ISOLATION AND SELECTION OF HIGH ACTIVITY ACETIC ACID BACTERIA FROM SHREDDED GREEN TEA KOMBUCHA FERMENTED BY SOME COMMERCIAL SCOBY

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ABSTRACT

Vietnam stands as the seventh-largest tea producer globally, offering a wide variety of commercial tea products. During the production of dry tea, approximately 10% of the annual yield consists of shredded tea, which has low economic value and is typically discarded. This type of tea is an abundant resource for producing fermented beverages such as Kombucha, which is produced by fermentation of sugared tea using a tea fungus. This is a symbiotic colony of bacteria and yeast (SCOBY) that converts sucrose into acetic acid. Among the microorganisms participating in this process, acetic acid bacteria take precedence, playing a pivotal role in organic acid production. In this study, six distinct commercial SCOBYs were cultivated in sugared tea broth, and the resulting fermented broth was utilized to isolate acetic acid bacteria. Ten strains were isolated and subjected to an analysis of their colony morphology and biochemical properties. These bacteria were preliminarily categorized into three genera: *Acetobacter*, *Gluconobacter*, and *Komagataeibacter*. Through screening for fermentation capability on YPGD agar medium supplemented with 5 g/L $CaCO_3$ and 40 mL/L ethanol, *Acetobacter* sp. LDK-A2 displayed the highest acidogenic ability, with a halo zone diameter ratio of 2.1, which was significantly different from other strains. Phylogenetic tree analysis using the 16S rRNA gene sequence identified that strain LDK-A2 belongs to *Acetobacter tropicalis* species.

Keywords: *Kombucha, acetic acid bacteria*, *Acetobacter stropicalis, shredded tea, tea fungus.*

1. INTRODUCTION

Shredded green tea is often removed during the processing of dry tea. During fermentation process, some broken tea leaves and buds fall to the bottom and are removed from commercial tea by mechanical sieving (accounting for 6 - 10% of the finished product volume). By comparison, this type of tea is only inferior in appearance but its nutritional value and chemical composition are equal to commercial tea (Diep, L.T.B. 2010). The chemical composition of shredded green tea generally contains polyphenols, caffeine, amino acids, minerals, flavonoids, and volatile organic compounds. Shredded green tea has high polyphenol content, with catechins being the most abundant. Epigallocatechin gallate is a particularly notable catechin with strong antioxidant properties and contributes to its numerous health benefits (Nasehi, M. *et al*. 2017). However, the prices of this tea have been reduced from 30% to 90 % compared with commercial products and have been used for animal feed, desiccant, tea bag and others (Loan, N.T.P. 2016). In Vietnam, tea by-products are always available and have large quantities in the tea-growing provinces in the Northern mountainous region. According to a report from the General Statistics Office, the output of shredded tea in 2018 was 9.6 thousand tons in the above areas (An, L.T. *et al.* 2020), which showed as an abundant material resource for use. Using cheap materials to create new products with high nutritional and economic value is a research trend in the world and Vietnam.

Kombucha is a fermented tea beverage that has been used for a long time in countries around the world. Kombucha is consumed as a daily popular beverage because it provides many health benefits such as antioxidants, digestive support, immune system stimulation, antibacteria, and others (Dufresne, C. and Farnworth, E. 2000). Kombucha is the product of the fermentation of sugared tea broth at room temperature for 5 to 10 days by microorganisms. The substrate commonly used is green or black tea due to its high content of specific chemical components such as phenolic compounds and and hydrolyzable tannins. In addition to the inherent astringency of tea, Kombucha exhibits a gentle vinegary fragrance, a sweet and sour flavor profile, and a subtle spiciness derived from alcohol, reminiscent of sparkling apple cider. The diverse chemical composition of Kombucha, encompassing organic acids, ethanol,

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vitamins, and polyphenols, results from microbial metabolism during the fermentation process. Consequently, these characteristics are contingent upon the specific original strain used. (Jayabalan, R. *et al.* 2014).

In Kombucha fermentation, the microflora, known as tea fungus (or SCOBY), includes acetic acid bacteria and yeast, which collaborate to convert sugar into acetic acid. The role of yeast is to break down sucrose into glucose and fructose for utilizing and generating ethanol and CO_2. Together with that, bacteria have the role of converting part of ethanol into acetic acid. In addition to creating the product's taste, acetic acid reduces the pH for controlling contamination and extending storage time (Tran, T. *et al.* 2020). The amount of acid depends on the dominant bacterial strain in the microflora. The group of acetic acid bacteria commonly belonged to four main genera *Acetobacter, Gluconobacter*, *Gluconoacetobacter*, and *Komagaeibacter* (Jayabalan, R. *et al.* 2014). Some studies showed that the two dominant genus frequently present in Kombucha fermentation were *Acetobacter* and *Gluconobacter* (Chakravorty, S. *et al.* 2016, Wang, B. *et al.* 2022). Liu, C.-H. *et al.* (1996) found four bacterial species in Kombucha, which included *Acetobacter xylium*, *Acetobacter pasteurianus*, *Acetobacter aceti*, and *Gluconobacter oxydans*. *Acetobacter intermedius* isolated from tea fermentation by Boesch, C. *et al.* (1998), was a new species belonging to the *Acetobacter* genus. However, the presence of acetic acid bacteria depends on the original strain, geographical location, climatic conditions, and the diversity of local microorganisms. In another study, Marsh, A.J. *et al.* (2014) reported that the dominant bacteria from five Kombucha samples from Canada, Ireland, the USA, and the United Kingdom was *Gluconobacter*. In Vietnam, Kombucha fermentation is commonly carried out on a small, household artisanal scale using SCOBY of unknown origin available in the market. Hence, microorganisms are often not identified, resulting in low fermentation efficiency and possible contamination (Phuong, N.T. and Huong, N.T. 2018). Moreover, the number of research on selecting acetic acid bacteria for Kombucha fermentation is still limited. This study aims to isolate and select acetic acid bacteria with high acid production capability for Kombucha fermentation from shredded green tea.

2. MATERIALS AND METHODS

2.1. Preparation of sample and isolation of acid acetic bacteria

Six distinct SCOBY products, namely Jun's SCOBY, Bao Nguyen's SCOBY, Tuyet Mai's SCOBY, Nana's SCOBY, Dat Viet's SCOBY, and Kefir's SCOBY, were purchased from Kombucha shops at local markets in Hanoi, with three samples per product (n = 3 samples per product). The samples were stored at 4°C and maintained until used for fermentation. The samples were cut into small pieces (10 g) and inoculated into 500 mL of sugared tea medium in a 1 L glass bottle. The medium was prepared using (10 g/L) shredded tea steeped in hot water (85°C) for 20 minutes, followed by the addition of 100 g/L of sucrose after removing tea residues. The bottle was incubated at 30 ^{o}C for 7 days in an incubator. After incubation, the fermentation broth was dillution and spread in Petri dishes containing YPGD agar medium (yeast extract 5 g/L, peptone 10 g/L, glycerol 5 g/L, D-glucose 5 g/L and agar 20 g/L). Bacteria were isolated base on colony morphology then stock solution were prepared in 30% glycerin and kept at -80^{o}C for longtime storage use.

2.2. Gram stain

The experiment was conducted following the method described by Smith (2005).

2.3. Biochemical properties of bacteria

Biochemical tests were conducted on isolated bacteria. Oxidation of ethanol to acetic acid by isolated bacteria was tested in a basal medium (10 g/L yeast extract and 0.04% bromophenol blue) (Dung, N.L. *et al.* 2002). Catalase activity was confirmed by the evolution of air bubbles on purified colonies after placing a drop of 3% H_2O_2 (v/v, Sigma-Aldrich, USA) (Reiner, K. 2010). A cellulose test was performed by reaction of Lugol's iodine stain with pellicles from liquid cultures (Romero-Cortes, T. *et al.* 2012). The oxidase test was carried out using 1% solution of tetramethyl-p-phenylene diamine dihydrochloride for a colour change for 10s as described by Apagu, B. *et al.* (2017). Growth at different temperatures (25^{o}C, 30^{o}C, and 37^{o}C) was conducted on YPGD agar medium incubated aerobically for 3 – 7 days (Kanchanarach, W. *et al.* 2010) . Growth in 0.35% acetic acid and 30 % D-glucose medium (w/v) were carried out according to the method described by Wang, B. *et al.* (2022). Growth in methanol medium was conducted following the method of Hanmoungjai, W. *et al.* (2007). The alcohol tolerance test was carried out using a basal medium consisting of 5 g/L yeast extract, 20 g/L agar, and ethanol at concentrations of 2%, 4%, 6%, and 10% (v/v) respectively. The acetic acid produced from various sources was determined

by observing the halo zone in a basal medium (5 g/L yeast extract, 10 g/L peptone, 5 g/L $CaCO_3$, and 20 g/L agar) with a tested carbohydrate source (glucose, sucrose, glycerol, fructose, lactose, maltose and trehalose) added at a concentration of 10 g/L (Wang, B. *et al.* 2022).

2.4. The ability of acid acetic production

1 µl of the bacterial suspensions was pointed on the surface of the YPGD agar medium that added 5 g/L $CaCO_3$ and 40 ml/L ethanol. The dish was incubated at 30°C for 72 h in an incubator then the halo-degradation ring was determined for comparison. The strain showed the highest halo zone that will be selected (Quang, P.H. *et al.* 2014).

2.5. Molecular characterization

Total DNA of bacteria was extracted using the Bacterial genomic DNA purification kit (ELPIS-Biotech, Daejeon, South Korea). 16S rRNA gene was amplified by PCR using specific primer pair sets and sequenced (27F–AGAGTTTGATCMTGGCTCAG and 1492-RTACGGYTACCTTGTTACGACTT). Cycles consisted of initial denaturation at 95 °C for 1 min, followed by 40 cycles (95 °C for 30s, 60 °C for 30s, 72 °C for 40s), and a final extension at 72 °C for 10 min. The obtained gene sequence was compared with the database from GenBank using BioEdit version 5.0.9.1. The phylogenetic tree was contructed by the neighbor-joining method in MEGA version X with 1000 bootstrap replicates.

2.6. Statistical analysis

The halo zone diameter was analyzed by one-way analysis of variance (ANOVA) using SPSS software version 26.0 (SPSS Inc. Chicago, IL, United States) with Duncan multiple range test *post hoc* analysis to determine significant differences of the mean.

3. RESULT AND DISCUSSION

3.1. Morphological characteristics of isolated bacteria

A total of 18 bacterial strains were isolated from the tea fermentation solution, with 10 of them identified as acetic acid bacteria. They were classified based on detecting a yellow color on the culture medium added with bromophenol blue (0,04%). All isolated bacteria are rod-shaped and gram-negative and have circular colonies but differences in surface morphology and color of the colonies. Most bacterial colonies are white while others are yellow, beige, or brown color. Together with that, some outstanding characteristics of the colony surface morphology such as smooth, convex, rough, shiny, or umbonate in the center were also summarized in Table 1. Many previous studies also reported the prevalence of acetic acid bacteria in traditional fermented tea (Chakravorty, S. *et al.* 2016, Gomes, R.J. *et al.* 2018).

3.2. Biochemical characteristics

According to the results, ten strains were catalase-positive and oxidase-negative and were able to grow at temperatures ranging from 25 to 37 °C. All strains were able to grow on culture medium supplemented with ethanol at a concentration of 2 to 8%. However, most isolates were not grown at a concentration of 10% ethanol except LDK-A2 and LDK-A10, indicating that the maximum tolerance ethanol for them was from 8 to 10%.

Table 1. Morphology of bacterial clononies isolated grown on YPGD agar medium.

SCOBY source	Strain	Colony morphology	Gram
Jun	LDK-A1	Circular, not high, white creamy color	-
	LDK-A2	Circular, convex, glossy surface, off-white color	-
Bao Nguyen	LDK-A3	Circular, shiny, light-yellow color	-
	LDK-A4	Circular, umbonate center, glossy surface, light-brown color	-
Tuyet Mai	LDK-A5	Circular, shiny, light-begie color	-
Nana	LDK-A6	Circular, smooth surface, light-brown color	-
	LDK-A7	Circular, rough surface, creamy color	-
Dat Viet	LDK-A8	Circular, umbonate center, creamy color	-
Kefir	LDK-A9	Circular, smooth surface, transparent white	-
	LDK-A10	Circular, shiny, creamy color	-

Note: -: negative

All strains were able to oxidize ethanol to acetic acid and showed growth on 0.35% acetic acid and YPGD medium. Most colonies did not produce pigment and were not able to grow on a 30% D-glucose medium. Additionally, some strains were able to grow on a methanolic agar

medium, and two showed capable of producing cellulose. Ten isolates were preliminarily classified into three groups of acetic acid bacteria based on their morphology and biochemical characteristics (Table 2). Bacteria belonging to group I were not able to use any carbohydrate as a substrate for production of acetic acid that was presumed to belong to the genus *Acetobacter* (Yamada, Y. and Yukphan, P. 2008, Wang, B. *et al.* 2022). Next, group II involved bacteria that were able to use some carbohydrates and did not grow on a culture medium containing methanol, which may belong to the genus *Gluconobacter* (Dwivedi, M. 2020, Wang, B. *et al.* 2022). Finally, bacteria were able to use diverse carbon sources as substrates and were capable of producing cellulose, which may belong to the *Komagataeibacter* genus and classified into group III (Volova, T.G. *et al.* 2018).

Table 2. The biological characteristic of the isolated strains

Characteristic	Group I *Acetobacter*					Group II *Gluconobacter*			Group III *Komagataeibacter*	
						LDK-				
	A1	A2	A3	A5	A10	A4	A6	A9	A7	A8
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
Growth at different temperature										
25 °C	+	+	+	+	+	+	+	+	+	+
30 °C	+	+	+	+	+	+	+	+	+	+
37 °C	+	+	+	+	+	+	+	+	+	+
Ethanol tolerance (v/v)										
4%	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+
8%	+	+	+	+	+	+	+	+	+	+
10%	-	+	-	-	W	-	-	-	-	-
Oxidation of ethanol to acetic acid	W	+	+	+	+	+	+	+	+	+
Pigment production	-	-	-	-	-	+	+	-	-	-
Cellulose production	-	+	-	-	-	-	-	-	+	+
Growth on										
Methanol medium	-	+	+	W	+	-	-	-	W	-
0,35% (w/v) acid acetic medium	+	+	+	+	+	+	+	+	+	+
30 % (w/v) D-glucose medium	-	-	-	-	-	-	-	-	-	-
YPGD medium	+	+	+	+	+	+	+	+	+	+
Production of acid from										
D-glucose	-	-	-	-	-	+	W	W	+	+
Glycerol	-	-	-	-	-	W	+	+	-	-
D-Fructose	-	-	-	-	-	+	W	W	+	W
D-Lactose	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	+	W	+	+	+
D-Maltose	-	-	-	-	-	-	-	-	+	+
D-Trehalose	-	-	-	-	-	-	-	-	-	-

*Note: * +: positive; -: negative; W: weak positive; each experiment was conducted three times.*

3.3. Acetic acid production

A high level of acetic acid in tea fermentation raises acidity, which is important to inhibit harmful microorganisms during fermentation process and

storage and maintain beverage taste (Gomes, R.J. *et al.* 2018). The ability to produce acetic acid of isolates was evaluated basing on ratio of halozone per bacterial colony diameter on YPGD agar medium supplemented with 4% ethanol. All strains exhibited a halo zone, with diameter ratios ranging from 1.1 to 2.1, on YPGD agar medium after 72 hours of incubation. Among them, LDK-A2 strain showed the highest acetic acid production with a ratio of 2.1, followed by LDK-A10 strain, which significantly differed from other strains (Figure 2). Both strains belonged to the *Acetobacter* genus following the above classification (Table 2). In the group of acetic bacteria, the genus *Acetobacter* is usually the most commonly, dominant, and capable of producing the highest amount of acetic acid (Jayabalan, R. *et al.* 2014). The LDK-A2 strain exhibited a greater capacity for acetic acid production compared to the bacterial strain isolated by Quang, P.H. *et al.* (2014). Their study reported that the majority of the thirty-three isolated bacteria from fermented tea were classified within the *Acetobacter* genus. Among them, *Acetobacter aceti* exhibited the highest acetic acid production, with a halo-zone ratio of 1.7. In another study, *Acetobacter tropicalis* strain ITV61 exhibited the highest capable acetic acid production among six acetic acid bacteria isolated from fermented cocoa (Romero-Cortes, T. *et al.* 2012). The production of acetic acid by these bacteria helps regulate the pH of the Kombucha. This acidic environment is essential for inhibiting the growth of harmful microorganisms while promoting the growth of beneficial bacteria and yeast. The acidic nature of acetic acid not only enhances flavor but also acts as a natural preservative, helping to prevent spoilage and extending the shelf life of Kombucha (Gomes, R.J. *et al.* 2018). According to the results, strain LDK-A2 was the most potential candidate for fermentation beverage that was selected for further studies.

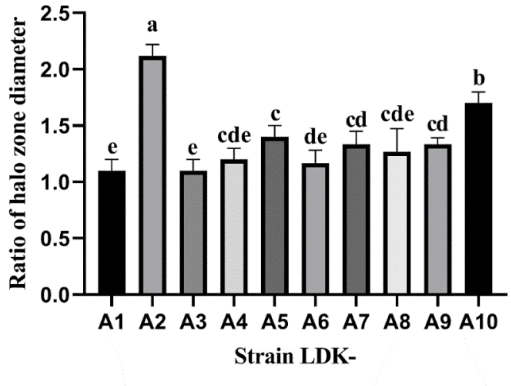

Figure 2. The ratio of halo zone diameter: colony diameter of selected strains at 72 hours of incubation.

Note: Values are presented as the mean ± SD (n=3), with three replicates each. Bars with the same letters are not significantly different between groups (p<0.05, Duncan's test).

3.4. Identification of acetic acid bacteria by 16S rRNA sequences

The partial 16S rRNA (1349 bp) gene sequence of LDK-A2 strain was deposited in the GenBank with the following accession number **OQ788359.**

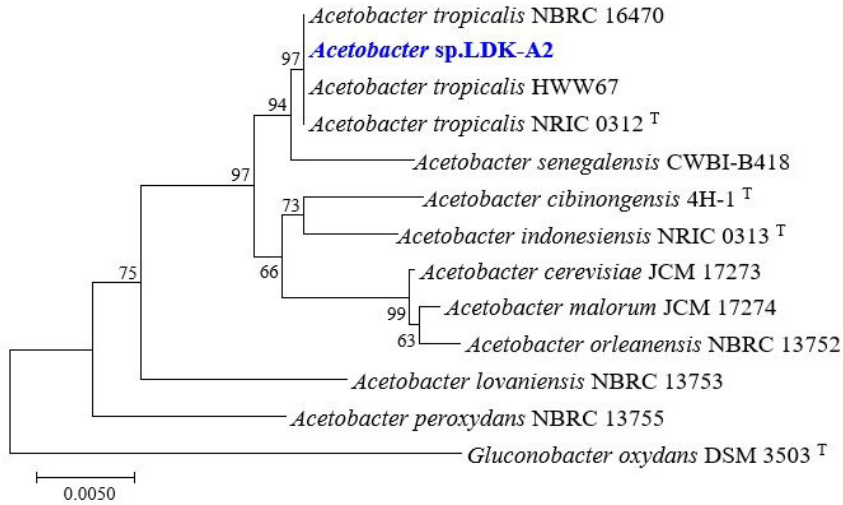

Figure 3. Phylogenetic relationships between LDK-A2 strain and related Acetobacter strains represented as a neighbor-joining (NJ) tree based on 16S rRNA gene sequences.

Note: The evolution history was determined using the NJ method with a Jukes-Cantor model. The numbers at the branching points represent the bootstrap values from 1,000 replications. Gluconobacter oxydans DSM 3503^T was used as the outgroup. Bar = 0.0050 substitutions per nucleotide position.

In a comparison of 16S rRNA sequences based on BLAST analysis, LDK-A2 showed the highest similarity with *Acetobacter tropicalis* HWW67 (100%) and *Acetobacter tropicalis* BCH750 (100%) sequences reported in the NCBI database. Phylogenetic tree analysis suggested that isolated strain LDK-A2 located close to *Acetobacter tropicalis* strain (NBRC 16470, HWW67 and NRIC 0312T) (**Figure 3**). In addition, the biochemical characteristic of LDK-A2 was similar to *A. tropicalis* following Bergey's bacterial classification (Romero-Cortes, T. *et al.* 2012, Sievers, M. and Swings, J. 2015). Therefore, strain LDK-A2 was identified *as Acetobacter tropicalis* species.

Acetobacter tropicalis is a type of acetic acid bacteria commonly found in fermented beverages, such as Kombucha, and in the fermentation of coconut, cacao, and apple products. (Savary, O. *et al.* 2021, Mas, P. *et al.* 2022). In a study by Coton et al.,(2017) *A. tropicalis* was the most dominant bacteria strain identified in green tea fermentation (Coton, M. *et al.* 2017). Also, another study showed that *A. tropicalis* ITV61 produced an abundance of acid acetic production (Romero-Cortes, T. *et al.* 2012). Likewise, *A.tropicalis* LDK-A2 in this study was also a dominant bacteria with the most ability to produce acid acetic compared with other strains.

4. CONCLUSIONS

In this investigation, six distinct commercial SCOBYs were cultured in sugared tea broth, and the resulting fermented broth was used for bacterial isolation. Eighteen bacterial strains were obtained from the fermented tea broth, with ten of them identified as acetic acid bacteria. These ten strains underwent examination for biochemical characteristics and colony morphology. Preliminary classification placed them within the genera *Acetobacter*, *Gluconobacter*, and *Komagataeibacter*. Assessment of acetic acid production on YPGD agar medium revealed that the *Acetobacter* sp. LDK-A2 strain demonstrated the highest acid production, with a halo-diameter ratio of 2.1. A phylogenetic tree, constructed using the 16S rRNA sequence, indicated that the LDK-A2 strain was associated with *Acetobacter stropicalis*.

PHÂN LẬP VÀ TUYỂN CHỌN CHỦNG VI KHUẨN AXÍT ACETIC HOẠT LỰC CAO TỪ KOMBUCHA TRÀ XANH VỤN ĐƯỢC LÊN MEN BỞI MỘT SỐ NGUỒN SCOBY THƯƠNG MẠI

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TÓM TẮT

Việt Nam là nước sản xuất chè lớn thứ bảy trên toàn cầu, cung cấp nhiều loại sản phẩm chè thương mại. Trong quá trình sản xuất chè khô, khoảng 10% sản lượng hàng năm là chè vụn, có giá trị kinh tế thấp và bị loại bỏ. Loại chè này là nguồn nguyên liệu dồi dào để sản xuất các loại đồ uống lên men như là trà Kombucha, được sản xuất từ việc lên men nước trà đường bởi nấm trà. Đây là một quần thể cộng sinh phức tạp giữa vi khuẩn và nấm men (SCOBY), giúp chuyển đổi sucrose thành axit axetic. Trong số các vi sinh vật tham gia quá trình này, vi khuẩn axit axetic chiếm ưu thế, đóng vai trò then chốt trong sản xuất axit hữu cơ. Trong nghiên cứu này, sáu loại SCOBY thương mại riêng biệt được nuôi cấy trong nước trà đường và dịch lên men được sử dụng để phân lập vi khuẩn axit axetic. Mười chủng vi khuẩn đã

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được phân lập và phân tích về hình thái khuẩn lạc và các đặc tính sinh hóa. Những vi khuẩn này được phân loại sơ bộ thuộc ba chi: Acetobacter, Gluconobacter và Komagataeibacter. Qua sàng lọc về khả năng lên men trên môi trường thạch YPGD bổ sung 5 g/L CaCO3 và 40 mL/L ethanol, *Acetobacter* sp. LDK-A2 thể hiện khả năng sinh axit cao nhất, với tỷ lệ đường kính vùng phân giải là 2,1, khác biệt đáng kể so với các chủng còn lại. Cây phát sinh loài sử dụng trình tự gen 16S rRNA đã xác định rằng chủng LDK-A2 thuộc loài *Acetobacter tropicalis*.

Từ khóa: *Kombucha, acetic acid bacteria*, *Acetobacter stropicalis, shredded tea, fermentation*

REFERENCES

Apagu, B. *et al.* (2017). 'Phytochemical and in vitro antibacterial properties of leaves of some selected plant species in Maiduguri, Nigeria', *The Pharmaceutical and Chemical Journal*, 4, pp. 5-14.

Boesch, C. *et al.* (1998). 'Acetobacter intermedius, sp. nov', *Systematic and applied microbiology*, 21, pp. 220-229. doi: 10.1016/S0723-2020(98)80026-X.

Chakravorty, S. *et al.* (2016). 'Kombucha tea fermentation: Microbial and biochemical dynamics',. *International journal of food microbiology*, 220, pp.63-72. doi: 10.1016/j.ijfoodmicro.2015.12.015.

Coton, M. *et al.* (2017). 'Unraveling microbial ecology of industrial-scale Kombucha fermentations by metabarcoding and culture-based methods', *FEMS microbiology ecology*, 93, fix048. doi: 10.1093/femsec/fix048.

Dufresne, C. and Farnworth, E. (2000). 'Tea, Kombucha, and health: a review', *Food Research International*, 33, pp. 409-421. doi: 10.1016/S0963-9969(00)00067-3.

Dung, N.L. *et al.* (2002). 'Vi sinh vật học', Nhà Xuất Bản Giáo Dục.

Dwivedi, M. (2020). 'Gluconobacter', *Beneficial Microbes in Agro-Ecology*, Academic Press,pp. 521-544. doi: 10.1016/B978-0-12-823414-3.00025-3.

Gomes, R.J. *et al.* (2018). 'Acetic acid bacteria in the food industry: systematics, characteristics and applications', *Food technology and biotechnology*, 56(2), pp.139-151. doi: 10.17113/ftb.56.02.18.5593.

Hanmoungjai, W. *et al.* (2007). 'Identification of acidotolerant acetic acid bacteria isolated from Thailand sources', *Research Journal of Microbiology*, 2(2), pp. 194-197. doi: 10.3923/jm.2007.194.197.

Jayabalan, R. *et al.* (2014). 'A review on Kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus', *Comprehensive reviews in food science and food safety*, 13(4), pp. 538-550. doi: 10.1111/1541-4337.12073

Kanchanarach, W. *et al.* (2010). 'Characterization of thermotolerant Acetobacter pasteurianus strains and their quinoprotein alcohol dehydrogenases', *Applied microbiology and biotechnology*, 85, pp.741-751. doi: 10.1007/s00253-009-2203-5.

An, L.T. *et al.* (2020). 'Đánh giá nguồn phụ phẩm chè sau chế biến khô làm thức ăn bổ sung trong chăn nuôi bò', *Tạp chí Khoa học công nghệ chăn nuôi*, 109, pp. 60-72.

Liu, C.-H. *et al.* (1996). 'The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation', *Food microbiology*, 13(6), pp.407-415. doi: 10.1006/fmic.1996.0047.

Marsh, A.J. *et al.* (2014). 'Sequence-based analysis of the bacterial and fungal compositions of multiple Kombucha (tea fungus) samples', *Food microbiology*, 38(4), pp.171-178. doi: 10.1016/j.fm.2013.09.003.

Mas, P. *et al.* (2022). 'Evolution in composition of Kombucha consortia over three consecutive years in production context', *Foods*, 11(4), pp. 614. doi: 10.3390/foods11040614.

Loan, N.T.P. (2016). 'Nghiên cứu sinh thái nhân văn làng nghề sản xuất chè Shan Tuyết tại xã Nà Chì, huyện Xín Mần, tỉnh Hà Giang', *VNU Journal of Science: Earth and Environmental Sciences*, 32(1S), pp. 267-273.

Nasehi, M. et al. (2017). 'Effect of polyethylene glycol addition on nutritive value of green and black tea co-products in ruminant nutrition', *Asian Journal of Animal and Veterinary Advances*, 12(5), pp. 254-260. doi: 10.3923/ajava.2017.254.260.

Phuong, N.T. and Huong, N.T. (2018). 'Phân lập và tuyển chọn hệ cộng sinh giữa nấm men và vi khuẩn trong lên men trà thủy sâm (Kombucha) nhằm nâng cao hàm lượng acid glucuronic', *Tạp chí Khoa học Đại học cần Thơ*, 54(1), pp.13-19. doi: 10.22144/ctu.jvn.2018.003.

Quang, P.H. *et al.* (2014). 'Phân lập, tuyển chọn nấm men và vi khuẩn acid acetic thử nghiệm lên men trà thủy sâm (Kombucha)', *Tạp chí Khoa học Đại học cần Thơ*, 34, pp. 12-19.

Reiner, K. (2010). 'Catalase test protocol', *American society for microbiology*, 1-6.

Romero-Cortes, T. *et al.* (2012). 'Isolation and characterization of acetic acid bacteria in cocoa fermentation', *African Journal of Microbiology Research*, 6(2), pp. 339-347. doi: 10.5897/AJMR11.986.

Savary, O. *et al.* (2021). 'Tailor-made microbial consortium for Kombucha fermentation: Microbiota-induced biochemical changes and biofilm formation', *Food Research International*, 147, pp. 110549. doi: 10.1016/j.foodres.2021.110549.

Sievers, M. and Swings, J. (2015). 'Acetobacteraceae', *Bergey's manual of systematics of Archaea and Bacteria*, 1-20. doi:10.1002/9781118960608.fbm00174.

Tran, T. *et al.* (2020). 'Microbiological and technological parameters impacting the chemical composition and sensory quality of Kombucha', *Comprehensive reviews in food science and food safety*, 19(4), pp. , 2050-2070. doi: 0.1111/1541-4337.12574.

Diep, L.T.B. (2010). 'Nghiên cứu hoàn thiện quy trình tinh chế polyphenol từ chè xanh vụn phế liệu', *Trường Đại học Bách Khoa Hà Nội: Luận văn thạc sĩ.*

Volova, T.G. *et al.* (2018). 'Production and properties of bacterial cellulose by the strain Komagataeibacter xylinus B-12068', *Applied microbiology and biotechnology*, 102(17), pp. 7417-7428. doi: 10.1007/s00253-018-9198-8.

Wang, B. *et al.* (2022). 'Isolation and characterisation of dominant acetic acid bacteria and yeast isolated from Kombucha samples at point of sale in New Zealand', *Current Research in Food Science*, 5, pp. 835-844. doi: 0.1016/j.crfs.2022.04.013.

Yamada, Y. and Yukphan, P. (2008). 'Genera and species in acetic acid bacteria', *International journal of food microbiology*, 125(1), pp. 15-24. doi: 0.1016/j.ijfoodmicro.2007.11.077.