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Characterization of lactic acid bacteria isolated from stinky tofu and suan-tsai using API 50 CHL

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A B S T R A C T

Stinky tofu and suan-tsai are traditional Taiwanese fermented foods produced through spontaneous fermentation involving lactic acid bacteria (LAB). This study aimed to explore the diversity of lactic acid bacteria present in suan-tsai and stinky tofu through the isolation, characterization, and identification of bacterial strains based on characteristics and the API 50 CHL Kit with 16S rRNA gene sequencing considered for future confirmation. Samples were diluted and cultured on MRS agar with the addition of $CaCO_3$. The 20 colonies forming clear zones were selected for morphological and biochemical characterization including microscopy, gram staining, motility, and catalase activity. Two isolates exhibiting clear zone formation, Gram-positive staining, non-motile behavior, cocci or rod-shaped morphology, and negative catalase activity were selected for identification using the API 50 CHL test kit. One isolate from stinky tofu (A11) was identified as *Leuconostoc mesenteroides* with 99.8% similarity and, one isolate from suan-tsai (C9) was identified as *Lactobacillus casei* with 99.4% similarity. Both strains were gram-positive, non-motile, and catalase-negative, indicating LAB characteristics. *L. mesenteroides* is a heterofermentative bacterium that produces lactic acid, CO_2, and ethanol contributing to creating an acidic condition and flavor development. *L. casei* is a homofermentative bacterium that support maintenance of low pH, inhibits pathogenic microorganisms, and supports food preservation. The probiotic potential of these strains was based on literature reports describing the antimicrobial and health promoting properties. These findings highlight representative LAB from Taiwan traditional fermented foods and its potential role in preservation, quality, and functionality.

Keywords: Fermentation; Lactic acid bacteria; Stinky tofu; Suan-tsai

1. INTRODUCTION

Fermentation is a biochemical process that changes complex organic compounds such as carbohydrates into simpler compounds (Behera *et al*., 2019). Spontaneous fermentation is widely used in the production of traditional foods, relying on natural microorganisms in the raw material or microorganisms that develop due to the environmental conditions which leads to the presence of lactic acid bacteria (LAB) that contribute to preservation and sensory characteristic development (Gänzle *et al*., 2023). On the other side, non-spontaneous fermentation occurs when there is an addition of starter or yeast to control the fermentation process. Both methods will support microbial metabolism that transforms substrates into fermentation products.

In the fermentation process, Lactic acid bacteria (LAB) are often used in spontaneous fermentation by generating lactic acid as the primary metabolic product of carbohydrate fermentation. There are two metabolic pathways of lactic acid bacteria fermentation, such as heterofermentative and homofermentative. Metabolic pathways of heterofermentative bacteria will produce CO_2, lactate, and ethanol. Moreover, the metabolic pathways of homofermentative bacteria will produce lactate as the main end product (Ganzle, 2015).

The Non-pathogenic genera of lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, and *Oenococcoccus* are commonly found in fermented beverages and foods like beer, wine, sourdough, pickled vegetables, etc (Liu, 2014). Salting and drying are common methods in fermentation to

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preserve foods with alcohol and organic acid, desirable sensory features like texture and flavor, and reduce toxicity. These methods will make a proper condition for LAB to grow. Taiwan has many traditional fermented foods such as suan-tsai and stinky tofu.

Taiwan is known for its variety of traditional fermented foods, including *suan-tsai* and *stinky tofu*. *Suan-tsai* is a spontaneously fermented vegetable product made from mustard greens (*Brassica juncea*) that develops a sour flavor through LAB activity (Liou et al., 2019). The process typically involves salting, which draws water from the vegetables with osmosis, creating a natural brine that favors LAB growth while inhibiting undesirable microorganisms (Anggraeni, 2021). In contrast, *stinky tofu* is a fermented soybean product characterized by a strong, distinctive aroma resulting from microbial metabolism during brining (Xie & Gänzle, 2023; Tian et al., 2024). It can be produced through either spontaneous fermentation or controlled fermentation with added starter cultures (Benucci et al., 2022). LAB in *stinky tofu* contributes to pH reduction through the production of lactic and acetic acids, as well as CO_2, which creates anaerobic conditions (Gu et al., 2018).

Previous studies have reported various LAB species in these products. In *suan-tsai*, isolates such as *Pediococcus pentosaceus*, *Tetragenococcus halophilus*, *Enterococcus faecalis*, *Lactobacillus alimentarius*, *L. brevis*, *L. coryniformis*, *L. farciminis*, *L. plantarum*, *L. versmoldensis*, *Leuconostoc citreum*, *L. mesenteroides*, and *L. pseudomesenteroides* have been identified (Liou et al., 2019; Swain et al., 2014). In *stinky tofu*, species including *Leuconostoc lactis*, *Lactobacillus paracasei*, *L. mixtipabuli*, *L. plantarum*, and *L. coryniformis* have been reported (Chen & Ying, 2017; Gu et al., 2018).

According to Liu *et al*. (2021), homemade sauerkraut samples were collected from three provinces in Southwest China, such as Sichuan, Guizhou, and Yunnan. These differences were primarily associated with regional climatic conditions, the variety and origin of raw vegetables, and traditional production procedures applied by local producers. Climatic factors such as ambient temperature and humidity during fermentation influence the growth rate and competitive interactions of LAB species, thereby shaping the final microbial profile. In addition, variation in raw materials can lead to differences in microbial communities, as vegetable varieties cultivated in different regions exhibit distinct physicochemical characteristics and associated surface microbiota. Variations in production methods, including salt concentration, fermentation time, and the type of fermentation methods, also contribute in microbial population. From this study, Lactobacillus plantarum being consistently detected across all sampling locations, the dominant LAB species varied among provinces. In certain regions, *Pediococcus* was more prevalent, whereas *Leuconostoc spp*. or *Lactobacillus brevis* predominated in others. These observations highlight the significant role of geographical origin and local production practices in shaping LAB biodiversity, even within the same type of fermented product.

Considering these findings, assessing the diversity of LAB isolates from specific geographical origins is essential for characterizing the microbial biodiversity of traditional fermented foods. This study focuses on Taiwanese fermented products such as stinky tofu and suan-tsai sourced from Taichung city, aiming to isolate and identify LAB strains present in these samples and to compare the results with previous identification studies on similar products. Such a comparison provides insights into potential geographical variations in LAB composition and contributes to a broader understanding of LAB diversity in Taiwanese fermented foods.

2. Material and Methods

2.1 Study area

This research project was held in Applied Microbiology Lab (D301) at D building of Hungkuang University, Taichung city, Taiwan from March 20 2024 until April 18 2024.

2.2 Materials

The ingredients needed in this experiment are 25g stinky tofu and suan-tsai, 450ml saline water, and MRSA + $CaCO_3$ media. The equipment needed in this experiment are laminar air flow, autoclave, alcohol 70%, micropipette, blue and yellow tip, incubator, petri plates, erlenmeyer, blender, light microscope Nikon E100 by (Nikon Corporation Japan), object glass, ose needle, test tubes, hot plate, stirrer, L spreader, bunsen

burner, vortex, pipette, crystal violete, iodine lugol, alcohol 95%, safranin, Biomérieux API 50 CHL kit test by (BioMérieux SA, USA).

2.3 Isolation and culturing of LAB

Every 0.1 ml of diluted samples from each serial dilution (10^{-1} to 10^{-9}) was transferred into MRSA + $CaCO_3$ media with duplicate replications by the spread plate method. Then all media were stored in an airtight container and placed in an incubator chamber for 48 hours at 37°C. Subsequently, 20 isolates from each sample showing a clear zone were selected for morphological and biochemical identification.

2.4 Morphological and biochemical characterization

The colony were observed based on morphological characteristics including microscopy by a light microscope Nikon E100 by (Nikon Corporation Japan) with a magnification of 10x10, gram staining, and biochemical test by catalase test. The isolates with the most characteristic features of LAB were identified using the API Kit test. (Khusbhoo *et al*., 2023).

2.5 Identification using API 50 CHL

Identification of LAB strains was performed using API 50 CHL Kit assays. One colony from each sample were diluted in 2 ml saline water. The bacterial solution was added until it matched the concentration of 2 McFarland standard. Then twice the number of counted droplets into 50 CHL Medium until homogenized. The homogenized suspensions of the cells were suspended in the medium and then transferred into each of the 50 wells on the API 50 CH strips. All wells on the plate were overlaid with sterile oil to create an anaerobic condition. The API Kit was then incubated at 37 °C and the results were read after 48hours. Fermentation of carbohydrates was identified by a yellow color except for middle cell (dark brown). The results were analyzed using the API WEB (Bio-Merieux) (Sukrama *et al*., 2018).

3. Results and Discussion

3.1 Isolation of lactic acid bacteria

There were more than 350 colonies in the stinky tofu and 122 colonies in the suan-tsai showing clear zone growth on the deMan-Rogosa Sharpe Agar (MRSA) + $CaCO_3$ medium. MRSA + $CaCO_3$ medium is a selective medium that supports the growth of certain bacteria such as Lactic Acid Bacteria (LAB). These colonies were suspected as LAB, as LAB produces lactic acid that binds $CaCO_3$ into Ca-lactate creating a clear zone around the colonies. The presence of a clear zone indicates the presence of LAB in the samples. Selecting a reasonable number of colonies, such as 20 is usually sufficient to identify the sample's most dominant and relevant bacteria. To avoid identifying the same species, each of the 20 selected colonies are chosen based on the different growth morphologies (color and shape) on MRSA + $CaCO_3$ medium (Lawalata *et al*., 2020). The 20 colonies from stinky tofu (A1, A2, A3, A4, A6, A7, A8, A9, A11, A13, A14, A15, A16, A17, A18, A28, A46, A61, A69, A79) and the 20 colonies from suan-tsai (C1, C2, C9, C10, C20, C27, C28, C29, C30, C34, C35, C40, C41, C49, C54, C56, C57, C59, C83, C90) will go through screening stage by identifying based on their morphology, motility, gram stain, catalase test and enzyme reaction through API 50 CHL Kit Test.

3.2 Characterization and identification of lactic acid bacteria

The characterization of every colony from stinky tofu and suan-tsai are given in Table 1. Characteristic of Lactic Acid Bacteria in Stinky Tofu and Suan-tsai. According to Swain *et al*., 2014, LAB is a group of gram-positive, non-motility, negative in catalase test, and has cocci or rod shapes. The results of the characteristics of isolated Lactic Acid Bacteria in Stinky Tofu and Suan-tsai are shown in Table 1.

Table 1. Characteristic of isolated lactic acid bacteria in stinky tofu and suan-tsai

Samples	Gram stain	Morphology	Motility	Catalase
A1	+	Cocci	-	-
A2	+	Rods	-	-
A3	+	Cocci	-	-
A4	+	Rods	-	-
A6	+	Cocci	-	-
A7	+	Rods	-	-

A8	+	Rods	-	-
A9	+	Cocci	-	-
A11	+	Cocci	-	-
A13	+	Cocci	-	-
A14	+	Rods	-	-
A15	+	Cocci	-	-
A16	+	Cocci	-	-
A17	+	Cocci	-	-
A18	+	Cocci	-	-
A28	+	Cocci	-	-
A46	+	Cocci	-	-
A61	+	Cocci	-	-
A69	+	Cocci	-	-
A79	+	Cocci	-	+
C9	+	Rods	-	-
C10	+	Rods	-	-
C20	+	Rods	-	-
C27	+	Rods	-	-
C28	+	Rods	-	-
C29	+	Cocci	-	-
C30	+	Rods	-	-
C34	+	Rods	-	-
C35	+	Rods	-	-
C40	+	Cocci	-	-
C41	+	Rods	-	-
C54	+	Cocci	-	-
C56	+	Rods	-	-
C57	+	Rods	-	-
C59	+	Rods	-	-
C83	+	Rods	-	-

Characteristic of LAB = Gram stain: positive (+); shapes: rods/cocci; motility: negative (-); catalase: negative (-)

From Table 1. shows that all stinky tofu and suan-tsai colony samples are gram-positive, have cocci or rod shape, and have no motility. Samples A79 and C90 showed a positive catalase result, a positive reaction is marked by the presence of bubbles. On the other side, the four samples from Suan-tsai (C1, C2, C49, C90) are not qualified as LAB because it has negative gram stain (C1, C2, C49). Due to the 50 CHL API Kit Test limitations, only two isolates, one from each product (A11 from stinky tofu and C9 from suan-tsai) were selected for identification because their morphological and biochemical profiles were similar to other isolates obtained from the same sample. Also, this isolate was selected based on its characteristics, as it exhibited a larger clear zone compared to the other isolates that grew. This approach was intended to avoid redundant characterization. However, it also limits the scope of the results, as other potentially significant bacterial strains present in the samples may not have been detected or characterized.

3.3 Gram staining

The results of A11 and C9 as shown in Fig. 1, indicate that both samples have positive gram stains due to their purple coloration. In gram staining, crystal violet acts as the primary dye while safranin acts as the secondary dye. LAB has thick peptidoglycan cell walls that can bind crystal violet as the primary stain. In gram staining, there is a washing process with alcohol or acetone solution. During the washing process, the thick peptidoglycan layer will retain the purple color of crystal violet. This happens because the thick peptidoglycan layer binds the primary dye strongly (Wulandari & Purwaningsih, 2019).

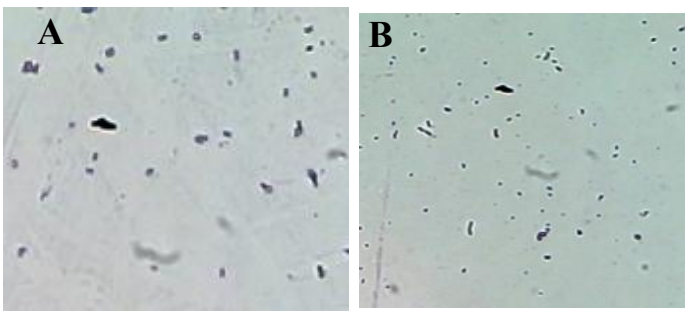

Figure 1. Gram staining result of stinky tofu and suan-tsai with 10x10 magnification: A. Isolate A11; B. Isolate C9

3.4 Catalase test

The results of A11 and C9 catalase tests show negative results. According to Khushboo *et al*. (2023), LAB can not break down the hydrogen peroxide (H_2O_2) into oxygen and water. Since LAB is an anaerobic and microaerophilic bacterium, it does not produce significant amounts of reactive oxygen species like hydrogen peroxide, which requires the catalase enzyme for the decomposition process. The absence of bubbles indicates the lack of catalase activity, which is a characteristic of LAB from other bacteria with high catalase activity.

3.5 Isolate identification with API 50 CHL kit test

The LAB strain was identified using a 50 CHL API Kit test. Every cell suspension was suspended and transferred in every 50 wells on API 50 CH strips. Mineral oil was added to create an anaerobic condition for LAB to grow during the incubation. This method involves the manual identification of microorganisms at the species level. The 50 CHL API identification method is quite accurate and fast because it is based on the carbohydrate fermentation pattern of bacteria. The 50 CHL API Kit identification test works by detecting the biochemical process of 49 carbohydrate substrates fermentation process, indicated by a color change in every strip. No color change indicates a negative result. On the other hand, A positive outcome of carbohydrate fermentation (+) is marked by a color change on the strip from purple to yellow, except for the middle section which should turn into dark brown (Sukrama *et al*., 2018; Karyantina *et al*., 2020).

The results of the biochemical processes based on carbohydrate fermentation from each sample are presented in Figure 2 and Figure 3. For the next step, identification was performed by API Web analysis (https://apiweb.biomerieux.com) to determine the name of LAB species that shown in Figure 4 and Figure 5. The identification of LAB is considered acceptable and valid when the similarity score is above 60%, as this shows a reliable match with the reference data (Sukrama *et al*., 2018).

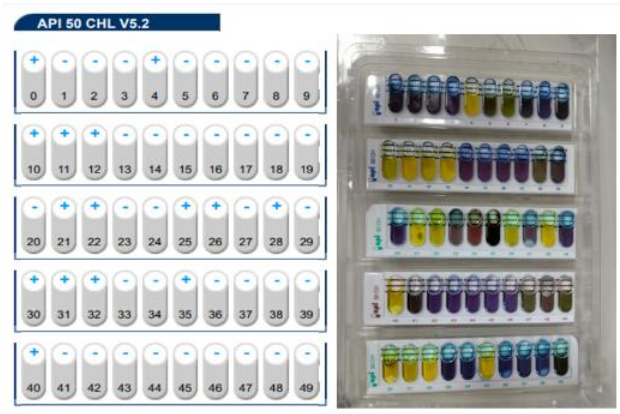

Figure 2. The biochemical results of carbohydrate fermentation from stinky tofu (A11)

Figure 3. The biochemical results of carbohydrate fermentation from suan-tsai (C9)

REFERENCE **DATE** 4/17/24

COMMENT

STINKY TOFU (A11) - 1

DOUBTFUL PROFILE

Strip	API 50 CHL V5.2
Profile	+---+-----+++---------++--+-+-+-+++--+----+---------
Note	

Significant taxa	**% ID**	**T**	**Tests against**			
Leuconostoc mesenteroides ssp mesenteroides/dextranicum 2	**99.8**	**0.31**	**0 0%**	**RIB 75%**	**DXYL 75%**	**MNE 100%**
			SAL 10%			

Next taxon	**% ID**	**T**	**Tests against**			
Leuconostoc mesenteroides ssp mesenteroides/dextranicum 1	**0.1**	**0.01**	**0 0%**	**DXYL 80%**	**MNE 99%**	**AMY 80%**
			ARB 95%	**CEL 99%**	**GEN 90%**	

Figure 4. The identification result of lactic acid bacteria from stinky tofu (A11)

REFERENCE **DATE** 4/17/24

COMMENT

PICKLED MUSTARD GREEN (C9) - 1

DOUBTFUL PROFILE

Strip	API 50 CHL V5.2
Profile	+----+----++++----++--++++++++-+++-+----+--+----+--
Note	POSSIBILITY OF Lactobacillus casei

Significant taxa	**% ID**	**T**	**Tests against**			
Lactobacillus paracasei ssp paracasei 1	**89.4**	**0.64**	**0 0%**	**TUR 80%**		
Lactobacillus paracasei ssp paracasei 3	**10.4**	**0.52**	**0 0%**	**SOR 20%**	**MLZ 20%**	**GNT 20%**

Next taxon	**% ID**	**T**	**Tests against**			
Lactobacillus plantarum 1	**0.1**	**0.36**	**0 0%**	**MEL 94%**	**TAG 7%**	

Figure 5. The identification result of lactic acid bacteria from suan-tsai (C9)

From Figure 4 and Figure 5, every sample has its description of percentages of possible similarity bacteria. Figure 14. The identification result of lactic acid bacteria from stinky tofu shows that the result of lactic acid bacteria from stinky tofu is *Leuconostoc mesenteroides* with 99.8 % similarity. According to Guan *et al.* (2024), *L. mesenteroides* is also found in traditional fermented soybean products known as mapo tofu or "hairy tofu" in China. Both stinky tofu and hairy tofu share the same similarities, including spontaneous fermentation and main raw material which is tofu. In spontaneous fermentation, natural microorganisms present in the raw material have a crucial role in driving the fermentation process, also contributing to the development of the sensory profile and preservation of the product. This statement is supported by Rossi *et al*. (2016), who

reported the presence of *L. mesenteroides* in fresh tofu due to its high moisture content and protein. These characteristics create a highly favorable environment for the growth of lactic acid bacteria. Furthermore, *L. mesenteroides* is often found at the beginning of fermentation, suggesting that it may act as an early colonizer that initiates the process, supports the growth of other microorganisms, and subsequently dominates the microbial population throughout fermentation. This dominance can be significantly influenced by the selection of raw materials used in the product (Yang *et al*., 2019).

On the other side, Figure 15. The identification result of lactic acid bacteria from suan-tsai shows that the result of lactic acid bacteria from suan-tsai is *Lactobacillus casei* with 99.4% similarity. As reported by Liu *et al.* (2021), *Lactobacillus casei* was also identified in a vegetable fermented product named sauerkraut. The presence of *Lactobacillus casei* isolate in both suan-tsai and sauerkraut can be attributed to similarities in fermentation methods, main raw material, and environmental conditions. Both products are prepared from leafy vegetables, such as cabbage or mustard greens, which provide fermentable sugars, vitamins, and minerals that support the growth of *L. casei*. The fermentation process in both cases is typically spontaneous with the addition of salt at some concentrations that inhibit spoilage microorganisms while favoring salt-tolerant lactic acid bacteria. Furthermore, *L. casei* exhibits strong acid tolerance and can thrive in the low-pH environments that develop during vegetable fermentation (Morales-Ríos et al., 2023). These shared substrates and selective fermentation conditions likely contribute to the consistent identification of *L. casei* in both suan-tsai and sauerkraut.

Leuconostoc mesenteroides is a heterofermentative lactic acid bacteria that produces lactic acid, CO_2, and ethanol as its products. Usually, Lactobacillus is the common strain found in stinky tofu such as *Lactobacillus paracasei*, *L. mixtipabuli*, *L. plantarum,* and *L. coryniformis*. Apart from the Lactobacillus strain, *Weisella confuse* and *Bacillus cereus* was also found in stinky tofu. Bacillus cereus was found in the first month of the fermentation process (Gu *et al*., 2018). However, it is still possible to find *Leuconostoc* strain in stinky tofu. Sticky tofu can be made using a non-spontaneous fermentation method with the addition of a starter. *Leuconostoc* is often used as a starter for the fermentation process. Using *Leuconostoc* as a starter for making stinky tofu increases the chances of it being the dominant strain in the stinky tofu samples. Based on Tian *et al* (2024) research, *Leuconostoc mesenteroides* are frequently found in the early-stage fermentation of stinky tofu. Another study by Gu *et al*. (2018) states that Lactobacillus strains were more commonly found in the later stage of fermentation. This suggests that the selected sample is still in the early phase of the fermentation process.

L. mesenteroides is a facultative anaerobic bacterium, meaning it can grow with or without the presence of oxygen. This trait is essential during the initial phase of fermentation process to starts the process. As a heterofermentative bacteria, *L. mesenteroides* produces lactic acid, CO_2, and ethanol to create a proper fermentation environment under low pH and anaerobic conditions to support the growth of other microorganisms during the next stage of the fermentation process (Jeon *et* al., 2017, Yang *et al*., 2019). These characteristics of *L. mesenteroides* support its role as a starter in fermentation**.** According to Tian *et al* (2023), *L. mesenteroides* is a starter in stinky tofu productions and significantly affects stinky tofu flavor. The main flavor substances are ethyl acetate, dimethyl disulfide, benzaldehide, dimethyl trisulfide, 1- heptanol, *n*-hexane, 2,3-pentanedione, isobutyl acetate, and 2,3-butanedione. Besides that, *L. mesenteroides* can be a probiotic that has many benefits for human health. Probiotics are living microorganisms that offer health benefits for the 'host' when consumed in sufficients quantities. Probiotic bacteria help to maintain the natural balance of gut microbiota. A healthy gut microbiota protects against gastrointestinal disease and infections. On the other side, probiotics can also be used as an antibiotic in the treatment of enteric infections (Swain *et al*., 2014). *L. mesenteroides* has the potential as biosorption to remove non-biodegradable heavy metals such as lead (Pb) (Yi *et al*., 2017).

Lactobacillus casei is a heterofermentative lactic acid bacteria that produces lactic acid, CO_2, and ethanol, as its products. The production of lactic acid will maintain the low pH of suan-tsai and inhibit the growth of pathogen microorganisms (Morales-Ríos et al., 2023). During fermentation, *L. casei* will grow and survive in

acid conditions (Guo *et al*., 2015). *L. casei* has also been found in other fermented vegetables such as Jiangshui-cai (Ullah *et al*., 2017), Gundruk, and Sinki (Swain *et al*., 2014). Frequently, *Lactobacillus alimentarius*, *Lactobacillus brevis*, *L. coryniformis*, *L. farciminis*, *L. versmoldensis*, *L. plantarum, Leuconostoc citreum*, *L. mesenteroides*, and *L. pseudomesenteroides, Enterococcus faecalis* are main LAB in suan-tsai (Liou et al., 2019). A high concentration of lactic acid bacteria will create an acidic condition. In these conditions, LAB will inhibit the growth of some bacteria such as *Bacillus spp.* and *Pseudomonas spp*. Furthermore, it will be difficult for another microorganism to grow (Zhao *et al*., 2016, Lv *et al*., 2018). According to Hill *et al* (2018), *L. casei* is a probiotic that can be used to treat or prevent various diseases including cancer and diarrhea. This statement has been proven by *in vivo* test that showed there is 80% reduction of tumor volume in mice fed by *L. casei* for 13 days. The cancer treatment methods can weaken the human immune system and cause some side effects such as diarrhea. Probiotics may help lower the risk of diarrhea caused by radiation during treatment procedures. Besides that, *L. casei* also produces caseicin which acts as a bacteriocin that can prevent the growth of pathogen bacteria such as *S. aureus*, *L. monocytogenes*, *Salmonella spp*, and *E. coli* (Cerón-Córdoba *et al*., 2024).

4. CONCLUSION

This study successfully isolated and identified lactic acid bacteria (LAB) from two traditional fermented products: stinky tofu and suan-tsai. Identification was performed using the API 50 CHL kit, which revealed that the predominant LAB strain in stinky tofu was *Leuconostoc mesenteroides*, with 99.8% similarity. L. mesenteroides was characterized as Gram-positive, cocci-shaped, non-motile, and catalase-negative. This species plays a crucial role in the initial stages of fermentation by creating an environment conducive to subsequent microbial activity through its heterofermentative metabolism, producing lactic acid, carbon dioxide, and ethanol.

In suan-tsai, the dominant LAB strain identified was *Lactobacillus casei*, with a 99.4% similarity. *L. casei* is also gram-positive, rod-shaped, non-motile, and catalase-negative. As a homofermentative bacterium, L. casei contributes to maintaining the low pH environment necessary to suppress the growth of pathogenic microorganisms during fermentation.

However, stinky tofu and suan-tsai samples also exhibited traits inconsistent with typical LAB characteristics, including catalase-positive reactions and Gram-negative results. These discrepancies suggest potential limitations of the API 50 CHL kit, which revealed that the predominant LAB strain in stinky tofu was *Leuconostoc mesenteroides*, with 99.8% similarity. *L. mesenteroides* was identified as Gram-positive, cocci-shaped, non-motile, and catalase-negative. .

In suan-tsai, the dominant LAB strain identified was *Lactobacillus casei*, with a 99.4% similarity. *L. casei* is also gram-positive, rod-shaped, non-motile, and catalase-negative. However, stinky tofu and suan-tsai samples also exhibited traits inconsistent with typical LAB characteristics, including catalase-positive reactions and Gram-negative results. These discrepancies suggest potential limitations of the API 50 CHL system and indicate the need for additional confirmatory analyses. Only two isolates (one from each product) were characterized in this study. These isolated LAB were selected because their morphological and biochemical identification data showed similar characteristics to other isolates within the same sample. As only two isolates were identified using the API 50 CHL system, the findings show a limited representation of the microbial diversity and should be interpreted with caution. Future research should incorporate molecular identification techniques, such as 16S rRNA gene sequencing, to enhance the accuracy of LAB identification. Moreover, further studies are recommended to explore the application of these LAB strains in improving fermentation efficiency and flavor development across various fermented food products.

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