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RESEARCH ARTICLE

Comparative analysis of microbial diversity in various kombucha starter cultures in Malaysia using the random amplified polymorphic DNA (RAPD) approach

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Abstract

Kombucha is sweetened black tea fermented with symbiotic culture of bacteria and yeasts (SCOBY) and has been widely consumed for its purported health benefits. The microbial consortium in kombucha is dominated by yeasts such as *Brettanomyces* sp. and *Zygosaccharomyces* sp., as well as acetic acid bacteria (AAB), *Komagataeibacter* sp. and *Acetobacter* sp. However, the source of SCOBY and substrates used may affect the microbial diversity as well as the biochemical, and flavour of the kombucha. Identifying the microbial population is important as the use of undefined starter cultures may lead to variable metabolite production and increase the risk of food pathogen contamination which can pose harm to human health. This study aims to isolate the microbes from kombucha in Malaysia for the future development of a new starter culture with specific species for the safe consumption of kombucha. Briefly, a total of 100 colonies were isolated from nine kombucha starter cultures with selective culture plates. Differences in colony morphology were observed based on their colours, surface texture, elevation, and margin. Their phenotypic morphology and genetic diversity were screened using microscopic examination, coupled with a catalase test and random amplified polymorphic DNA (RAPD), respectively. Based on the data, 51% of the isolates showed yeasts morphology under microscopic examination, while the rest were bacteria. Additionally, 55.79% of the isolates showed distinct banding profile patterns in the RAPD assessments. In conclusion, the data of this study shows that there is a diverse microbial consortium in different starter cultures of kombucha from Malaysia, mainly predominated by Gram-negative AAB and Gram-positive yeasts. Identification at the species level is to be conducted in the future. By understanding the microbiota diversity in kombucha, it contributes to the development and production of a safe functional drink.

Keywords: isolation, kombucha, microbial diversity, RAPD, starter cultures

INTRODUCTION

Kombucha's origins date back to ancient times, with roots in Asia and Eastern Europe (Alp Arici, 2021). Although its origin remains unclear, it is believed to have first appeared in China around 220 BCE, where it was called the "Tea of Immortality" (Wang *et al.*, 2022). From China, kombucha spread to other parts of Asia, such as Korea and Japan, before eventually reaching the European region and the rest of the world (Yang *et al.*, 2022). The fermentation process and the use of a symbiotic culture of bacteria and yeasts (SCOBY) were likely discovered through trial and error and were passed down through generations (Wang *et al.*, 2022). The global kombucha market has experienced significant growth in recent years. It was valued at USD 2.56 billion in 2021, and by 2025 it is expected to reach 3-5 billion USD, with a forecasted growth of up to 11.4 billion USD for another five years (Napojów & Polsce, 2024). Aside from

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increasing the value of kombucha in the global market, research conducted on kombucha also showed significant growth throughout the years (Ahmed *et al.*, 2020; Kim & Adhikari, 2020; Sogin & Worobo, 2024; Wang *et al.*, 2016; Xia *et al.*, 2019). With the rise of the commercial kombucha industry, both homebrewing methods and mass production have been developed to meet consumer demand (Vitas *et al.*, 2021). Consequently, kombucha is now widely available in various forms, including bottled and canned beverages, as well as on tap in cafes and restaurants (Atkinson *et al.*, 2023).

Kombucha is traditionally made of black tea, sugar, and SCOBY. It has a specific refreshing, sweet, and slightly sour flavour that tastes like sparkling cider (Wang *et al.,* 2022). The microbial consortium of kombucha is mainly dominated by yeasts such as *Zygosaccharomyces* spp., *Brettanomyces* spp. and acetic acid bacteria (AAB) of the genera *Komagataeibacter,* and *Acetobacter*, and although not always dominant, lactic acid bacteria (LAB) such as *Lactobacillus* also help in the fermentation process of kombucha (Tran *et al.,* 2020). During fermentation, yeasts hydrolyse sucrose to ethanol, while AAB will then synthesize acetic acid from ethanol (Nyhan *et al.*, 2022). The microbial composition of kombucha is complex, with a core microbial community essential for fermentation control and potential standardization of kombucha quality (Coton *et al.*, 2017). It can vary based on several factors such as the type of substrate used, the source of starter culture, the production environment, and the presence of probiotics in commercial products such as *Bacillus coagulans* (Yang *et al.*, 2022).

Kombucha is now consumed worldwide as an alternative to soda drinks due to its fizzy and effervescent taste, aside from being a healthy functional drink (Azfaralariff *et al.*, 2022). In the 2000s, kombucha experienced a resurgence in popularity as a health beverage, and it is now widely produced both industrially and through homebrewing methods across the globe (Diguță *et al.*, 2020). Its popularity as a lifestyle product and health beverage can be attributed to its reputed health benefits, including antioxidant and probiotic potential, as well as its antibacterial activity (Gaggìa *et al.*, 2019). Furthermore, studies have shown that kombucha confers beneficial effects, such as anti-cancer properties (Taupiqurrohman *et al.*, 2024), hepatoprotective effects (Murugesan *et al.*, 2009), cardiovascular protection, and immune system strengthening (Czarnowska-Kujawska *et al.*, 2024). The health benefits of kombucha are largely attributed to their bioactive compounds such as D-saccharic acid-1,4-lactone, tea polyphenols, glucuronic acid, gluconic acid, enzymes, and vitamins produced during the fermentation process (Jayabalan *et al.*, 2014; Mousavi *et al.*, 2020; Ojo & de Smidt, 2023). A study by Bhattacharya *et al.* (2013) reported that D-saccharic acid-1,4-lactone was able to inhibit glucuronidase, an enzyme indirectly associated with certain types of cancer. Aside from that, studies have shown that glucuronic acid demonstrated hepatoprotective effects by eliminating toxins, which supports liver health and detoxification (Murugesan *et al.*, 2009; Ojo & de Smidt, 2023). A study by Isakov *et al.* (2023) also exhibited that kombucha showed a positive effect on digestion, specifically in regulating bowel movements. On the other hand, kombucha has also been reported to contain organic acids such as acetic, lactic, malic, gluconic, tartaric, and citric acids in substantial amounts, which contribute to its low pH, which can influence intestinal motility and reduce water absorption, thus helping to prevent constipation (Isakov *et al.*, 2023; Wang *et al.*, 2020).

Despite the numerous health benefits and popularity of kombucha, undefined starter cultures may have variability in metabolite production and excessive consumption may cause potential dangers to human health. Studies have shown that the microbial consortium in kombucha is complex, varying across different batches and starter cultures (Sharifudin *et al.*, 2021; Villarreal-Soto *et al.*, 2020). This diversity in microbes can lead to differences in chemical composition, including variations in metabolite production, as well as in acid and alcohol levels (Landis *et al.*, 2022; Yang *et al.*, 2022). Risk factors regarding kombucha toxicity and its potential harm are largely due to human errors during fermentation, which can lead to poor-quality products (Ojo & de Smidt, 2023). A study conducted by Sogin & Worobo (2024) revealed that several lawsuits were filed against kombucha producers due to the content of alcohol in commercial non-alcoholic kombucha, which exceeds the legally allowable limit for non-alcoholic beverages (>0.5% alcohol by volume (ABV) in many countries). This is particularly concerning for consumers who are sensitive to alcohol, including those who are pregnant, taking certain medications, or managing underlying health conditions (Jang *et al.*, 2021).

There is limited understanding of the microbial consortium across different starter cultures of kombucha. One of the strategies used to investigate the microbial diversity of kombucha is isolating microbes from the starter cultures. The isolation of yeasts and bacteria through precise approaches is important for fundamental studies to assess the genetic diversity among starter cultures. This provides essential information on suitable species for future kombucha production, ensuring both quality maintenance and safer consumption (Tullio, 2022; Wang *et al.*, 2022). Comparing a large number of

microbial species from different sources of starter cultures using conventional phenotyping methods may yield ambiguous results and be time-consuming (Gholami *et al.*, 2021). One of the approaches to study the genetic diversity of the microbial consortium in kombucha is using random amplified polymorphic DNA (RAPD). RAPD analysis which involves the amplification of random fragments of a target microbes genome using short single primers (up to 10 nucleotides) under flexible conditions can be implemented to acquire more detailed information on the diversity of microbial species, particularly, in understanding whether they can be differentiated depending on their specific provenance (Urshev *et al.*, 2024), which is also the focus of this study. The isolation of microbes from kombucha cultures will provide insights into their biochemical composition and functional roles during fermentation. For this purpose, in this study, the species diversity of microbes from several starter cultures of local kombucha in Malaysia has been isolated and studied using microscopic examination, catalase tests, and RAPD.

MATERIALS AND METHODS

Preparation of kombucha

Nine starter cultures of kombucha were purchased online from local sellers in Malaysia. The black tea and sugar were purchased from a local seller in Malaysia. Kombucha was prepared following the method described by Zubaidah *et al.* (2018) with slight modifications. In brief, about 10 g of black tea (granulated leaves) was added to one litre of sterile water containing 10% sucrose (w/v) and boiled for 15 minutes. The brewed tea was then transferred to sterile glassware and allowed to cool to room temperature before adding 10% of the starter culture (v/v). Subsequently, the glass beaker was covered with a sterile cloth and left to ferment at room temperature for seven days under dark conditions (Coelho *et al.*, 2020).

Isolation of acetic acid bacteria and yeasts

Kombucha broth (50 mL) was centrifuged at 11,000 x*g* (Eppendorf Centrifuge 5804 R, Germany) for 20 minutes. The supernatant was discarded, and the pellets were resuspended in 1X sterile phosphate-buffered saline (pH=7.4). A serial dilution up to 10^{-7} was prepared from the solution and plated on two different selective media. The isolation was conducted according to Wang *et al.* (2022) for AAB and Gaggìa *et al.* (2019) for yeasts, with slight modifications. In brief, about 0.1 mL of the kombucha solution was plated onto glucose yeast extract calcium carbonate agar (GYC) (5% D-glucose, 1% yeast extract, 0.5% $CaCO_3$, 2% agar w/v) (Himedia, India) and consisting of 2% cycloheximide (Sigma-Aldrich, St. Louis, Missouri, USA) to inhibit the growth of yeasts. The AAB was cultivated at 28 °C for five to seven days under aerobic conditions. Colonies with different morphology based on their colours, surface texture, elevation, and margin shape were then isolated and sub-cultured using the same media for another 48 hours to obtain pure colonies. Isolates displaying halo zones around their colonies on GYC agar can be hypothesized as purified AAB (Sharifudin *et al.*, 2021). On the other hand, Sabouraud Dextrose Agar (SDA) (Himedia, India) was used for the isolation of yeasts. The yeasts were incubated at 30 °C for five to seven days under aerobic conditions. Then, colonies of interest were purified by successive streaking on the same media for another 48 hours. Both AAB and yeasts isolated cultures were cultivated on the GYC and SDA media containing 50% glycerol (w/w), respectively, and were kept at -80 °C.

Phenotypic characteristics of AAB and yeasts

Phenotypic characterization of AAB and yeasts from kombucha was assessed using Gram staining and the catalase test (Abdel-Salam *et al.*, 2012; Claus, 1992; Reiner, 2013; Wang *et al.*, 2022). A loopful of pure AAB and yeasts cultures was used for the microscopic examination and catalase testing. Gram stains of the isolates were prepared, and their cell morphology was examined under 100X magnification using a fluorescent microscope with oil immersion (Olympus BX53F, Tokyo, Japan). Catalase activity was confirmed by the rapid formation of air bubbles on young, purified colonies after adding two drops of 3% H_2O_2 (v/v) (Sigma-Aldrich, St. Louis, Missouri, USA).

Random amplified polymorphic DNA (RAPD)

A loopful of a single colony was resuspended in 30 µL of sterile distilled water before being heated at 90 °C for 10 minutes. The solution was then centrifuged at maximum speed to obtain the supernatant. RAPD was performed through amplification with primers rapd-4 (5'-AAGAGCCCGT-3') (Urshev *et al.*, 2024) or RP (5'-CAGCACCCAC-3') (Abbès *et al.*, 2013) separately, with slight modification. A total volume of 10 µL of the PCR mixture was prepared using 1X *GoTaq* PCR buffer (Promega, Madison, USA), 3.0 mM of $MgCl_2$, 0.4 mM of dNTPs, 1.25 units of *GoTaq* polymerase (Promega, Madison, USA), 1 µM of primers, and 2 µL of supernatant as the template. The thermocycling program was set at 94 °C for 4 minutes; 40 cycles of 94 °C for 30 seconds, 38 °C for 45 seconds, and 72 °C for 1 minute, and a final

elongation at 72°C for 2 minutes using an Eppendorf C1000 Thermal Cycler (Eppendorf, Germany). The RAPD products were separated in 1.5% (w/v) of agarose gel stained with ethidium bromide (0.5 µg/mL), with a 1kb ladder (Promega, USA) as the marker. Gel visualization was performed using the Alpha Image gel documentation system (Alpha Innotech, USA). Gel photographs were then analysed manually to observe the dissimilarity and similarity between band patterns. Clustering was performed to distinguish between overlapping strains.

RESULTS

Isolation of yeasts and acetic acid bacteria

A total of 100 isolates were obtained from nine starter cultures of kombucha. All isolates were recovered from 10^{-1} to 10^{-5} of dilutions of kombucha, but none were recovered after 10^{-6} dilution. The colonies were randomly selected based on their differences in colour, surface texture, elevation, and margin shape from each sample for pure microbial cultures, as shown in Figure 1. Table 1 shows the number of isolates selected from each sample.

The isolated colonies showed various morphologies with distinct characteristics. Figure 2 shows the distinct characteristics of AAB on GYC agar with the presence of halo zones around their colonies. On the other hand, strongly identifiable features between microbes can be observed among the pure colonies as depicted in Figure 3. Based on the observation, they are distinct in colours (beige, white, creamy, yellow, and brownish pink) while some colonies are rough and opaque, the others have a sticky and translucent texture with a smooth and shiny surface. A detailed list of the morphological descriptions of the isolates is presented in Supplementary Table 1.

Cellular morphology and catalase activity

Microscope observations of the cellular morphology of the isolates grown on both GYC agar and SDA agar showed that the isolates represented 32% Gram-negative-short rod bacteria and 51% Gram-positive-yeasts, respectively, and only 7% of the isolates were categorized as Gram positive-rod shaped, 5% as Gram negative-round shaped, and 5% of the isolates showed Gram positive-round shaped features. Differences in the cellular morphology of a few isolates are shown in Figure 4, while a complete list of the cellular morphology of the isolates is presented in Supplementary Table 1. All isolates showed positive catalase activity except for isolates 29-D, 30-D, 33-H, 35-I, 36-I, 41-F, 42-A, 45-F, 65-G, 65-E, 70-I, 63-I, and 77-A (Supplementary Table 1).

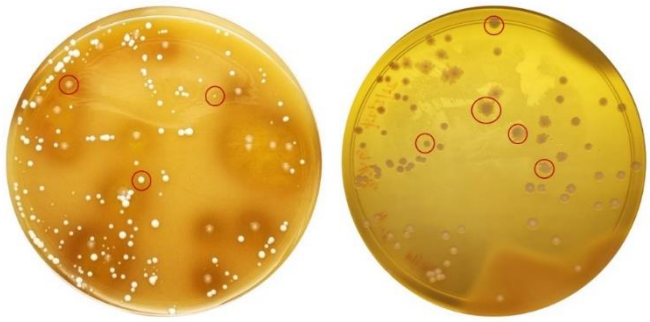

Figure 1. Random selection of colonies with different features based on their colours, surface texture, elevation, and margin shape on agar spread plate

Table 1. Random selection of isolates from each starter cultures of kombucha

Sample	Isolates no.
A	7, 22, 39, 40, 42, 48, 49, 74, 75, 76, 77, 82, 83, 84, 85
B	9, 10, 19, 20, 23, 24, 37, 92, 93
C	1, 2, 3, 4, 5, 6, 11, 12, 13, 14, 25, 26, 47, 50, 51, 52, 86, 87, 88, 89
D	27, 28, 29, 30, 99
E	15, 16, 17, 18, 31, 32, 43, 44, 66, 67, 68, 69, 80, 81, 94, 95
F	8, 41, 45, 46
G	55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 96, 97
H	33, 34, 38, 53, 54, 90, 91
I	21, 35, 36, 70, 71, 72, 73, 78, 79, 98, 100

*Abbreviation of isolation numbering will be used based on this table for this paper, as example: 7-A, 1-C, and 55-G

Figure 2. Spread plate of culture on GYC agar. Arrow indicates the halo clear zone surrounding the colonies

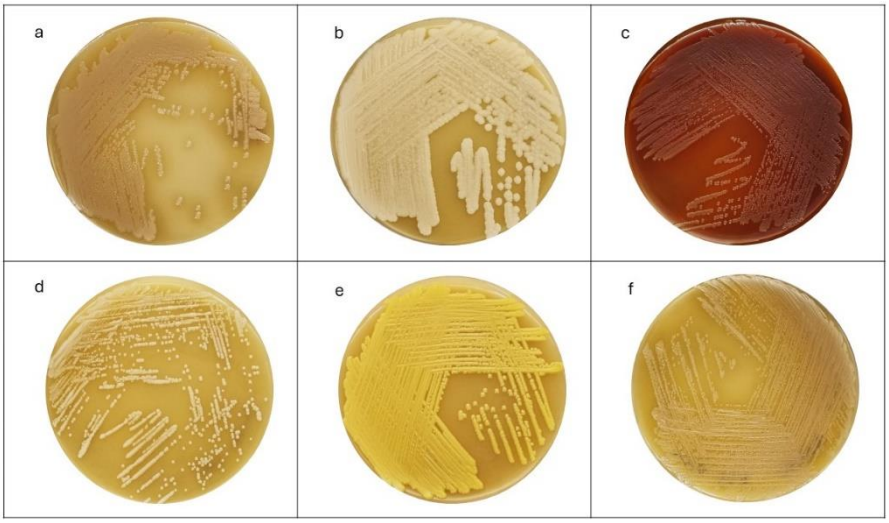

Figure 3. A representative picture showing pure culture of isolates. a) 64-G, b) 2-C, c) 65-G, d) 82-A, e) 39-A, f) 49-A with distinct colony morphology from each other

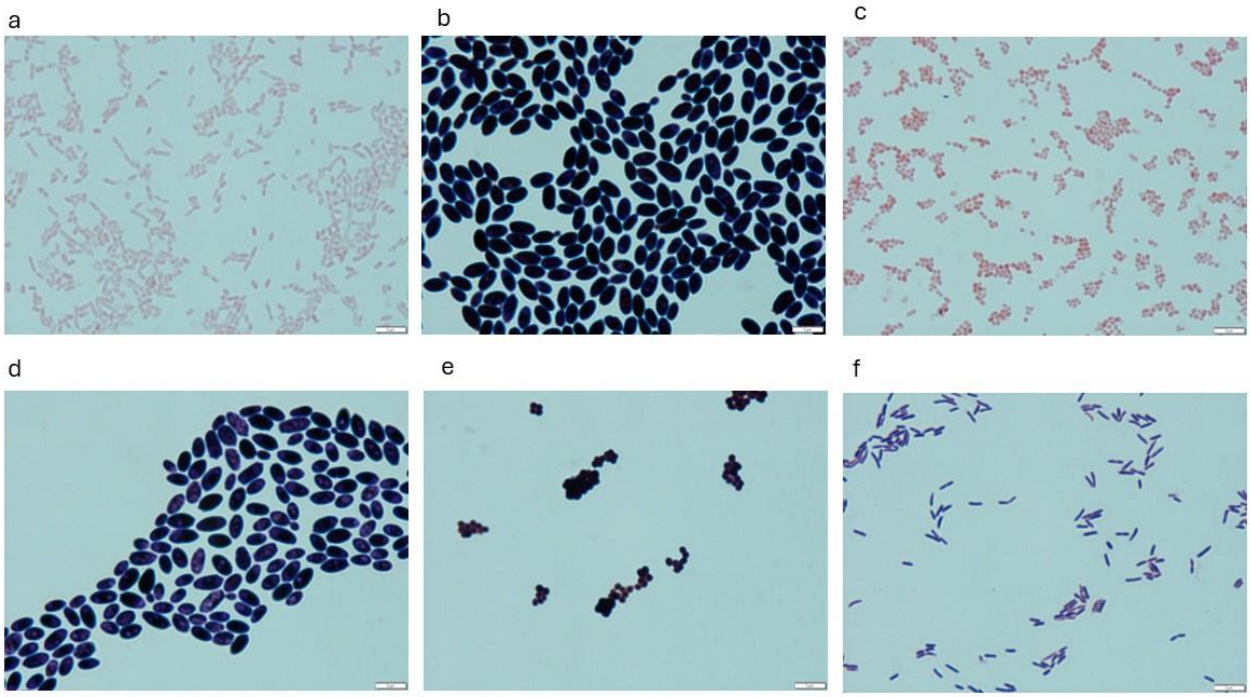

Figure 4. Cellular morphology of the isolates from kombucha SCOBY. a) 64-G: Gram-negative-short rod, b) 2-C: Gram-positive-ellipsoidal shape and monopolar budding, c) 63-G: Gram-negative-cocci, d) 82-A: Gram-positive-ellipsoidal shape and bipolar budding, e) 39-A: Gram-positive-cocci in cluster, f) 31-E: Gram-positive-rod. Gram staining, 100X magnification. Bar = 5µm

Genotypic profile differentiation by RAPD

Genotypic profile differentiation was performed by RAPD for 95 isolates with distinct colony morphologies. Two isolates that showed mixed cultures and three isolates with similar characteristics based on morphological assessment (Supplementary Table 1) were omitted from RAPD. PCR amplification with rapd-4 and RP primers revealed clear discriminatory DNA banding patterns (Figure 5). A total of 53 different banding patterns were observed, with both primers producing similar results. Based on the RAPD data and morphological assessment, 49.1% of the observed isolates are yeasts while the rest are bacteria. Only 10 isolates (4-C,6-C, 17-E, 18-E, 21-I, 25-C, 26-C, 27-D, 86-C, 88-C) produced no detectable DNA bands. Based on the similarity of band patterns, 49 out of 95 isolates were clustered into 17 groups (Table 2), while 36 isolates with distinct bands were not classified into any groups (Table 3).

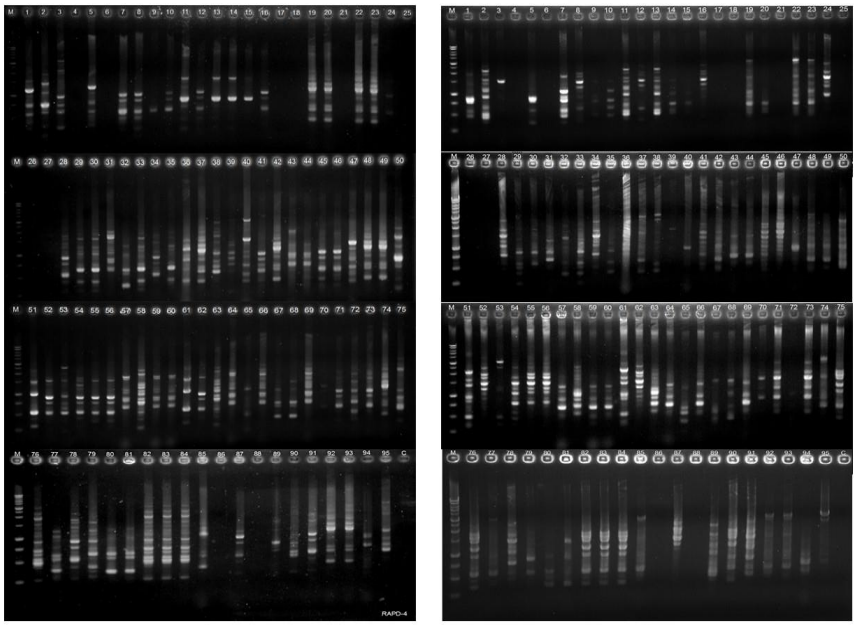

Figure 5. RAPD profiles of 95 isolates obtained with primer RAPD-4 (A) and RP (B)

Table 2. Clustering of 49 out of 95 isolates with similar band patterns.

Group No.	*Isolates*	*Group No.*	*Isolates*
1	7-A, 8-F	*10*	52-C, 55-G, 56-G, 62-G
2	11-C, 13-C, 14-C	*11*	57-G, 59-G, 60-G
3	12-E, 16-E	*12*	58-G, 63-G
4	19-B, 20-B, 22-A, 23-B, 37-B, 42-A, 48-A, 49-A, 74-A	*13*	67-E, 68-E
5	28-D, 34-H	*14*	64-G, 66-E, 69-E
6	29-D, 30-D, 33-H	*15*	80-E, 81-E
7	43-E, 44-E	*16*	82-A, 83-A, 84-A
8	45-F, 46-F, 71-I	*17*	92-B, 93-B
9	51-C, 61-G		

Table 3. List of isolates with distinct band patterns

Isolates
1-C, 2-C, 3-C, 5-C, 9-C, 10-B, 15-E, 24-B, 31-E, 32-E, 35-I, 36-I, 38-H, 39-A, 40-A, 41-F, 47-C, 50-C, 53-H, 54-H, 65-G, 70-I, 72-I, 73-I, 75-A, 76-A, 77-A, 78-I, 79-I, 85-A, 87-C, 89-C, 90-H, 91-H, 94-E, 95-E

DISCUSSION

In this study, a total of 53 isolates with distinct morphologies (Tables 2 and 3) were successfully isolated and differentiated using both phenotypic morphology assessments and the molecular approach, RAPD. According to Gholami *et al.* (2021), conventional techniques alone are often insufficient for accurately distinguishing between closely related microbial strains, so RAPD was used to effectively analyse genetic diversity and differentiate between strains using a short single primer. This method is advantageous because it does not require any prior knowledge of DNA sequence information, is cost-effective, and provides quick results. Notably, the use of RAPD in comparing the microbial strains in kombucha, particularly in Malaysia, represents a novel approach, offering fresh insights into the genetic diversity of local kombucha microbiota.

As AAB and yeasts are the pre-dominant microbes in kombucha, GYC and SDA media were used to isolate the microbes from the starter cultures. GYC and SDA are the common and ideal media to be used as they can effectively support the growth of most types of AAB and yeasts (Gullo *et al.*, 2006; Sengun & Karabiyikli, 2011; Sharafi *et al.*, 2010; Wang *et al.*, 2022). AAB are crucial in the fermentation process due to their ability to oxidize sugars and alcohols to organic acids as the byproducts of the fermentation (Zhang *et al.*, 2023). According to Yuan *et al.* (2013) and Sharifudin *et al.* (2021), halo formation on the GYC medium can be observed where this condition is caused by the production of acetic acid from the bacteria which lowers the pH, causing calcium carbonate to dissolve and form a clear zone around the colonies as shown in Figure 2.

The morphology of bacterial and yeast colonies was observed visually and based on the observations, the isolates show a variety of colony characteristics, including differences in colour, surface texture, form, and elevation, which is similar to *Acetobacter*, *Glucoacetobacter, Komagataeibacter, Gluconobacter* and yeasts. Wang *et al.* (2022) and Mamlouk and Gullo (2013) described *Acetobacter* colonies as having a circular shape and a colour of cream to beige on GYC agar, with some isolates showing brown tones and producing a brownish, water-soluble pigment which is similar to *Glucoacetobacter,* these characteristics are similar to several isolates in this study including 49-A and 59-G, respectively. On the other hand, *Komagataeibacter* species are reported to have a circular shape with a smooth or rough surface and raised or umbonate elevation (Wang *et al.*, 2022), which is similar to the morphology of isolates 44-E and 64-G. Meanwhile, *Gluconobacter* has a smooth, glistening entire margin, and appears pink (Wang *et al.*, 2022; Yamada & Yukphan, 2008), these morphologies can be observed in isolates 1-C and 5-C. Overall, this indicates that the microbial consortium in kombucha of Malaysia is diverse, evidenced by the presence of *Acetobacter*, *Glucoacetobacter, Komagataeibacter,* and *Gluconobacter* in the starter cultures.

In contrast, yeasts morphology typically varies, with colonies often appearing white to creamy, with a glossy and shiny, or dull and smooth surface (Wang *et al.*, 2022). The form of yeast colonies also differs, with margins that can be entire, lobate, or erose, and elevations that range from raised to convex and umbonate (Wang *et al.*, 2022). In general, the yeast morphologies formed in this study are creamy to white in colour with some colonies having a shiny surface and entire margin, such as 18-E and 82-A, while some colonies showed a dull and dry surface with umbonate in the centre, such as 55-G and 50-C which is similar to those previously reported by Wang *et al.* (2022). According to Coton *et al.* (2017), yeasts community dynamics are complex, with the dominant species shifting over time during the fermentation process. Initially, osmotolerant species like *Zygosaccharomyces, Torulaspora delbrueckii,* and *Schizosaccharomyces pombe* dominate, hydrolysing sugars into ethanol and lowering the pH value. As the environment becomes more acidic, acid-tolerant yeasts such as *Brettanomyces* and *Candida stellata* then take over, continuing the fermentation and contributing to the flavour of kombucha. Understanding this complexity makes it valuable to identify the yeast species involved.

Besides their unique colony characteristics, the cellular morphology of the microbes was also examined using Gram-staining and catalase activity tests. The AAB, which are Gram-negative bacteria, have ellipsoidal to rod-shaped cells that can appear singly, in pairs, or in chains, and they do not form spores (Mamlouk & Gullo, 2013). Their cell dimensions range from 0.4 to 1 μm in width and 0.8 to 4.5 μm in length and are catalase positive (Wang *et al.*, 2022). Meanwhile, the cell morphology of yeast is typically Gram-positive with an oval or irregular shape under the microscope (Kurtzman *et al.*, 2011). They reproduce primarily through budding, which can be monopolar, bipolar, or multipolar, and the cell dimensions range from 2 to 10 μm in diameter or length (Kurtzman *et al.*, 2011). Our data showed that, out of 100 isolated colonies, 51 colonies are Gram-positive while the rest are Gram-negative. All these observed details of the isolated microbes (Supplementary Table 1) are consistent with the characteristics of microbes mentioned above. The almost similar ratio of Gram-positive and Gram-negative microbes in the starter cultures further supports the diverse microbial consortium of

kombucha in Malaysia, providing insights into isolating specific microbes for the development of safer functional drinks in the future.

Genotype analysis of a total of 95 isolates by RAPD showed patterns consisting of numerous bands with distinct intensity and size. Both rapd-4 and RP primers revealed genetic differences among the microbial isolates of kombucha starter culture, as shown in Figure 5. RP and rapd-4 primers were chosen for this study as these primers have been commonly used for RAPD fingerprinting of bacteria in fermented foods (Berthold-Pluta *et al.*, 2017; Maleki Kakelar *et al.*, 2019; Putri *et al.*, 2011; Sieladie *et al.,* 2011; Urshev *et al.*, 2024). Our data revealed that, after clustering the microbes into the same band patterns, 53 out of 95 (55.79%) are distinct from each other with a nearly equal ratio of yeasts to AAB (26 yeast: 27 AAB). For safer functional drink development, the use of specific species of yeasts or AAB is undoubtedly essential. Therefore, the identification of these yeasts and AAB down to species level using sequencing in the next phase of the study is proposed.

CONCLUSION

A total of 53 isolates, each with distinct profiles in both morphological assessments and RAPD analysis, were obtained from nine kombucha starter cultures from Malaysia, indicating a diverse microbial consortium. Identifying these isolates is crucial for understanding the microbial diversity within kombucha. This study observed two predominant types of microbes in the kombucha: the Gram-negative AAB and Gram-positive yeasts. Additionally, a few isolates exhibited different morphological characteristics from these two typical kombucha microbes, which are worth further examination. This study's findings will enhance our knowledge of the microbial community in kombucha, providing valuable information for identifying unknown species. This, in turn, could contribute to the future development of kombucha by incorporating suitable species, thereby ensuring safer consumption.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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