast Extract Glucose Chloramphenicol Agar (YGCA)(ISO and IDF 2004). The plates were incubated at 25°C for 2-5 days and counted. Colonies with distinct colour, size and morphology were picked and stained using methylene blue. The colonies with yeast morphology were purified by streaking in the Malt Extract Agar(MEA). The purified isolates were stored at 4°C in MEA slants and in 70% glycerol stock at -20°C.

### 2.3 Determination of acidity and pH of samples

For acidity 10 g of curd was mixed with 10 ml distilled water and titrated against N/10 NaOH using phenolphthalein indicator till the attainment of pale pink colour. pH was determined using pH meter by directly inserting the probe to the homogenized sample.

### 2.4 Identification of yeast

The selected yeast isolates were identified based on the carbohydrate fermentation pattern using API20C AUX Test Kit (Biomérieux, France), according to manufacturer's instructions. The strips had 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation tests. The ability of yeast to utilize the substrate as sole carbon source was evaluated. The reactions were compared with the control cupule and identified based on the numeric profile in apiweb<sup>TM</sup> identification software.

## 3. RESULTS AND DISCUSSIONS

The yeast count in the samples ranged from 5.0 to 6.7 log CFU/g with an average of  $5.89 \pm 0.38$  log CFU/g. Average acidity and pH of the samples were  $1.92 \pm 0.34$  % LA and  $3.59 \pm 0.60$  respectively. A total of 23 yeast isolates were identified based on morphology and carbohydrate fermentation pattern using API 20C AUX kit (Biomérieux, France). The isolates belonged to *Candida kefyr* (8 isolates), *Candida spherica* (3 isolates), *Candida parapsilosis* (3 isolates), *Rhodotorulamucilogenosa* (3 isolates), *Candida rugosa* (1 isolate), *Candida colliculosa* (1 isolate), *Candida lusitanae* (1 isolate), *Candida zeylanoides* (1 isolate), *Candida krusei* (1 isolate) and *Candida famata* (1 isolate). (Table.1)

Comment [SdS5]: Only one space between words is better.

Comment [SdS6]: Check if this is correct.

Comment [SdS7]: It's better to have no space after this punctuation.

Table.1 Identification of yeast isolates by sugar fermentation pattern using API 20C AUX Kit

| Yeast isolate | Control | D-Glucose | Glycerol | Calcium 2-L-Arabinose | D-Xylose | Adonitol | Xylitol | D-Galactose | Inositol | D-Sorbitol | Methyl- $\alpha$ -D-N-Acetyl-D-Cellobiose | D-lactose | D-Maltose | D-Saccharose | D-Trehalose | D-Melezitose | D-Raffinose | Identified         |
|---------------|---------|-----------|----------|-----------------------|----------|----------|---------|-------------|----------|------------|---|-----------|-----------|--------------|-------------|--------------|-------------|--------------------|
| Y01           | -       | +         | +        | -                     | -        | -        | -       | -           | -        | -          | +   | +         | -         | +            | -           | -            | -           | <i>C. krusei</i>   |
| Y06           | -       | +         | -        | -                     | -        | -        | -       | +           | -        | +          | -   | +         | -         | +            | -           | -            | +           | <i>C. kefyr</i>    |
| Y07           | -       | +         | +        | -                     | +        | -        | +       | +           | -        | +          | -   | -         | -         | +            | +           | -            | +           | <i>nosa</i>        |
| Y08           | -       | +         | +        | -                     | -        | -        | +       | +           | -        | +          | +   | +         | +         | +            | -           | +            | -           | <i>C. spherica</i> |
| Y09           | -       | +         | +        | +                     | -        | +        | -       | -           | -        | +          | +   | -         | +         | +            | +           | -            | -           | <i>e</i>           |
| Y10           | -       | +         | +        | -                     | +        | -        | +       | +           | -        | -          | -   | +         | -         | +            | -           | -            | +           | <i>C. kefyr</i>    |

| Y22               | Y21               | Y20               | Y19               | Y17               | Y16               | Y15             | Y14            | Y13            | Y12             | Y11             |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----------------|----------------|----------------|-----------------|-----------------|
| -                 | -                 | -                 | -                 | -                 | -                 | -               | -              | -              | -               | -               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| -                 | -                 | -                 | -                 | -                 | -                 | -               | -              | -              | -               | -               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| -                 | -                 | -                 | -                 | -                 | -                 | -               | -              | -              | -               | -               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| -                 | -                 | -                 | -                 | -                 | -                 | -               | -              | -              | -               | -               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| -                 | -                 | -                 | -                 | -                 | -                 | -               | -              | -              | -               | -               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| +                 | +                 | +                 | +                 | +                 | +                 | +               | +              | +              | +               | +               |
| <i>R.muciloge</i> | <i>R.muciloge</i> | <i>C.spherica</i> | <i>C.zeylanoi</i> | <i>C.parapsil</i> | <i>C.parapsil</i> | <i>C.rigosa</i> | <i>C.kefyr</i> | <i>C.kefyr</i> | <i>C.famata</i> | <i>spherica</i> |



can assimilate lactose and metabolise citrate to ethanol, glycerol, lactic acid and propionic acid in milk (Fleet, 1990). *Candida krusei/Pichia kudriavzevii* is a potential probiotic yeast isolated from fermented dairy and non dairy food products (Alkalbaniet al., 2022). This species is reported to be acid-resistant and thermotolerant and doesnotextert any extracellular toxins and can be used in the synthesis of single cell protein (Yadav et al., 2014). The presence of *C.lusitancae* and *C.colliculora* have been previously reported in Zimbabwean fermented milk (Gadagaet al., 2000). *Candida rugosa* and *Rhodotorulamucilogenosa* are usually associated with milk fat causing spoilage in milk and milk products (Jakobsen and Narchus,1996). Both these species were also detected in this study. Many of these yeasts are capable of causing spoilage in dairy products and in plankton form non *Candida-albicans* species like *C.krusei*, *C.lusitaniae*, *C.famata*, *C.parapsilosis* and *C.tropicalis* can produce biotypes and virulence factors similar to *C.albicans* showing the risk of their delivery through fermented foods. But the contribution of yeast in enhancement of flavour and other technological properties in traditional fermented milks like kefir, kumiss and fermented tea Kombucha have been well studied (Kruk et al., 2021). Thorough animal model studies have to be carried out to prove the invasiveness of these isolates before recommending them as starter cultures (de Melo Pereiraet al., 2022).

Comment [SdS9]: Metabolize

Comment [SdS10]: These words work better combined into one word.

Comment [SdS11]: Separate the words

Comment [SdS12]: extert? What does it mean? Is this correct?

Comment [SdS13]: Check if this correct

Comment [SdS14]: Separate the words

#### 4. CONCLUSION

Traditional fermented milks have been evolved through the various cultural and culinary practices and hence the microflora in these products are unique in contributing to the artisanal sensory attributes. The microflora of fermented milk can be affected by hygiene in the production and processing conditions. Environment plays a significant role in contributing yeast and nonstarter microorganisms to these products. At the same time several technologically relevant and probiotic yeast strains co exist with lactic acid bacteria in fermented milks that can be potentially exploited as starrer cultures for industrial applications. For this molecular characterization and safety assessment yeasts isolated from fermented milks need to be studied.

Comment [SdS15]: is

Comment [SdS16]: co-exist

#### REFERENCES

1. Mutukumira AN. Properties of amasi, a natural fermented milk produced by smallholder milk producers in Zimbabwe. *Milchwissenschaft*. 1995. 50: 201-205

2. Chandran A, Beena AK, Baghya S, Rathish RL, Rahila MP. Probiotic characterisation of *Enterococcus faecalis* strain isolated from a household dahi sample of Wayanad district, Kerala. *J.Vet.Ani.Sci.* 2022. 53 (1): 70-78
3. Krishna AM, James L, Beena AK, Rajakumar SN, Mercey KA. Changes in microbiological parameters, pH and titratable acidity during the refrigerated and room temperature storage of dahi prepared from the milk of Vechur cows: an indigenous cattle breed of Kerala. *J. Dairy Res.* 202. 88(4):436-9.
4. Gadaga TH, Mutukumira AN, Narvhus JA. Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. *Int. Dairy J.* 2000 10(7):459-66.
5. Alvarez-Martin P, Florez AB, Hernández-Barranco A, Mayo B. Interaction between dairy yeasts and lactic acid bacteria strains during milk fermentation. *Food Control.* 2008 19(1):62-70.
6. Zohre DE, Erten HÜ. The influence of *Kloeckeraapiculata* and *Candida pulcherrima* yeasts on wine fermentation. *Process Biochemistry.* 2002. 38(3):319-24.
7. RajkowskaK, Kunicka-Styczynsk A. Typing and virulence factors of food-borne *Candida* spp. Isolates. *Int.J.FoodMicrobiol.* 2018 279: 57-63
8. Augustin M, Majesté PM, Hippolyte MT. Effect of manufacturing practices on the microbiological quality of fermented milk (Pendidam) of some localities of Ngaoundere (Cameroon). *Int J CurrMicrobiol App Sci.* 2014. 3(11):71-81.
9. Maoloni A, Blaiotta G, Ferrocino I, Mangia NP, Osimani A, Milanović V, Cardinali F, Cesaro C, Garofalo C, Clementi F, Pasquini M. Microbiological characterization of Gioddu, an Italian fermented milk. *Int.J.FoodMicrobiol.* 2020. 23: 71-81
10. Fleet G.H. Yeasts in dairy products—a review. *J. App. Bacteriol.* 1990. 68:199-211
11. Alkalbani NS, Osaili TM, Al-Nabulsi AA, Obaid RS, Olaimat AN, Liu SQ, Ayyash MM. In Vitro Characterization and Identification of Potential Probiotic Yeasts Isolated from Fermented Dairy and Non-Dairy Food Products. *J. Fungi.* 2022. 8(5):544.
12. Yadav JS, Bezawada J, Ajila CM, Yan S, Tyagi RD, Surampalli RY. Mixed culture of *Kluyveromycesmarxianus* and *Candida krusei* for single-cell protein production and organic load removal from whey. *Bioresource Technol.* 2014. 64:119-27.
13. Jakobsen M, Narchus J. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *Int. Dairy J.* 1996. 6: 755–768.

14. Kruk M, Trzaskowska M, Ścibisz I, Pokorski P. Application of the “SCOBY” and kombucha tea for the production of fermented milk drinks. *Microorganisms*. 2021. 9(1):123.
15. de Melo Pereira GV, Maske BL, de Carvalho Neto DP, Karp SG, De Dea Lindner J, Martin JGP, de Oliveira Hosken B, Soccol CR. What Is Candida Doing in My Food? A Review and Safety Alert on Its Use as Starter Cultures in Fermented Foods. *Microorganisms*. 2022; 10(9):1855.

UNDER PEER REVIEW