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Nutritional Requirement of Newly Isolated Wild Strain *Acetobacter tropicalis* for the Improvement of Acetic Acid Production

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ABSTRACT

The aim of this study was to determine the best nutritional requirements, focusing on sugar concentration and nitrogen sources, for a newly acetic acid strain *Acetobacter tropicalis* isolated from MD2 pineapple peels. To this aim, the identification of isolated strains from spontaneous fermentation of pineapple peel was done through PCR amplification and sequencing of the 16S rRNA gene. Identified *Acetobacter tropicalis* shows characteristics and biochemical traits align with *Acetobacter* genus. The study delves into the carbon and nitrogen source requirements for MD2 pineapple peel juice with varying sugar concentrations. The study explores how acidity, yield, and microbial growth are affected, uncovering optimal outcomes with particular sugar concentrations and levels of nitrogen sources. Notably, the addition of 0.25 g/L yeast extract resulted in the highest acidity of 36.03 g/L and product yield of 0.643 g acid/ g substrate and 0.011 g acid/ g biomass. Surprisingly, this new strain flourishes without a need of additional sugar. The findings contribute valuable information for optimizing acetic acid fermentation processes utilizing *Acetobacter tropicalis*, highlighting the importance of carbon and nitrogen sources for this strain.

1. Introduction

Acetic Acid Bacteria (AAB) represent an extensively researched bacterial group, owing to their distinctive metabolic capabilities at low pH, which make them highly adaptable than other bacterial cohorts [1]. They play a pivotal role in vinegar production and the development of chocolate flavor precursors during fermentation, primarily due to their capacity to convert alcohol (ethanol or wine) into acids [2]. Among the AAB group, *Acetobacter* sp. stands out as a prominent strain, commonly found in sourdoughs, cocoa fermentation [3], and spontaneously fermented wine [4]. Each AAB strain exhibits distinct nutritional requirements, including carbon and nitrogen sources. For example, a co-

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culture of *Saccharomyces cerevisiae* and *Acetobacter aceti* necessitates 80 g/L of glucose to achieve a maximum acetic acid yield of 57 g/L, while pure culture of *Acetobacter pasteurianus* under similar conditions, requires 200 g/L of total sugar to produce 47.9 g/L of acetic acid [5]. These variations in nutrient requirements and acetic acid production can be attributed to the specific strains employed [6].

Carbon sources are essential nutrients that support strain growth by consuming glucose through the glycolytic pathway (EMP) and the hexose monophosphate shunt (HMS), ultimately leading to pyruvate production. Pyruvate is then converted into acetaldehyde by terminal oxidase (PDB) [7]. Nitrogen, on the other hand, is vital for providing building blocks for microbial growth and enhancing enzyme complexes responsible for catalyzing acetic acid production [8]. A study by Román-Camacho *et al.,* [9] has demonstrated that the growth of AAB is optimal in the presence of a nitrogenous mixture. For instance, yeast extract, often referred to as "yeast food," is rich in essential nutrients such as free alpha-amino nitrogen, minerals, and vitamins, in addition to its antioxidative properties [8]. Furthermore, yeast extract could stimulate the growth of cells [10]. Simultaneously, peptone, a mixture of proteins and amino acids, contributes to enzymatic stability and promoting acetic acid production [11]. The combination of both compounds in the media results in optimal fermentation performance.

To date, no comprehensive study has investigated the nutritional requirements of the *Acetobacter tropicalis* strain isolated from MD2 pineapple peel. This research focuses on examining the sugar and nitrogen requirements of the newly isolated strain for acetic acid production. Prior to this, the *Acetobacter tropicalis* strain was isolated from spontaneously fermented MD2 pineapple peel and subsequently characterized through physical and biochemical analyses.

2. Methodology

2.1 Materials

MD2 pineapple was collected from a local market in Kuantan, Pahang, Malaysia. The clean peels were liquidized using an electrical blender (Panasonic MX-900M) at a ratio of 1:1 (w/v) with sterile distilled water based on the assumption of equal density [12]. The resulting pineapple peel juice was filtered through a 20 µm pore size coffee filter [13], and the juice was kept at -20 ℃ for further usage as fermentation media, also known also original pineapple peel juice (OPPJ).

Acetic acid of 99.8% purity, 99.7% ethanol, calcium carbonate, yeast extract, glucose, nutrient agar, nutrient broth, sucrose, and sodium hydroxide were obtained from R&M Chemicals (Malaysia). Other chemicals and standards were purchased from Sigma-Aldrich (USA).

2.2 Isolation, Identification and Characterization of AAB

Enrichment of pineapple peel juice was performed aerobically for 72 h at 30 ℃ with agitation at 200 rpm. The potential AAB isolates were identified in a selective Carr medium (0.04 g/L bromothymol blue, 20 g/L agar, 10 g/L yeast extract, 3 g/L glucose, 17.5 g/L (v/v) ethanol, and 10 g/L calcium carbonate) [14] and glucose-yeast extract-calcium carbonate (GYC) (50 g/L glucose, 10 g/L yeast extract, 5 g/L $CaCO_3$) [15]. The potential AAB strain was further assessed for acid production capability by single-stage fermentation using glucose-yeast extract-ethanol (GYE) medium (10 g/L yeast extract, 50 g/L glucose or 70 g/L (v/v) ethanol) at 30 ℃ with 200 rpm agitation for five days [15,16]. Further evaluation on acid-producing ability was evaluated on potency index (PI) using modified GYC agar comprised of 50 g/L glucose, 10 g/L yeast extract, 5 g/L $CaCO_3$, and 20 g/L agar in distilled water [15,17]. Selected strains were subjected to molecular identification using the universal

primers 27F and 1492R, PCR amplification of the 16S rRNA gene [18,19], and morphological (physical appearance and Gram stain) [18] and biochemical traits (catalase, oxidase, motility, and indole test) [14].

2.3 Carbon and Nitrogen Source Requirement

Pineapple peel juice prepared earlier was used as fermentation media, with sugar adjustment. Total sugar concentration of the juice was adjusted using glucose to reach five different concentrations: original pineapple peel (33 g/L sugar), 46, 57, 66, and 71 g/L. Ethanol (7 % v/v) and inoculum (5 % v/v) were also added, and the final working volume was 50 mL.

The nitrogen source used was peptone and yeast extracts. Four combinations were tested: no additional nitrogen source, 0.25 g/L yeast extract, 0.50 g/L peptone, and 0.25 g/L yeast extract + 0.50 g/L peptone. Ethanol (7 % v/v) and inoculum (5 % v/v) were also added, and the final working volume was 50 mL of the pineapple peel juice.

All fermentations were carried out at 30 ℃ for five days with agitation at 200 rpm. Sample was taken at the beginning and the end of the fermentation course and undergo centrifugation at 4000 rpm for 15 min for analytical methods. Sample for biomass quantification was without centrifugation.

2.4 Analytical Methods

2.4.1 Quantification of sugar and ethanol with high performance liquid chromatography

Standard sugar solutions were prepared for xylose, glucose, fructose, and sucrose. These represent the sugar composition in the pineapple peel juice. High performance liquid chromatography (HPLC) 1260 Infinity II (model number G7111B, serial number DEAET 00386, Agilent Technologies) system equipped with refractive index detector (RID) was employed to quantify the sugars according to the method provided by SUPELCOGEL (Cat. No. 59320-U).

Sugar separation was performed in C-610H column (SUPELCOGEL) with 280 mm length and 4.6 mm diameter injected with 10 µL of sample. 0.1 % of phosphoric acid at 0.50 mL/min constant flowrate was used as the mobile phase and the column temperature was set to 30 ℃. Ethanol separation was achieved using the same column and mobile phase, but at 0.70 mL/min constant flowrate with 50 ℃ column temperature. The mobile phase was filtered and degassed for 30 minutes before analysis [20].

2.4.2 Determination of total nitrogen

Total nitrogen content in the fermentation culture was determined using a total nitrogen kit by HACH, USA. All procedure was following the instructions given by the manual from the company. Sample digestion was done in a DR1900 HACH digital reactor block and optical density was read using DR900 HACH portable colorimeters with 394 N, Total HR TNT program.

2.4.3 Acidity determination

Total acidity in the samples was estimated using titration [21-23]. About 1.0 mL sample was mixed with a few drops of phenolphthalein and titrated using 0.1 M NaOH as the neutralizer. Each sample was titrated twice and the mean of the NaOH volume used was used to calculate the acidity following Eq. (1).

$$\text{Total acid } (g/L) = \left(\frac{0.1\text{ M NaOH } \times \text{Vol. of NaOH (L)}}{\text{Vol. of sample (L)}}\right) \times \text{Dilution Factor} \times 60.052 \text{ g/mol} \quad (1)$$

2.4.4 Biomass quantification

Cell growth was measured either as optical density (OD) at 600 nm with an ultraviolet spectrophotometer (UV Vis) or by measuring the cell dry weight (CDW). CDW was performed following method by Ming *et al.,* [24] dried for 24 h at 60 ℃.

2.5 Statistical Analysis

The experiment was conducted in duplicates. Microsoft Excel 2010 was applied to perform One-way Analysis of variance (ANOVA) with confidence interval of 95%.

3. Results

3.1 Isolation, Identification and Characteristic of AAB

Twenty-five bacterial isolates were retrieved from MD2 pineapple peel and were identified as acetic acid bacteria (AAB) based on their capability to grow as green colonies [25] on blue Carr agar, as depicted in Figure 1.

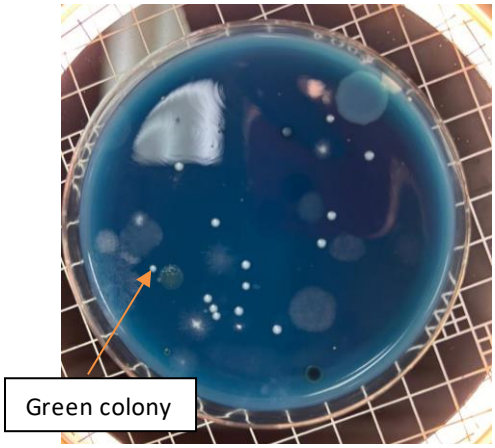

Fig. 1. Shades of green colonies formed on Carr agar with sample dilution of 10^{-7}

Figure 2 shows the acidification capacity based on acid concentration and potency index (PI). Out of the 25 strained tested, three isolates, 5, 19, and 22 displayed the highest acidification capacity, with the maximum acid production up to 6.76 g/L and maximum PI values of 6.2. These three isolates were further characterized for their morphology and biochemical traits.

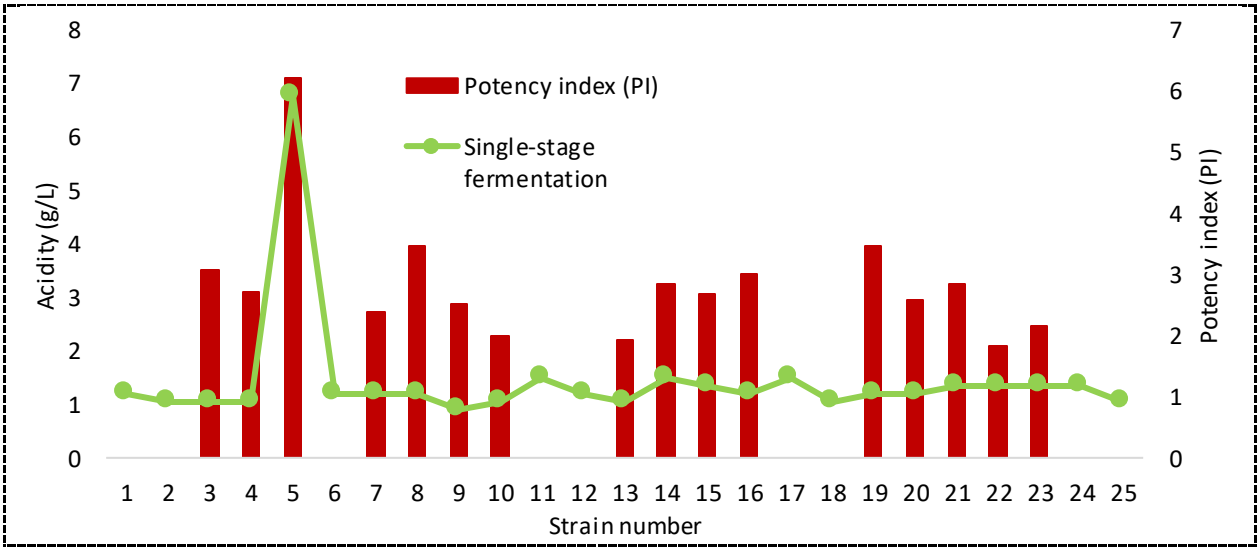

Fig. 2. Acidification capability of 25 isolates from MD2 pineapple peel

Microscopic examination of isolates 5, 19, and 22 on nutrient agar followed by Gram staining revealed the presence of an ellipsoidal rod-shaped cell with Gram-negative bacteria occurring in pairs and chains (Table 1). Biochemical tests confirmed that these isolates were catalase-positive, oxidase-negative, indole-negative and motility test-negative. These characteristics corresponded to the isolates' profile belonging to genus *Acetobacter*, as reported by El-Askri *et al.,* [18]. Current findings were consistent with those studies, which reported the presence of *Acetobacter fabarum* and *A. pasteurianus* displaying the same morphological traits as the isolate 5.

Table 1
Cell morphology and biochemical characteristics of selected isolates

Isolate number		5	19	22
Morphology characteristics	Cell shape	Rod	Cocci	Cocci
	Gram's stain	-	-	-
Biochemical characteristics	Catalase	+	+	+
	Oxidase	-	-	-
	Production of acetic acid from ethanol	+	+	+
	Motility test	-	-	-
	Indole test	-	-	-

PCR amplification and sequencing of the 16S rRNA gene were performed, and the species of isolates 5, 19, and 22 were determined. The nucleotide sequences of the isolates were compared to the NCBI library by constructing a phylogenetic tree [26]. The sequence of 16S rRNA gene from isolate 5 showed 100 % similarity with *Acetobacter tropicalis* Ni-6b strain, while isolate 19 had 98 % similarity with *Staphylococcus haemolyticus* and isolate 22 had 98 % identity with *Staphylococcus ureilyticus*. As a result, only isolate 5 was confirmed as AAB and selected for nutritional requirement study.

3.2 Carbon Requirement

Total sugar content in MD2 pineapple peel juice (OPPJ) was 32.79 g/L, comprising 8.66 g/L sucrose, 9.21 g/L glucose, and 14.91 g/L fructose. A different study by Tanamool *et al.,* [27] reported a lower sugar content (16.69 g/L) consist of 3.56 g/L sucrose, 6.08 g/L glucose, and 7.05 g/L fructose from a random pineapple species. In the process of fermentation, carbon sources are necessary to facilitate the initiation of yeast activity, leading to the production of ethanol during alcoholic

fermentation [28]. Nevertheless, in this study, a single-stage fermentation was performed which is an acetous fermentation, leaving out the alcoholic fermentation stage. Instead, ethanol was directly incorporated into the media. As a result, the carbon source was mainly utilized for the growth of isolate 5, *Acetobacter tropicalis*. In this way, ethanol undergoes an incomplete oxidation process through the catalysis of the ADH enzyme, resulting in the production of acetaldehyde. Consequently, acetaldehyde is converted into acetic acid by ALDH. The effect of different sugar concentration to acidity and yield is shown in Figure 3.

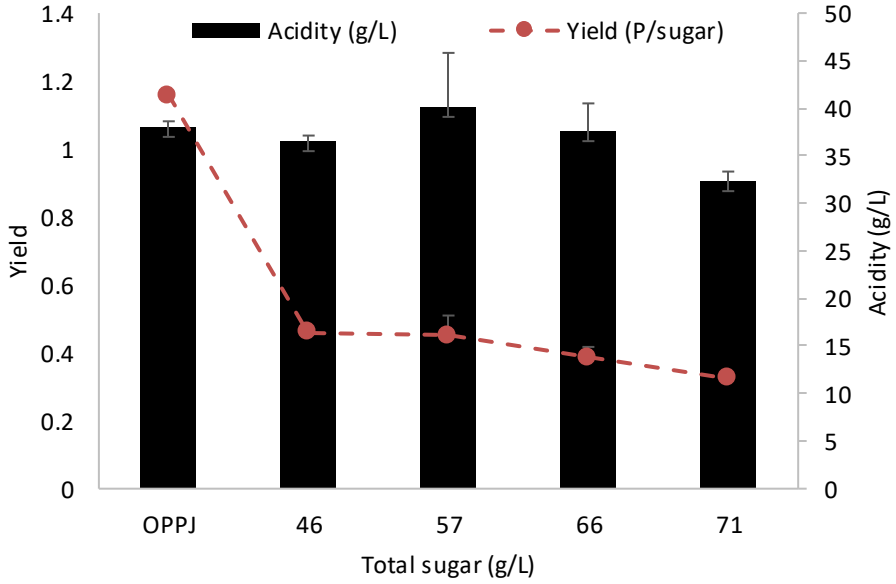

Fig. 3. Acidity and Yield (YP/S) as a function of different sugar concentration in the media. OPPJ: Original Pineapple Peel Juice

The highest level of acidity (40 g/L acid) was observed at a sugar concentration of 57 g/L while the yield was the highest in the OPPJ substrate (1.16 g acid/g sugar). From the figure, sugar addition into OPPJ fairly improved the acid production until certain level (57 g sugar/L). At higher sugar concentration, it was detrimental to acid production, as the acidity reduced to a minimum of 32.28 g acid/L at 71 g sugar/L. On contrary, study by Fronteras *et al.,* [5], shows a high requirement of sugar in their fermentation. Their study utilized calamansi and mango peel in a two-stages fermentation using co-culture of *Saccharomyces cerevisiae* and *Acetobacter aceti*. By using up to 250 g/L of sugar, both peel media produced 47.90 and 34.20 g/L of acetic acid for calamansi and mango, respectively. Another study by Tanamool *et al.,* [27], produces 72 g acid/L from 140 g sugar/L using co-culture of *Saccharomyces cerevisiae* and *Acetobacter pasteurianus*. These findings show a variety of carbon requirements, specifically sugar, will depend on the media and strain also the fermentation strategy involved, single or two-stages fermentation.

Another point to ponder is that acid yield, based on sugar consumption exhibits a negative effect as sugar concentration increased in the OPPJ media. Figure 3 shows that the highest yield, $Y_{p/s}$, was obtained in the original OPPJ media (1.158 g acid/g sugar). The yield was sharply decreased by more than 50 % as the total sugar increased up, to 71 g/L (0.324 g acid/g sugar). A similar trend was observed in a study by Fronteras *et al.,* [5], as the acetic acid yield was steadily decreased from 0.16 to 0.14 g acid/g sugar as the sugar concentration increased from 15 to 25 %. The reduced yield resulting from an increase of sugar concentrations suggests that the conditions were not optimized. Thus, the acidity generated ought to be proportional to the quantity of sugar necessary to achieve a higher yield.

3.3 Nitrogen Requirement

The study investigates the role of nitrogen as an essential nutrient in acetic acid fermentation, focusing on the influence of yeast extract and peptone on microbial growth and acetic acid production. The provided information by Sankuan *et al.,* [29] emphasizes the importance of nitrogen in the biosynthesis of key cellular components, including amino acids, proteins, and nucleotides. Yeast extract and peptone, rich in essential nutrients, are commonly used in food industries and bacterial culture media [8].

The nitrogen content was tested in four conditions which were the original OPPJ without nitrogen source addition, 0.25 g/L yeast extract, 0.50 g/L peptone, and a combination of 0.25 g/L yeast extract and 0.50 g/L peptone. The results, illustrated in Figure 4, indicate that the addition of 0.25 g/L yeast extract produced the highest acidity at 36.03 g/L, suggesting an enhance acetate assimilation and microbial growth [30]. The original OPPJ media produces the second-best acid (34.98 g/L) and a remarkable product over substrate yield, $Y_{P/S}$ (3.18 g acid/g sugar) than the other media formulation. Moving on to another nitrogen source addition, OPPJ with 0.50 g/L peptone produced 34.68 g/L acid and the lowest was in OPPJ with a mixture of 0.25 g/L yeast extract and 0.50 g/L peptone (33.78 g/L acid).

Acetobacter possesses the ability to utilize acetic acid through the action of acetyl-CoA synthetase (acs), which converts acetate into acetyl-CoA and citrate synthase (aarA), thereby allowing acetyl-CoA to enter the TCA cycle. This process facilitates the removal of acetic acid from the cytoplasm via the TCA cycle, thereby reducing its deleterious effects on the cytoplasm. The fact that *Acetobacter* can acidify its cytoplasm suggests that it contains substances that can adapt to an acidic environment, which aids in the production of high concentrations of acetic acid and decreases the growth doubling time [7]. Another point to note is that cell over substrate yield, $Y_{X/S}$ for all fermentation media, were barely changing in a range from 0.005 to 0.099 g/g (Figure 4). This finding aligns with Nutongkaew *et al.,* [31] study, which demonstrated optimal acetic acid production (2.87 g/L) was without nitrogen addition in a co-culture fermentation using oil palm trunk residues hydrolysate.

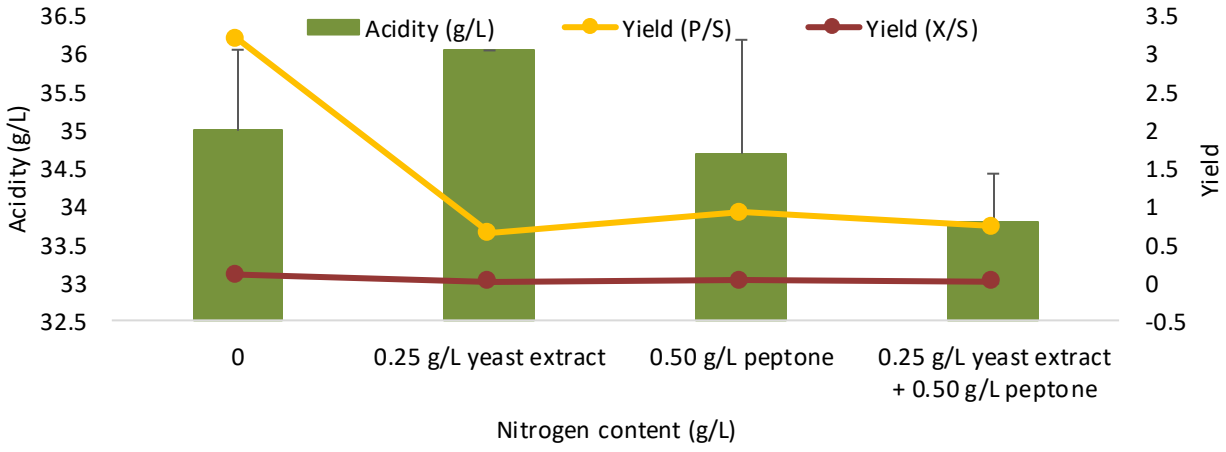

Fig. 4. Acidity and product yield as a function of different nitrogen sources in OPPJ

4. Conclusions

The newly isolated strain from MD2 pineapple peel was identified as *Acetobacter tropicalis*, exhibited the traits of AAB. This strain shows an affinity with the original pineapple peel juice (OPPJ)

media with a maximum acid production of 37.98 g acid/L, without any additional sugar and nitrogen source enhancement. This early investigation provides valuable insights into the isolation, identification and characteristics of new AAB strain from MD2 pineapple peel and its nutrition requirements to grow and produce acid. Further research can build upon these results for fermentation processes to maximize the cell growth and acetic acid production.

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References

[1] Jamsheera, C.P. and B.V. Pradeep. "Antibacterial Activity of Silver Functionalized Bacterial Cellulose isolated from *Acetobacter Tropicalis* Jcp OK356208.1 from Rotten Orange." *Research Journal of Biotechnology* 18, no. 7 (2023): 105-120. https://doi.org/10.25303/1807rjbt1050120

[2] Hommel, R.K. "Encyclopedia of Food Microbiology (Second Edition)." *Reference Module in Food Science* 1 (2014): 3-10. https://doi.org/10.1016/B978-0-12-384730-0.00001-X

[3] Robledo-Márquez, Karina, Victoria Ramírez, Aarón Fernando González-Córdova, Yadira Ramírez-Rodríguez, Luis García-Ortega, and Joyce Trujillo. "Research opportunities: Traditional fermented beverages in Mexico. Cultural, microbiological, chemical, and functional aspects." *Food Research International* 147 (2021): 110482.

[4] Silhavy, K. A. R. I. N., and K. A. R. I. N. Mandl. "Acetobacter tropicalis in spontaneously fermented wines with vinegar fermentation in Austria." *Mitteilungen Klosterneuburg* 56 (2006): 102-107.

[5] Fronteras, Jennifer P., Dominica DM Dacera, Daisic D. Bello, and Kathleen Joy L. Delos Santos. "Utilization of pesticide-free calamansi (Citrus microcarpa) and mango (Mangifera indica) peels for the production of acetic acid with potential industrial application." *Bioresource Technology Reports* 15 (2021): 100806. https://doi.org/10.1016/j.biteb.2021.100806

[6] Selvanathan, Yashini, Saleha Shamsudin, Norazwina Zainol, and Nasratun Masngut. "Nutritional Requirements and Fermentation Condition for Acetic Acid Production From Agricultural Fruit Waste." *Chemical Engineering & Technology*: e202300581.

[7] Qiu, Xiaoman, Yao Zhang, and Housheng Hong. "Classification of acetic acid bacteria and their acid resistant mechanism." *Amb Express* 11, no. 1 (2021): 29. https://doi.org/10.1186/s13568-021-01189-6

[8] Jacob, F. F., M. Michel, M. Zarnkow, M. Hutzler, and F. J. Methner. "The complexity of yeast extracts and its consequences on the utility in brewing: A review." *BrewingScience* 72 (2019): 50-62.

[9] Román-Camacho, Juan J., Inés M. Santos-Dueñas, Isidoro García-García, Jaime Moreno-García, Teresa García-Martínez, and Juan C. Mauricio. "Metaproteomics of microbiota involved in submerged culture production of alcohol wine vinegar: A first approach." *International Journal of Food Microbiology* 333 (2020): 108797. https://doi.org/10.1016/j.ijfoodmicro.2020.108797

[10] Nasrah, Nur Syazana Muhamad, Mior Ahmad Khushairi Mohd Zahari, Nasratun Masngut, and Hidayah Ariffin. "Statistical optimization for biobutanol production by clostridium acetobutylicum ATCC 824 from Oil Palm Frond (OPF) juice using response surface methodology." In *MATEC Web of Conferences*, vol. 111, p. 03001. EDP Sciences, 2017. https://doi.org/10.1051/matecconf/201711103001

[11] Nancib, N., C. Branlant, and Joseph Boudrant. "Metabolic roles of peptone and yeast extract for the culture of a recombinant strain of Escherichia coli." *Journal of industrial microbiology* 8, no. 3 (1991): 165-169. https://doi.org/10.1007/BF01575849

[12] Selvanathan, Yashini, and Nasratun Masngut. "A statistical study of factors affecting natural biovinegar fermentation from pineapple peel waste." In *IOP Conference Series: Materials Science and Engineering*, vol. 1092, no. 1, p. 012004. IOP Publishing, 2021. https://doi.org/10.1088/1757-899X/1092/1/012004

[13] Ridzuan, N. A. M., S. M. Shaarani, Z. I. M. Arshad, N. Masngut, N. Zainol, and J. H. Shariffuddin. "Study on enzyme activities in pineapple fruit and pineapple waste to be applied as poultry supplement." In *IOP Conference Series: Materials Science and Engineering*, vol. 991, no. 1, p. 012064. IOP Publishing, 2020. https://doi.org/10.1088/1757-899X/991/1/012064

[14] Mathew, Bernadette, Shaily Agrawal, Nandita Nashikkar, Sunita Bundale, and Avinash Upadhyay. "Isolation of acetic acid bacteria and preparation of starter culture for apple cider vinegar fermentation." *Advances in Microbiology* 9, no. 6 (2019): 556-569. https://doi.org/10.4236/aim.2019.96034

[15] Kim, Dong-Hyeon, Jung-Whan Chon, Hyunsook Kim, and Kun-Ho Seo. "Development of a novel selective medium for the isolation and enumeration of acetic acid bacteria from various foods." *Food Control* 106 (2019): 106717. https://doi.org/10.1016/j.foodcont.2019.106717

[16] Boonsupa, W. "Chemical properties, antioxidant activities and sensory evaluation of mango vinegar." (2019): 229-240.

[17] Islam, Tahsina, Farzana Diba, Roni Miah, Ayesha Siddiqa, Nafisa Azmuda, Shamsun Nahar, Nihad Adnan, Shuvra Kanti Dey, and Ali Azam Talukder. "Optimization of acetic acid production rate by thermotolerant Acetobacter spp." *Advances in Microbiology* 7, no. 11 (2017): 749-759. https://doi.org/10.4236/aim.2017.711060

[18] El-Askri, Taoufik, Meriem Yatim, Youness Sehli, Abdelilah Rahou, Abdelhaq Belhaj, Remedios Castro, Enrique Durán-Guerrero, Majida Hafidi, and Rachid Zouhair. "Screening and characterization of new Acetobacter fabarum and Acetobacter pasteurianus strains with high ethanol–thermo tolerance and the optimization of acetic acid production." *Microorganisms* 10, no. 9 (2022): 1741. https://doi.org/10.3390/microorganisms10091741

[19] Gerard, Liliana Mabel, Cristina Verónica Davies, Carina Alejandra Soldá, María Belén Corrado, and María Verónica Fernández. "Application of molecular methods for the identification of acetic acid bacteria isolated from blueberries and citrus fruits." (2020): 193-205. https://doi.org/10.4014/mbl.1912.12006

[20] Ariffin, K. K., N. Masngut, M. N. A. Seman, S. M. Saufi, S. Jamek, and M. S. M. Sueb. "Dilute acid hydrolysis pretreatment for sugar and organic acid production from pineapple residues." In *IOP Conference Series: Materials Science and Engineering*, vol. 991, no. 1, p. 012057. IOP Publishing, 2020. https://doi.org/10.1088/1757-899X/991/1/012057

[21] Selvanathan, Yashini, and Nasratun Masngut. "Optimization of process factor and characterization of vinegar-like beverage production via spontaneous fermentation from pineapple peel waste." *LWT* 182 (2023): 114818. https://doi.org/10.1016/j.lwt.2023.114818

[22] Selvanathan, Yashini K., Shalyda M. Shaarani, and Nasratun Masngut. "Factorial analysis on biovinegar production from pineapple waste using mixed strains." *Journal of Chemical Engineering and Industrial Biotechnology* 6, no. 1 (2020): 32-38. https://doi.org/10.15282/jceib.v6i1.4725

[23] Rahman, Mohd Nazri Abdul, Viha Vivilin Masawal, Rachel Yieng Qing Yii, Natasha Narudin, Rovina Kobun, Nor Hayati Muhammad, Edwin Yau Chen Ng, and Muh Ade Artasasta. "Innovative Development of Instant Kombucha Drink and Kombucha Ice Cream Using Spray Drying Techniques." *International Journal of Advanced Research in Food Science and Agriculture Technology* 2, no. 1 (2024): 8-30. https://doi.org/10.37934/fsat.2.1.830

[24] Ming, Yue, Guoqiang Li, Zhuangzhuang Shi, Xin Zhao, Yufei Zhao, Ge Gao, Ting Ma, and Mengmeng Wu. "Co-utilization of glucose and xylose for the production of poly-β-hydroxybutyrate (PHB) by Sphingomonas sanxanigenens NX02." *Microbial Cell Factories* 22, no. 1 (2023): 162. https://doi.org/10.1186/s12934-023-02159-2

[25] Selvanathan, Yashini, Nor Hasmaliana Abdul Manas, Norazwina Zainol, and Nasratun Masngut. "Endogenous ethanol-and thermo-tolerant strain from Malaysia pineapple peel: Screening and molecular characterisation." *Food Bioscience* 62 (2024): 105198. https://doi.org/10.1016/j.fbio.2024.105198

[26] Clarridge III, Jill E. "Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases." *Clinical microbiology reviews* 17, no. 4 (2004): 840-862. https://doi.org/10.1128/CMR.17.4.840-862.2004

[27] Tanamool, Varavut, Mallika Chantarangsee, and Wichai Soemphol. "Simultaneous vinegar fermentation from a pineapple by-product using the co-inoculation of yeast and thermotolerant acetic acid bacteria and their physiochemical properties." *3 Biotech* 10, no. 3 (2020): 115. https://doi.org/10.1007/s13205-020-2119-4

[28] Yerolla, Raju, Sri Harsha Nekkanti, Suhailam Pullanikkattil, and Chandra Shekar Besta. "Modeling and Control of Beer Fermentation for Optimal Flavor and Performance." *Chemical Engineering & Technology* 46, no. 8 (2023): 1554-1565. https://doi.org/10.1002/ceat.202200376

[29] Sankuan, Xie, Zhao Cuimei, Fan Bingqian, Zheng Yu, Xia Menglei, Tu Linna, Song Jia, Zhao Xinyi, and Wang Min. "Metabolic network of ammonium in cereal vinegar solid-state fermentation and its response to acid stress." *Food microbiology* 95 (2021): 103684. https://doi.org/10.1016/j.fm.2020.103684

[30] Davami, Fatemeh, Farnaz Eghbalpour, Leila Nematollahi, Farzaneh Barkhordari, and Fereidoun Mahboudi. "Effects of peptone supplementation in different culture media on growth, metabolic pathway and productivity of CHO DG44 cells; a new insight into amino acid profiles." *Iranian biomedical journal* 19, no. 4 (2015): 194.

[31] Nutongkaew, Tanawut, Poonsuk Prasertsan, Chonticha Leamdum, Supalak Sattayasamitsathit, and Pongsak Noparat. "Bioconversion of oil palm trunk residues hydrolyzed by enzymes from newly isolated fungi and use for ethanol and acetic acid production under two-stage and simultaneous fermentation." *Waste and Biomass Valorization* 11 (2020): 1333-1347. https://doi.org/10.1007/s12649-019-00678-x