Short Research Article

Prevalence of *Candida* species in 'tThairu' a traditional fermented milk

ABSTRACT

Aims: To determinestudy the prevalence of Candida species in 'tThairu', atraditional fermented milk prepared in the households of Kerala.

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

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

Methodology: A total of 30 household 'tThairu' 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 (Biomerieux, France).

Results:The yeast count in the samples ranged from 5.0 to 6.7 log CFU/g with an average of 5.89±0.38 log CFU/g. Average acidity and pH of the samples were 1.92±0.34 % LA and 3.59±0.60 respectively. A total of 23 yeast isolates were identified based on morphologyand carbohydrate fermentation pattern using API 20C AUX kit (Biomerieux, 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 'tThairu'. Predominant *Candida* species were lactose fermenters, but the presence of a 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 are 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 concentrations, which helps them to proliferate in fermented foods and cause spoilage. But in traditional fermented foodslike 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). The l~~L~~ack 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(Augustin*et al*., 2014). Theacidophilic character of yeast along with low sensitivity to the antagonistic activity of lactic acid bacteria can support the growth of yeast in fermented

milks. Hence the study on the prevalence of *Candida* species in traditionally fermented milks are important from a 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^{o}C during transportation and analysed within 12 h of sampling.

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^{o}C for 2-5 days and counted. Colonies with distinct colour, size and morphology were pickedand 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^{o}Cin MEA slants and in 70% glycerol stock at -20^{o}C.

2.3 Determination of acidity and pH of samples

Ten grams~~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 (Biomerieux, 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^{TM} 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±0.38 log CFU/g.Average acidity and pH of the samples were1.92±0.34 % LA and 3.59±0.60 respectively. A total of 23 yeast isolates were identified based on morphologyand carbohydrate fermentation pattern using API 20C AUX kit (Biomerieux, 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 lusitaniae* (1 isolate), *Candida zeylanoides* (1 isolate), *Candida krusei* (1 isolate) and *Candida famata* (1 isolate).(Table.1)

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-α -D-	N-Acetyl-	D-Cellobiose	D-lactose	D-Maltose	D-Saccharose	D-Trehalose	D-Melezitose	D-Raffinose	Identified
Y01	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	*C. krusei*
Y06	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+	*C. kefyr*
Y07	-	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	+	-	+	*nosa*
Y08	-	+	+	-	-	-	-	+	+	-	+	-	+	+	+	+	+	-	+	-	*C.spherica*
Y09	-	+	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	-	-	*e*
Y10	-	+	+	-	-	+	-	+	+	-	-	-	-	-	+	-	+	-	-	+	*C.kefyr*

Comment [ENA1]: Consider re-doing this table. It looks quite awkward

Y22	Y21	Y20	Y19	Y17	Y16	Y15	Y14	Y13	Y12	Y11
-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	+	+	-	-	-	-	-
+	+	-	-	+	+	-	+	-	+	-
+	+	+	-	+	+	+	-	+	+	+
-	-	-	-	+	+	-	-	-	+	-
+	+	-	-	-	-	-	-	+	+	-
+	+	+	-	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	-	+	+	+
-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	+	+	-	-	+	-
+	+	+	-	-	-	-	+	-	-	-
-	-	+	-	-	-	-	+	+	+	+
+	-	+	-	+	+	+	-	-	+	+
+	+	+	-	+	+	-	+	+	+	+
+	+	+	-	-	-	-	-	-	-	-
+	-	-	-	+	+	-	-	-	+	+
+	+	-	-	-	-	-	+	-	+	+
R.muciloge	*R.muciloge*	*C spherica*	*C.zeylanoi*	*C.parapsil*	*C.parapsil*	*C.rugosa*	*C. kefyr*	*C.kefyr*	*C.famata*	*spherica*

Y23	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	-	+	*C. kefyr*
Y24	-	+	+	-	+	-	-	-	+	-	-	-	-	+	+	-	+	-	-	+	*C. kefyr*
Y27	-	+	+	+	+	+	-	-	+	-	+	-	+	-	-	+	+	-	+	-	*C.parapsil*
Y28	-	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	*C.*
Y 29	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	-	+	*C. kefyr*
Y 30	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*C. kefyr*

Among these *Candida kefyr*, *Candida spherica*, *Candida krusei* and *Candida famata*were able to ferment lactose. Majority of isolates were able to ferment galactose.

The traditional fermented milk 'Thairu' was found to have comparatively higher acidity and low pH. The presence of yeast has been reported in an Italian fermented milk 'Gioddu' a product with~~that was having~~ pH in the range of 3.8-4.2. The strong acidity of that product was attributed to the presence of yeast along with lactic acid bacteria in that product (Maoloni*et al*., 2020). The occurrence of yeast in traditional fermented milks prepared under household conditions need to be thoroughly investigated as there is high microbial risk associated with some of these florae. In this study out of the 23 isolates 20 were belonging to *Candida* species showing a prevalence of 86.95 per cent. Among these the predominant species were *Candida kefyr/Klyveromycesmarxianus* and *Candida spherica/Klyveromyces lactis.*Both of these yeasts

can assimilate lactose and metabolise citrate to ethanol, glycerol, lactic acid ad propionic acid in milk (Fleet, 1990). *Candida krusei/Pichia kudriavzevii* is a potential probiotic yeast isolated from fermented dairy and non dairy food products (Alkalbani*et al*., 2022). This species is reported to be acid-resistant and thermotolerant and doesnot extert 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 Zimbabuan fermented milk (Gadaga*et 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*showng 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 Pereira*et al*., 2022).

Comment [ENA2]: Do you mean produce?

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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~~co-exist with lactic acid bacteria in fermented milks that can be potentially exploited as starer cultures for industrial applications. For this molecular characterization and safety assessment yeasts isolated from fermented milks need to be studied.

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