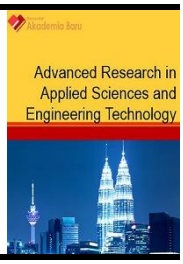
Penerbit Akademia Baru

Journal of Advanced Research in Applied Sciences and Engineering Technology

Journal homepage: www.akademiabaru.com/araset.html
ISSN: 2462-1943



Detection of Beneficial Lactic Acid Bacteria (LAB) and Yeast In Sarawak Fermented Food

Elexson Nillian[1,*], Nick Laurence Buyong[1], Dalene Lesen[1], Grace Bebey[1], Azham Zulkharnain[2]

[1] Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia
[2] Department of Bioscience and Engineering, Shibura Institute of Technology, Japan

ABSTRACT

Sarawak native's fermented food can be a catalyst for boosting the local economy in Sarawak. The Lactic Acid Bacteria (LAB) are generally regarded as safe, have a stability of usage, and originate from natural resources. Lactic acid bacteria and yeast work in synergy to provide a natural way to enhance the nutritive value and flavour of the food. The study aims to investigate the presence of potential probiotic Lactic Acid Bacteria (LAB) and yeast isolated from Sarawak fermented food. Two hundred fifty (n=250) of samples including fifty (n=50) each sample such as fermented shrimps (cencaluk), fermented mustard vegetables (kasam ensabii), fermented fish (kasam ikan), fermented dabai (Canarium odontophyllum) and fermented fish (rusip). Molecular identification of the bacteria and yeast isolates was carried out by PCR amplification of the 16S rRNA (27F and 1492-R) and ITS (ITS5-F and ITS4-R) rRNA region. The successfully amplified PCR products were sent for Sanger sequencing As a result, a total of 45.2% (113/250) Lactic Acid Bcateria (LAB) which are 96% (48/50) of W. paramesenteroides was detected in fermented dabai, 80% (40/50) S. pasteuri in fermented fish rusip samples and 50% (25/50) in P. agglomerans in cencaluk samples. Meanwhile, yeast Candida species; 90% (45/50) of C. magnoliae and 50% (25/50) of C. parapsilosis are detected in fermented dabai and ensabi respectively. Based on the findings, both yeast and bacteriocin-producing lactic acid bacteria found in the fermented food specifically in dabai. A better understanding of microbial ecology can help the food industry to improve the foods in terms of quality and safety. The good quality of the LAB and yeast in the food such as starter culture will enhance the texture and nutritional value and Sarawak fermented food product found enriched with LAB.

Keywords:
LAB, yeast, Sarawak, fermented, food

Received: 20 August 2021 **Revised: 27 September 2021** **Accepted: 27 September 2021** **Published: 4 October 2021**

1. Introduction

Continual efforts have been made by national organizations to discover new tourism products and activities that have the opportunity of being marketed and promoted in Sarawak. Gastronomy has been identified as one of the new tourism products which is directly relevant to the Sarawak context. At present, gastronomy is a valuable source of attracting tourists from all over the world and this has led to the empirical investigation on domestic tourist perception of Sarawak ethnic native's food such as fermented food including *Terubuk ikan masin*, *cencaluk, pekasam* and many more [1].

* *Corresponding author.*
E-mail address: nelexson@unimas.my

https://doi.org/10.37934/araset.24.1.19

Ethnic native food in fact is a window of the identity of the Sarawak cultures and a pleasurable experience for tourists.

Nest generation sequencing (NGS) has transformed from being solely a research tool to becoming routinely applied in many fields including diagnostics, outbreak investigations antimicrobial resistance, forensics, and food authenticity. The technology is developing at a rapid pace, with continuous improvement in quality and cost reduction. NGS in food microbiology is predominantly used in determination of the whole genome sequence of a single cultured isolate including a bacterial colony, a virus, or any other organism in food sample [2].

Fermented food is very popular among the people in Asia-Pacific region including Sarawak. Since this region is characterized by its tropical and subtropical climate, it is very important to preserve the harvest crops. Lactic acid bacteria or (LAB) is a group of related bacteria producing lactic acid as the result of carbohydrate fermentation [3]. LAB is widely used as starter cultures for dairy, meat, and vegetable fermentations [4]. In addition to flavour development and food preservation, they also produce variety of compounds with antimicrobial activity, including organic acids, hydrogen peroxide and bacteriocin [5,6].

Bacteriocin produced by LAB could inhibit not only closely related species, but also the growth of pathogenic bacteria [7,8,9]. Many LABs have important roles in the production of fermented foods, and some of the bacteria are capable of inhibiting the growth of a wide variety of food spoilage microorganisms [10]. Thus, LABs are an attractive source of inhibitory compounds with promising natural food preservatives for improved food quality and safety.

In recent years, there is an increase in detecting the presence of the microorganism that are present in the fermented food. Most of the research are using the cultural method since it is a long and well-established method to determine the presence of microorganisms [10]. However, according to Giraffa and Neviani [12], this method has limitation as it is difficult to obtain the true community of microbial present in the food. It is because only the cultivable microorganisms are detected which only present in a little portion from the total microbial population in food [12]. As the development of next generation sequencing (NGS) techniques, the whole microbial community can be determined. Metagenomic analysis is based on gene DNA fragments, in which the genetic materials are obtained directly from the environment [10]. Kergourlay et al. [11] also mentioned that up until recently, the metagenomic analysis used in food are less reported as there are considered low in term of diversity of microbial community in food compared to soil and human microbiota.

The study aims to investigate the presence of potential probiotic Lactic Acid Bacteria (LAB) and yeast isolated from Sarawak traditional fermented food including *cencaluk, kasam ensabi, kasam ikan*, fermented *dabai* and *rusip*. The findings reveal information regarding the bacterial diversity and their possible role in Sarawak fermented food using molecular approaches.

2. Methodology

2.1 Samples Collection

A total of 250 of samples including 50 each sample such as fermented shrimps (*cencaluk)*, fermented vegetables (*kasam ensabi*), fermented fish (*kasam ikan*),fermented dabai (*Canarium odontophyllum*) and fermented fish *(rusip*) were collected from grocery stores and wet market in Kuching, Sarawak, Malaysia. The processing of the samples was held in Microbiology Laboratory 2, Faculty of Resources Sciences and Technology, UNIMAS.

2.2 Isolation of Lactic Acid Bacteria and Yeast

A total of eleven gram (n=11) of food sample was homogenised with 99 mL of 1X phosphate buffered saline (PBS) for 2 minutes. Then, serial dilutions were done for each sample. LAB was isolated using the de Man, Rogosa and Sharpe (MRS) media, while yeasts were isolated using potato dextrose agar (PDA) media. The LAB was incubated anaerobically in anaerobic jar for 72 hours at 35℃. The yeast was incubated aerobically for 48 hours at 30℃. Morphology of the colonies formed on the media was observed using microscope by gram staining. A colony from each plate was used for DNA extraction.

2.3 DNA Extraction

The method used was based on DNA extraction method by Martin-Platero et al. [12]. One gram of each food samples was weighed. The food sample was finely ground before transferred into 1X PBS for homogenization. Pellet obtained was suspended in 100 µL lysozyme buffer (20 mM Tris-HCL, pH 8, 2 mM EDTA, 1% Triton X-100) and incubated at 37°C for 30 min. Next, 600 µL of lysis buffer was added to the mixture and incubation was done at room temperature for 15 min. Then, 10 µL of proteinase K (10 mg/mL) was added into the mixture before incubation at 37°C for 15 min. The incubation then continued to increased temperature of 80°C for 5 min. Then, cooling down was performance to room temperature for 10 min, and 200 µL of sodium acetate (3 M, pH 5.2) was added.

The mixture was vortexed briefly and placed on ice for 15 min. Subsequently, the sample was centrifuged for 10 min at 6,000 rpm. Supernatant was then transferred to a new tube and equal volume of isopropanol was added to precipitate the DNA. The tube was inverted several times before centrifugation for 5 min at 10,000 rpm to obtain the DNA pellet. The supernatant was discarded, and the pellet was washed with 1 mL of 70% ethanol and left to dry at room temperature. The dried DNA pellet was then suspended in 50 µL TE buffer.

2.4 PCR amplification

PCR amplification was performance in a thermocycler for 30 and 35 cycles using 16S rRNA primer set and ITS primer set, respectively. For lactic acid bacteria identification, 16S rRNA primer set, 27F (5’ – AGA GTT TGA TCM TGG CTC AG -3’) and 1492R (5’- TAC GGY TAC CTT ACGACTT-3’) [13] were used. The amplification was done under the following condition: initial denaturation at 95°C for 3 min, and denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 min and final extension at 72°C for 5 min.

For yeast identification, ITS universal primer set (ITS4-R: 5’-TCCTCCGCTTATTGATATGC and ITS5-F: 5’-GGAAGTAAAAGTCGTAACAAGG) [13] was used. The samples were preheated at 95°C for 2 min, followed by denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 min and final extension at 72°C for 10 min.

The amplified PCR products were visualized under UV transilluminator after running the 1.5% agarose gel. A successful PCR amplification were expected to produce ITS amplicons of sizes ranging from 400 to 600 bp and 16S rRNA amplicons of size 1500bp. This study was held in the Department of Bioscience and Engineering, Shibura Institute of Technology, Japan Laboratory.

2.5 DNA sequencing

The successfully amplified PCR products were sent for Sanger sequencing.

2.6 Data analysis

The raw sequences obtained were trimmed and assembled using BioEdit (version 7.2.5) software. All the 16S rRNA and ITS gene sequences were subjected to identity search using BLASTn tool from the National Center for Biotechnology Information (NCBI) website.

3. Results

3.1 Morphology of LAB and yeast

Through gram staining, LAB showed the gram-negative short rod shaped and cocci shaped for *W. paramesenteroides* and *S. pasteuri* respectively. However, *P. agglomerans* appeared as gram negative and cocci in shaped. The colony morphology of *C. magnoliae* and *C. parapsilosis* are quite similar with each other which are white, creamy, and shiny on potato dextrose agar and it is oval or round in shaped. Morphologies characterization has been performance and as a result, Figure 1 show *Weissella paramesenteroides* and Figure 2 show the *Staphylococcus pasteuri* were detected in fermented *dabai* and *rusip* respectively. Meanwhile, Figure 3 show *Pantoe agglomerans* was detected in the *cencaluk.*

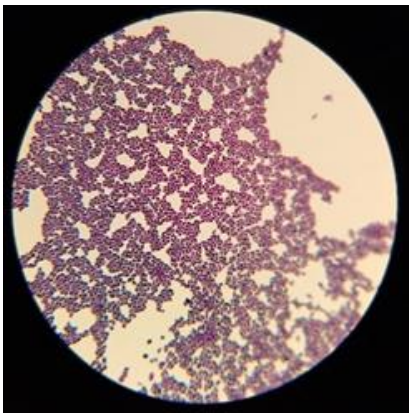


Fig. 1. The microscopic observation of *W. paramesenteroides* under microscope 40x magnification. These bacteria are Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology

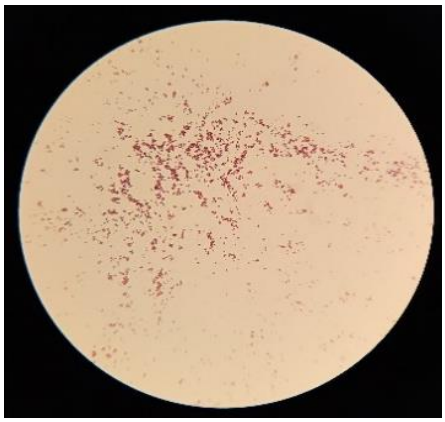


Fig. 2. The microscopic observation of gram-positive *S. pasteuri* using 40x magnification. *Staphylococcus pasteuri* is a coagulase-negative, Gram-positive bacterium of the *Staphylococcacea*e family

3.2 Molecular identification

The molecular identification of LAB and yeast was carried out using PCR amplification. 16S rRNA (27F and 1492-R) and ITS (ITS5-F and ITS4-R) was used for the detection of LAB and yeast respectively. The result for PCR amplification of 16S DNA of LAB was show in Figure 4 while the PCR of ITS rRNA was show in Figure 5.

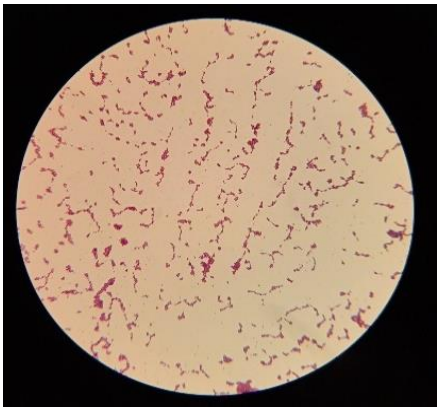


Fig. 3. Gram negative of *P. agglomerans* under the microscope using 40x magnification.
Pantoea agglomerans is a gram-negative aerobic bacillus that belongs to the family *Enterobacteriaceae*

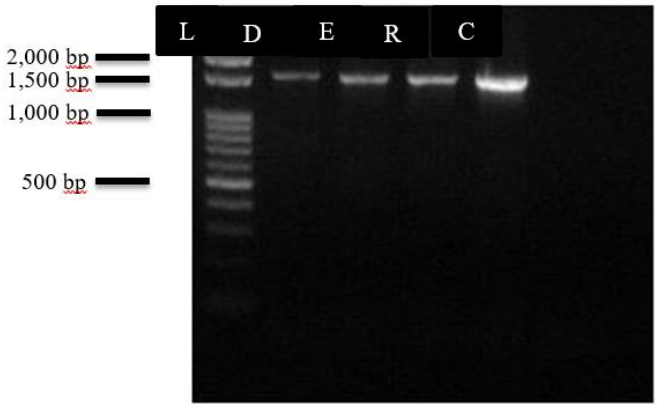


Fig. 4. PCR amplification map of LAB 16S DNA gene. Lane L: 100 bp ladder. Lane D: food sample from fermented *dabai.* Lane E: food sample from *Ensabi*. Lane R: food sample from *rusip.* Lane C: Positive control : Synthetic non-biological 16S DNA

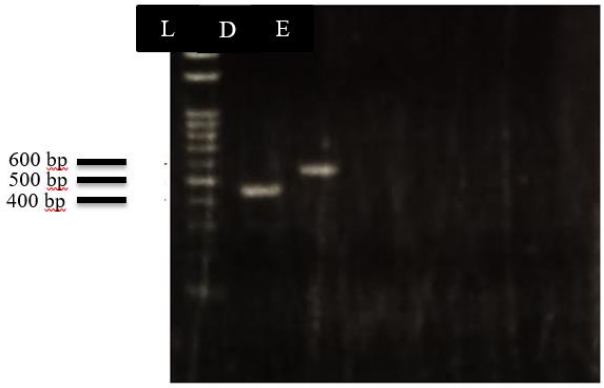


Fig. 5. ITS rRNA PCR amplification map of yeast isolates. Lane L: 100 bp ladder. Lane D: food sample from fermented *dabai.* Lane E: food sample from *ensabi.*

As been confirmed using DNA sequencing**,** a total of 45.2% (113/250) Lactic Acid Bcateria (LAB) which are 96% (48 /50) of *W. paramesenteroides* was detected in fermented *dabai,* 80% (40/50) *S. pasteuri* in fermented fish *rusip* samples and 50% (25/50) in *P. agglomerans* in *cencaluk* samples. Meanwhile there are no detection of LABs in fermented *ensabii* and fermented fish. The yeast *Candida species* confirmed with 90% (45/50) of *C. magnoliae* and 50% (25/50) of *C. parapsilosis* are detected in fermented *dabai* and *ensabi* respectively while there is no detection of it in fermented *fish,* fermented fish (*rusip)* can *cencaluk (shrimp).*

4. Discussion

Identification of the LAB and yeast was using a common method of using agar media to observe the morphology and gram staining of the microorganism. It is important to recognize the morphology of the microorganism to validate the behaviour, biological function, and interaction with another organism. Generally, LAB is a gram-positive bacterium, anaerobic and produce lactic acid. As a result, 45.2% bacteria are gram positive which are *W. paramesenteroides and S. pasteuri* as the staining shows the purple colour or rod or cocci shape.

Weissella sp. belong to phylum *Firmicutes,* class *Bacilli,* order *Lactobacillales* and family *Leuconostocaceae* [15,16]. This rods or ovoid-shaped cocci bacteria is difficult to differentiate with another genus such as Leuconostoc and *Lactobacillus* [17]. To get a precise identification of this species, it is only possible when using molecular identification such as 16S rRNA [16]. All *Weissella sp.* are catalase negative, non-endospore forming cell, obligately heterofermentative and fermentation of glucose producing carbon dioxide, ethanol and/or acetate depending on species [15,17].

W. paramesenteroides which is found in *dabai* is closely related to *Leuconostoc mesenteroides* which causes the re-classification of this group as a new species by Gavie in 1967. The advantage characteristic of this species is it can produce bacteriocins [16]. The production of the bacteriocin helps to inhibit the growth of other closed related bacterial strain especially the pathogenic microorganism in food. The configuration of lactic acid is either DL or D(-) *W. paramesenteroides* can be found in rhizosphere of olive tree, raw milk cheese and fermented sausage [16].

S. pasteuri is one of the *Staphylococcus* species which are gram positive and cocci in shaped. Other than detecting *S. pasteuri* in rusip, there is limited study in the ecology of the *S. pasteuri* but there is reported that this species presence in meat product such as sausage [18] (Hong et al., 2018). As mentioned by Savini et al. [19], despite its roles which can cause disease such as nosocomial infections, it remains controversial issues as this bacterium able to express resistance to several antibiotic compound. According to Hong et al. [20], *S. pasteuri* strains have the high antimicrobial activities in vegetables. This can be proven from the previous study by Hong et al. [20], that *S. pasteuri* RSP-1 have a strong antimicrobial activity against *Staphylococcus aureus,* a strong antibiotic resistance bacterium.

P. agglomerans originated from family *Enterobacteriaceae* is a bacterium that associated with plants and can cause infection to human and vertebrates [21]. *P. agglomerans* was enriched in healthy hepatopancreas of shrimp [22]. From the research by Cornejo-Granados et al. [22], the lipopolysaccharides of *P. agglomerans* boost the disease resistance against penaeid acute viremia in kuruma shrimp. This proves that the presence of *P. agglomerans* prevents the presence of food-borne pathogen in cencaluk. Other than that, during the fermentation of rye sourdoughs, *P. agglomerans* is also used prior the growth of lactobacilli to provide essential vitamin for the growth of lactobacilli [23]. In recent study, *P. agglomerans* plays role as an antibiotic for cancer patient as a biopesticide and food preservative [24].

Even though *P. agglomerans* does not included as LAB but it can produce bacteriocin same as *W. paramesenteroides* and *S. pasteuri*. All these species of microorganism have the high potential probiotic against food-borne pathogen. Bacteriocin producing bacteria have the high chance of survival as they can eliminate others microorganism. This indicates that the presence of these species in the Sarawakian fermented food will added values to the local product.

Other than the presence of LAB, yeast also presence in the fermented food to enhance more quality food. In this study two species of *Candida sp.* were found in *dabai* and *ensabi. Candida* sp. physiology appear in two forms either in yeast or filamentous form [25]. Gastrointestinal tract and mucosal surfaces are the main reservoirs of *Candida* sp. As for *C. magnoliae,* it appears in white to creamy coloured yeast form (oval-shaped budding cells) [26]. *C. magnoliae* are mostly found in honey and during fermentation of cocoa beans [27,28]. It also plays an important role in food and pharmaceutical industry as it produces erythritol and mannitol [27,29]. Erythritol and mannitol is a low-calorie sweetener is produced using biotechnology method and it is safe to consume by diabetic patient [30,26]. Both sugar alcohols also can be found naturally in fruits and vegetables and be consumed by *C. magnoliae* [31]. Another unique characteristics of *C. magnoliae* is its fructophilic behaviour. This characteristic made this species to consume fructose quickly compared to other

sugars. From this study, *C. magnoliae* consume the fructose from the dabai fruit. This *Candida* species never associated with human disease until a report made by Lo Cascio et al. [26], where a 42-year-old man was infected by *C. magnoliae.*

In addition, Candida *species* found in fermented food is *C. parapsilosis.* This type of species was dominantly found during the alkaline fermentation during the Spanish green olive processing to eliminate the bitterness of the fruit and *Kinema,* a traditional Indian fermented soybean [32,33]. Since *ensabi* is a mustard green leaf, this may be the reason *C. parapsilosis* found in the *kasam ensabi*. Other than can be found in environment such as soil, seawater and plant, *C. parapsilosis* can also be isolated form mucosal surface, skin, and nail [34]. According to Van Asbeck et al. [34], *C. parapsilosis* categorized as non-pathogenic organism in normal flora in human body. However, it is now known as a top opportunistic and nosocomial pathogen as it can cause wide range of infection from thrush to invasive disease such as fungemia and arthritis. From the statement above, there are no infection documented through the consumption of contaminated food from the *C. parapsilosis* [14].

Both mentioned yeasts share a common characteristic which both play important roles in providing good taste for the food. Even though some of the fermented food was dominated by LAB, yeast plays the secondary roles in ripening and flavour development [34]. Getting rid the off flavour from the food enhance the food acceptability for human consumption.

Another finding of this study is that LAB and yeast can cohabiting in a same food. Microbial community of LAB and yeast can contribute to texture and safety of the final products. Yeast is responsible for the production of ethanol while LAB produce lactic acid. *W. paramesenteroides* and *C. magnoliae* was found associated in fermented *dabai*. The presence of both bacteria and yeast in fermented *dabai* makes the smell of the food favours to human especially to Sarawak community. This can be proven from the previous study by Carvalho et al. [36], the mixed fermentation of the LAB, *Lactococcus lactis* and yeast, *Saccharomyces cerevisiae* in cachaça, a type of sugar cane spirit produced in Brazil. The study shows a significant levels of ethanol content in cachaça from the mixed fermentation. As the result from the tasting evaluation, the cachaça obtained the higher scores in aroma and appearance categories [36].

5. Conclusion

In this study, LAB and yeast have been identified in the Sarawak fermented food. The LAB species which are *W. paramesenteroides* and *S. pasteuri* and two different yeast strain, *C. magnoliae* and *C. parapsilosis* was found in the fermented food. The findings in this study revealed for the first time the Lactic Acid Bacteria (LAB) diversity and their possible role in Sarawak fermented food, besides, determine the beneficial bacterial in Sarawak fermented food through molecular approach. A further study on the microbial community in the fermentation is essential to observe the population and interaction between different species of microorganism. With the development of biotechnology in food industry can guarantee a better quality of food product to provide a high nutritional value for human consumption.

Acknowledgements

Research fund was sponsored by F07/FRGS/1873/2019 Universiti Malaysia Sarawak (Unimas) Kota Samarahan, Kuching Sarawak.

References

[1] Nading, N. and H. N. Ismail. 2015. “Domestic tourist's perception on Sarawak ethnic native's food” (Unpublished master's thesis). Universiti Teknologi Malaysia.

[2] Balamurugan Jagadeesana, Peter Gerner-Smidtb, Marc W. Allardc, Sébastien Leuilletd, Anett Winklere, Yinghua Xiaof, Samuel Chaffrong, Jos Van Der Vossenh, Silin Tangi, Mitsuru Katasej, Peter McClurek, Bon Kimural, Lay Ching Chaim, John Chapmann, Kathie Granto. 2019. The use of next generation sequencing for improving food safety:.Translation into practice. Food Microbiology 79 (2019) 96–115

[3] Ali, F. W., Saari, N., Bakar, F. A., Abdulamir, A., Mohammed, A. S. and A. H. Zulkifli. 2009. "Novel, Practical and Cheap Source for Isolating Beneficial γ-Aminobutyric Acid-Producing Leuconostoc NC5 Bacteria." *Research Journal of Medical Sciences* 3 (4): 146-153. http://docsdrive.com/pdfs/medwelljournals/rjmsci/2009/146-153.pdf.

[4] Jeppesen, V. F. and H. H. Huss. 1993. "Characteristics and antagonistic activity of lactic acid bacteria isolated from chilled fish products." *International Journal of Food Microbiology* 18 (4): 305-320. doi:10.1016/0168-1605(93)90153-8.

[5] Choi, H.J., Lee, H.S., Her, S., Oh, D.H. and S. S. Yoon. 1999. "Partial characterization and cloning of leuconocin J, a bacteriocin produced by *Leuconostoc* sp. J2 isolated from the Korean fermented vegetable Kimchi." *Journal of Applied Microbiology* 86 (2): 175-181. doi:10.1046/j.1365-2672.1999.00471.

[6] O'Sullivan, L., Ross, R. and C. Hill. 2002. "Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality." *Biochimie* 84 (5-6): 593-604. doi:10.1016/s0300-9084(02)01457-8.

[7] Jack, R. W., Tagg, J. R. and B. Ray, B. 1995. "Bacteriocins of gram-positive bacteria." *Microbiological Reviews* 59 (2): 171-200. doi:10.1128/mmbr.59.2.171-200.1995.

[8] Klaenhammer, T. R. 1988. "Bacteriocins of lactic acid bacteria." *Biochimie* 70: 337-349.

[9] Tagg, J. R., Dajani, A. S. and L. W. Wannamaker. 1976. Bacteriocins of gram-positive bacteria. *Bacteriological Reviews* 40 (3): 722-756. doi:10.1128/mmbr.40.3.722-756.1976.

[10] Lindgren, S. E. and W. J. Dobrogosz. 1990. "Antagonistic activities of lactic acid bacteria in food and feed fermentations." *FEMS Microbiology Letters* 87 (1-2): 149-164. doi:10.1111/j.1574-6968.1990.tb04885.

[11] Kergourlay, G., Taminiau, B., Daube, G. and M. C. Vergès. 2015. "Metagenomic insights into the dynamics of microbial communities in food." *International Journal of Food Microbiology* 213: 31-39. doi:10.1016/j.ijfoodmicro.2015.09.010.

[12] Giraffa, G. and E. Neviani. 2001. "DNA-based, culture-independent strategies for evaluating microbial communities in food-associated ecosystems." *International Journal of Food Microbiology* 67 (1-2): 19-34. doi:10.1016/s0168-1605(01)00445-7.

[13] Martín-Platero, A. M., Valdivia, E., Maqueda, M. and M. Martínez-Bueno. 2007. "Fast, convenient, and economical method for isolating genomic DNA from lactic acid bacteria using a modification of the protein "salting-out" procedure." *Analytical Biochemistry* 366 (1): 102-104. doi:10.1016/j.ab.2007.03.010.

[14] Banjara, N., Suhr, M. J. and H. E. Hallen-Adams. 2015. "Diversity of Yeast and Mold Species from a Variety of Cheese Types." *Current Microbiology* 70(6): 792-800. doi:10.1007/s00284-015-0790-1.

[15] Björkroth, J. A. and A. Endo. 2014. "The genus Weissella." In L. Dicks, W. Holzapfel, & B. Woods (Eds.), *Lactic Acid Bacteria, Biodiversity and Taxonomy* (pp. 418-428). Chichester: Wiley Blackwell.

[16] Fusco, V., Quero, G. M., Cho, G., Kabisch, J., Meske, D., Neve, H. and C. M. Franz. 2015. "The genus Weissella: Taxonomy, ecology, and biotechnological potential." *Frontiers in Microbiology* 6 (March). doi:10.3389/fmicb.2015.00155.

[17] Leblan, D. J. 2006. *The Prokaryotes: Vol. 4: Bacteria: Firmicutes, Cyanobacteria*. 3rd ed. NY, USA: Springer Science + Business Media. Retrieved from website: http://books.google.nl/books?id=C5tzLBabUh8C.

[18] Hong, J., Kim, J., Quan, L., Heu, S. and E. Roh. 2018. "Purification and Characterization of Pasteuricin Produced by *Staphylococcus pasteuri* RSP-1 and Active against Multidrug-Resistant *Staphylococcus aureus*." *Journal of Food Protection* 81 (11): 1768-1775. doi:10.4315/0362-028x.jfp-18-111.

[19] Savini, V., Catavitello, C., Bianco, A., Balbinot, A. and D. D'antonio. 2009. "Epidemiology, Pathogenicity and Emerging Resistances in *Staphylococcus pasteuri*: From Mammals and Lampreys, to Man." *Recent Patents on Anti-Infective Drug Discovery* 4 (2): 123-129. doi:10.2174/157489109788490352.

[20] Hong, J., Quan, L., Heu, S., Jung, K. S., Han, S., Moon, E. and E. Roh. 2014. "A new antimicrobial substance produced by *Staphylococcus pasteuri* isolated from vegetables." *Food Science and Biotechnology* 23 (3), 983-990. doi:10.1007/s10068-014-0133-1.

[21] Kaur, I. P., Inkollu, S., Prakash, A., Gandhi, H., Mughal, M. S. and D. Du. 2020. "*Pantoea agglomerans* Bacteremia: Is It Dangerous?." *Case Reports in Infectious Diseases* 2020 (September): 1–4. https://doi.org/10.1155/2020/7890305.

[22] Cornejo-Granados, F., Lopez-Zavala, A. A., Gallardo-Becerra, L., Mendoza-Vargas, A., Sánchez, F., Vichido, R. and A. Ochoa-Leyva. 2017. "Microbiome of Pacific Whiteleg shrimp reveals differential bacterial community composition between Wild, Aquacultured and AHPND/EMS outbreak conditions." *Scientific Reports* 7 (1): 1-15. doi:10.1038/s41598-017-11805-w2.

[23] Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L. and V. Piironen. 2006. "Effects of yeasts and bacteria on the levels of folates in rye sourdoughs." *International Journal of Food Microbiology* 106 (2): 137-143. doi:10.1016/j.ijfoodmicro.2005.06.013.

[24] Dutkiewicz, J., Mackiewicz, B., Lemieszek, M. K., Golec, M. and J. Milanowski. 2016. "*Pantoea agglomerans*: A mysterious bacterium of evil and good. Part IV. Beneficial effects." *Annals of Agricultural and Environmental Medicine* 23 (2): 206-222. doi:10.5604/12321966.1203879.

[25] Kadosh, D. and V. Mundodi. 2020. "A Re-Evaluation of the Relationship between Morphology and Pathogenicity in Candida Species." *Journal of Fungi* 2020 6 (1): 13. doi:10.3390/jof6010013

[26] La Cascio, G., Carbonare, L. D., Maccacaro, L., Caliari, F., Ligozzi, M., Cascio, V. L. and R. Fontana. 2007. "First Case of Bloodstream Infection Due to *Candida magnoliae* in a Chinese Oncological Patient." *Journal of Clinical Microbiology* 45 (10): 3470-3473. doi:10.1128/jcm.00934-07.

[27] Carvalho, C. M., Meirinho, S., Estevinho, M. L. F. and A. Choupina. 2010. "Yeast Species Associated with Honey: Different Identification Methods Especies De Levaduras Asociadas a La Miel: Métodos Diversos De Identificación." *Archivos de Zootecnia*, 59 (225): 103–113. https://scielo.isciii.es/pdf/azoo/v59n225/art11.pdf

[28] Miguel, M. G. da C. P., Reis, L. V. de C., Efraim, P., Santos, C., Lima, N. and R. F. Schwan. 2017. "Cocoa fermentation: Microbial identification by MALDI-TOF MS, and sensory evaluation of produced chocolate." *LWT - Food Science and Technology* 77: 362–369. https://doi.org/10.1016/j.lwt.2016.11.076.

[29] Saha, B. C. 2004. "Purification and Characterization of a Novel Mannitol Dehydrogenase from *Lactobacillus intermedius*." *Biotechnology Progress* 20 (2): 537-542. doi:10.1021/bp034277p.

[30] Kieliszek, M., Kot, A. M., Bzducha-Wróbel, A., Błażejak, S., Gientka, I. and A. Kurcz. 2017. "Biotechnological use of *Candida* yeasts in the food industry: A review." *Fungal Biology Reviews* 31 (4): 185-198. doi:10.1016/j.fbr.2017.06.001.

[31] Medeiros, A. S. S. 2014. "Fermentation of fruit juices by the osmotolerant yeast Candida magnoliae." Retrieved from https://run.unl.pt/bitstream/10362/13833/1/Medeiros_2014.pdf.

[32] Lara-Hidalgo, C. E., Hernández-Sánchez, H., Hernández-Rodríguez, C. and A. Dorantes-Álvarez. 2017. "Yeasts in Fermented Foods and their Probiotic Potential." *Austin Journal of Nutrition and Metabolism* 4 (4): 1045.

[33] Tamang, J. P. 2015. "Naturally fermented ethnic soybean foods of India." *Journal of Ethnic Foods* 2 (1), 8-17. doi:10.1016/j.jef.2015.02.003.

[34] Van Asbeck, E. C., Clemons, K. V. and D. A. Stevens. 2009. "*Candida parapsilosis*: A review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility." *Critical Reviews in Microbiology* 35 (4): 283-309. doi:10.3109/10408410903213393.

[35] Johnson, E. A. and C. Echavarri-Erasun. 2011. "Yeast biotechnology." In *The Yeasts* 1. https://doi.org/10.1016/B978-0-444-52149-1.00003-3.

[36] Carvalho, F. P., Duarte, W. F., Dias, D. R., Piccoli, R. H. and R. F. Schwan. 2015. "Interaction of Saccharomyces cerevisiae and Lactococcus lactis in the fermentation and quality of artisanal cachaça." *Acta Scientiarum - Agronomy* 37 (1): 51–60. https://doi.org/10.4025/actasciagron.v37i1.18397.