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Characterization of Thermotolerant Acetic Acid Bacteria Isolated from Various Plant Beverages in Thailand

Panadda Charee[1], Varavut Tanamool[2], Hirohide Toyama[3], Wichai Soemphol[1,4]*

1-Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai, Thailand 43000.

2-Chemistry Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand 30000.

3-Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan 093-0213.

4-Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand 40002.

Abstract

Background and objective: Thermotolerant acetic acid bacteria are more resistant to high temperatures than that other bacteria are. This difference includes 5-10ºC, which decreases high costs of cooling systems needed for the growth of these bacteria while preserving their high productivity. In this study, effective thermotolerant acetic acid bacteria isolated from various fermented plant beverages were characterized for their ability to produce acetic acid and their characteristics were investigated.

Materials and methods: Various thermotolerant acetic acid bacteria isolated from fermented plant beverages samples were investigated for their ability to produce acetic acid at high temperature. Two isolates of *Acetobacter pasteurianus* were selected and their growth and acetic acid production ability were assessed under various conditions. Furthermore, capsular polysaccharides of these strains were extracted and characterized using gas-liquid chromatography and Fourier-transform infrared spectroscopy.

Results and conclusion: In this study, *Acetobacter pasteurianus* and *Acetobacter tropicalis* were dominant thermotolerant acetic acid bacteria in the fermented plant beverage. Two isolates of *Acetobacter pasteurianus*, Fermented Plant Beverages 2-3 and 2-16, produced acetic acid effectively at 39°C, compared to that *Acetobacter pasteurianus* SKU1108 did as a superior control for thermotolerant acetic acid bacteria. Fermented Plant Beverage 2-3 was able to completely oxidize 5% v v^{-1} ethanol to acetic acid at 39°C, while a long lag time was observed at 6% ethanol. However, production of acetic acid was still up to 4% w v^{-1}. Moreover, this isolate exhibited excellent resistance to acetic acid at high temperatures, compared to 2 other strains in liquid and solid media. However capsular polysaccharides isolated from the 3 strains included glucose, rhamnose and galactose. Fourier-transform infrared spectroscopy spectra of capsular polysaccharides from Fermented Plant Beverage 2-3 at 1740 cm^{-1} identified a clearly different *O*-acetyl ester. This might be attributed to different *O*-acetyl ester contents of the capsular polysaccharides and also to resistance to acetic acid and high temperature.

Conflict of interest: The authors declare no conflict of interest.

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***Corresponding author:**
Wichai Soemphol,
Fermentation Research Center for Value Added Agricultural Products (FerVAAP), Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand 40002.

E-mail: wichso@kku.ac.th

1. Introduction

Acetic acid bacteria (AAB) are ubiquitous micro-organisms in natural alcohol and sugar-rich resources and various fermented products [1-4]. Furthermore, they are found as symbiotic microorganisms in insects [5]. In addition to well-recognized vinegar producers in Genera Acetobacter and Komagataeibacter (formally Gluconaceto-bacter), other AAB are capable of catalysing incomplete oxidation of sugar alcohols and sugar acids, resulting in elevated accumulation of the corresponding products in culture media [6,7]. Such an oxidation reaction, also called

oxidative fermentation, has been popular in industrial fermentations of various value-added biotechnological products [8]. During acetic acid fermentation, heat is generated through biological activities as well as fermentation processes. In general, fermentation needs a high-cost cooling control system. Thermotolerant AAB (TH-AAB) are bacteria resistant to temperatures, 5-10ºC higher than other bacteria within the same genera. This characteristic makes these bacterial strains appropriate bacteria to solve the high-cost problem of acetic acid production while preserving their high productivities. Several TH-AAB strains have been isolated and characterized for their properties in high-temperature fermentations [9-12]. The mechanism related to thermo-tolerance has been speculated to be a different mechanism from the common heat-shock mechanism, generating through adaptation under various sub lethal stress conditions and modification of genetic elements [13-15]. Therefore, strains with tolerance to such stress conditions may carry mutations that allow them to better tolerate high temperatures. Effective AAB can be isolated from natural sources that may be different from isolates from in vitro adaptations.

Recently, fermented plant beverages (FPBs) produced from a variety of plants have been favourite in Thailand because FPBs are publically believed to promote general health due to their high nutritional values and bioactive compounds derived from raw materials during the fermentation processes [16]. Moreover, FPBs are capable of inhibiting pathogenic bacteria and acting as probiotics [17,18]. Biotransformation of various FPBs has been investigated and revealed that two groups of micro-organisms contribute to fermentation, including yeasts and lactic acid bacteria (LAB) [19].

Thick pellicles of bacterial cellulose produced by AAB have been reported in most FPB products, which are partly similar to the tea fungus of Kombucha. Production of FPBs is frequently initiated at the start of a new batch of fermentation after supplying sufficient nutrients to culture media in the original container and setting them to ferment without controlling the temperature. These microorganisms must tolerate fluctuating temperatures, especially in summer. In addition to yeasts and LAB, AAB are speculated to possibly adapt after long successive fermentation processes.

In the current study, effective TH-AAB from various FPBs were screened. Various AAB were selected and assessed based on their ability to ferment acetic acid at high temperatures compared to TH-AAB *Acetobacter (A.*) *pasteurianus* SKU1108 and *A. tropicalis* SKU1100.

2. Materials and methods

2-1 Bacterial strains and culture conditions

Fermented plant beverage samples of individual plants were collected from Chonburi Province, Thailand. Most of these samples showed pellicle formation on top surface of the culture media. The *A. pasteurianus* SKU1108 (NBRC101655) and *A. tropicalis* SKU1100 (NBRC101654) were used as references for effective TH-AAB. Strains were pre-cultured in potato media (10 g of glucose, 20 g of glycerol, 5 g of peptone, 5 g of yeast extract and 100 ml of potato extract in 1 l of water) and incubated at 30ºC with shaking at 200 rpm (Vision Scientific, Korea). The YPGD media (5 g of glucose, 5 g of glycerol, 5 g of yeast extract and 5 g of peptone in 1 L of water) was used for comparing bacterial growth and acid production. The AAB strains were cultured on potato agar plates containing 1.5% w v^{-1} of agar and 0.5% w v^{-1} of $CaCO_3$ and incubated at 30°C for 24 h before storing at 4°C.

2-2 Screening of TH-AAB

Inoculating loopfuls of the samples were streaked on YPGE agar plates consisting of 2% of glycerol, 0.5% of yeast extract, 1% of peptone, 4% of ethanol v v^{-1}, 0.003% of bromocresol green and 0.06% of cycloheximide for antifungal activities. All colonies exhibiting yellow-halo zones were collected by patching on YPGE containing 0.5% of $CaCO_3$ instead of bromocresol green for verifications. Pure cultures were achieved by repeating streaks on the same agar media. A single colony was assessed for catalase activity using drops of 30% hydrogen peroxide (H_2O_2) to eliminate contaminations by LAB. All AAB isolates were replicated on YPGD containing 4% v v^{-1} of ethanol and their growth abilities were compared at 30, 37 and 39°C. After incubation for two days, isolates enable of growing at 39°C were selected as candidates for thermotolerant AAB. Pre-cultures were prepared by bacterial growing on potato media at 30ºC for 18 h and then transferring a colony to a test tube containing 5 ml of YPGD media and 4% v v^{-1} of ethanol and incubating at 39ºC with shaking at 200 rpm. Acetic acid production was monitored every 24 h and measured as total acidity using titration with 0.8 N NaOH against phenolphthalein as pH indicator [3,11].

2-3 Acetic acid production by TH-AAB at high temperatures

To assess acetic acid fermentation, 5 ml of pre-cultures were transferred to a 500-ml Erlenmeyer flask containing 100 ml of YPGD media supplemented with 4 or 6% v v^{-1} of ethanol. The culture was incubated at 39ºC with shaking at 200 rpm. Bacterial growth was monitored for turbidity at 24 h intervals using spectrophotometer (Shimadzu UV1800, Japan) at 600 nm and acetic acid was investigated using titration as previously described. Experiments were carried out in triplicate.

2-4 Comparison of acetic acid resistance at various temperatures

To prepare pre-cultures, bacterial cells were grown on potato media at 30ºC for 24 h. For dot plates, collected cells were diluted in 10-fold serial dilutions. Then, 5 µl of the cell suspensions were spotted on YPGD agar plates containing various concentrations of acetic acid followed by incubation at various temperatures for 48 h. To compare capability of acetic acid production under various acetic acid stress conditions, 5% of pre-cultures were transferred to YPGD media containing various concentrations of acetic acid and ethanol followed by incubation at 39°C with shaking at 200 rpm. Bacterial growth and acetic acid accumulation in culture media were assessed as previously described.

2-5 AAB identification using 16S rRNA sequencing

The TH-AAB isolates were cultured in potato media at 30°C with shaking at 200 rpm until the mid-log phase was reached (nearly 24 h). Cells were harvested using centrifugation and subjected to chromosomal DNA isolation based on a previous study [20]. Isolated DNAs were used as template for PCR using iTaq Kit and universal primer pair of 27f and 1492r. A PCR product of approximately 1.5 kb was generated and further purified using DNA clean-up kits before sequence analysis .Multiple alignments of the sequences were generated using LUSTALW program [21] and neighbour-joining phylogenetic trees were constructed using Mega Software v.6.0 with 1500 bootstrap replicates [22]. Sequences were deposited in DDBJ database under accession numbers of LC462259, LC462260, LC462261, LC462262, LC462263, LC462264, LC462265, LC462266, LC462267, LC462268, LC462269 and LC462270 for the isolates FPB1, FPB 2, FPB 5, FPB33, FPB35, FPB82, FPB1-98, FPB2-3, FPB2-6, FPB2-16, FPB2-17 and FPB3-20, respectively.

2-6 Extraction and partial purification of capsular polysaccharides (CPS)

The CPS of FPB2-3 strain was isolated and compared to those of two other strains of *A. pasteurianus*. Cells were achieved after cultivation on potato media containing 2% of ethanol at 37°C for 48 h. Isolation of CPS was carried out using sonication based on a previously described method [23]. A cell-free extract was achieved after removing the membrane fractions using ultracentrifugation. After treating with proteinase and DNase, the supernatant was dialyzed overnight using NaCl buffer and precipitated using 2-propanol. The precipitate was dried and used as crude CPS. The monosaccharide composition was analysed using trimethylsilyl (TMS) method [24]. Briefly, 2 mg of the CPS sample were hydrolysed by adding 200 µl of trifluoroacetic acid and incubated at 100°C for 16 h. This was dried using nitrogen stream by adding isopropyl alcohol (repeated three times). The sample was silylated using mixture of pyridine and N-trimethylsilylimidazole at 1:1 ratio. The reaction mixture was analysed using gas liquid chromatography (GLC) with a TC-1 Column (0.25 mm × 30 m) (GL Science, Japan) equipped with a gas chromatographer (Shimadzu GC8, Japan). Nitrogen was used as the carrier gas at a flow rate of 20 ml min^{-1} and an operating temperature of 180ºC.

2-7 FT-IR (Fourier-transform infrared spectroscopy) analysis of the partially purified capsular polysaccharides

The partially purified CPS was transformed to powder before analysis. The FT-IR spectrum was assessed at wavenumbers of 4000–400 cm^{-1} (16 scans with 4 cm^{-1} resolution) using Bruker TENSOR27 FT-IR Spectrometer (Bruker Optics, Germany) equipped with an ATR unit.

3. Results and discussion

3-1 Contribution of thermotolerant acetic acid bacteria in fermented plant beverages

The FPBs, collected from local brewers in Chonburi Province, Thailand, were prepared from individual plant sources and allowed to ferment for at least six months. The products included pH values of approximately 4-5 and total acidity values of 0.5-3.5% w v^{-1}. Most FPBs exhibited a thick pellicle floating on surface of the media, possibly produced by AAB. The microbial community in such products has mainly been reported as yeasts and LAB [19]. Based on a modified isolation procedure used in this study, AAB from FPBs were achieved from a single colony with a clear zone on agar plates containing $CaCO_3$ and ethanol. The colonies were subsequently assessed for their growth ability at various temperatures. Of a total of 300 colonies, 35 colonies showed growth at 39°C with 4% of ethanol. These colonies were selected for the comparison of acetic acid production in 5 ml of YPGD supplemented with 4% of ethanol v v^{-1}. Acetic acid was accumulated rapidly after 24 or 48 h with a maximum yield of 90%. The acetate over-oxidation resulted in a dramatic decrease in acetic acid of the culture media (data not shown). Within the bacterial isolates, 12 isolates could produce maximum acetic acid at 24 h, similar to *A. pasteurianus* SKU 1108, and were selected for acetic acid fermentation under shaking conditions. The pre-cultures of 12 isolates were transferred to 500-ml Erlenmeyer flasks containing 100 ml of YPGD media supplemented with 4% v v^{-1} of ethanol. All cultures were able to grow in liquid media at 39ºC. Acetic acid accumulation was similar and reached the highest value after fermentation for two days with a maximum acetic acid yield of 3.5% w v^{-1}. At this concentration, acetic acid could assimilate in cells and cells could enter the over-oxidation phase with no acetate resistant phase, as previously reported by other studies [3]. To assess effectiveness of TH-AAB, the bacterial growth abilities of TH-AAB isolates were

compared with two reference TH-AAB strains. The growth ability of TH-AAB isolates could be divided into at least two major groups; the first group showed a growth rate similar to that of *A. pasteurianus* SKU1108 with a slightly higher rate, while the second group demonstrated a growth rate similar to that of *A. tropicalis* SKU1100 with a slightly longer lag time (data not show). Other isolates showed less growth. Based on the results of the bacterial growth ability and acetic acid production, isolates of Group 1 (FPB1-98, FPB2-3, FPB2-6, FPB2-16, FPB2-17 and FPB3-20) were selected as well as six isolates of Group 2 (FPB1-2, FPB1-5, FPB1-7, FPB1-33, FPB1-35 and FPB1-82) for further identification. Based on the partial nucleotide sequences of 16S rRNA, all isolates belonged to the Genus Acetobacter; Group 1 was close to *A. pasteurianus*, while Group 2 was close to *A. tropicalis* (Figure 1). These results suggested that the two species of AAB were dominant TH-AAB found in FPBs, similar to AAB found in natural habitats of tropical areas and in fermented products [3,10,11,25]. The first group is well known as potential acetic acid-producing TH-AAB [11], while the second group includes TH-AAB producing pellicle polysaccharides at high temperatures [26].

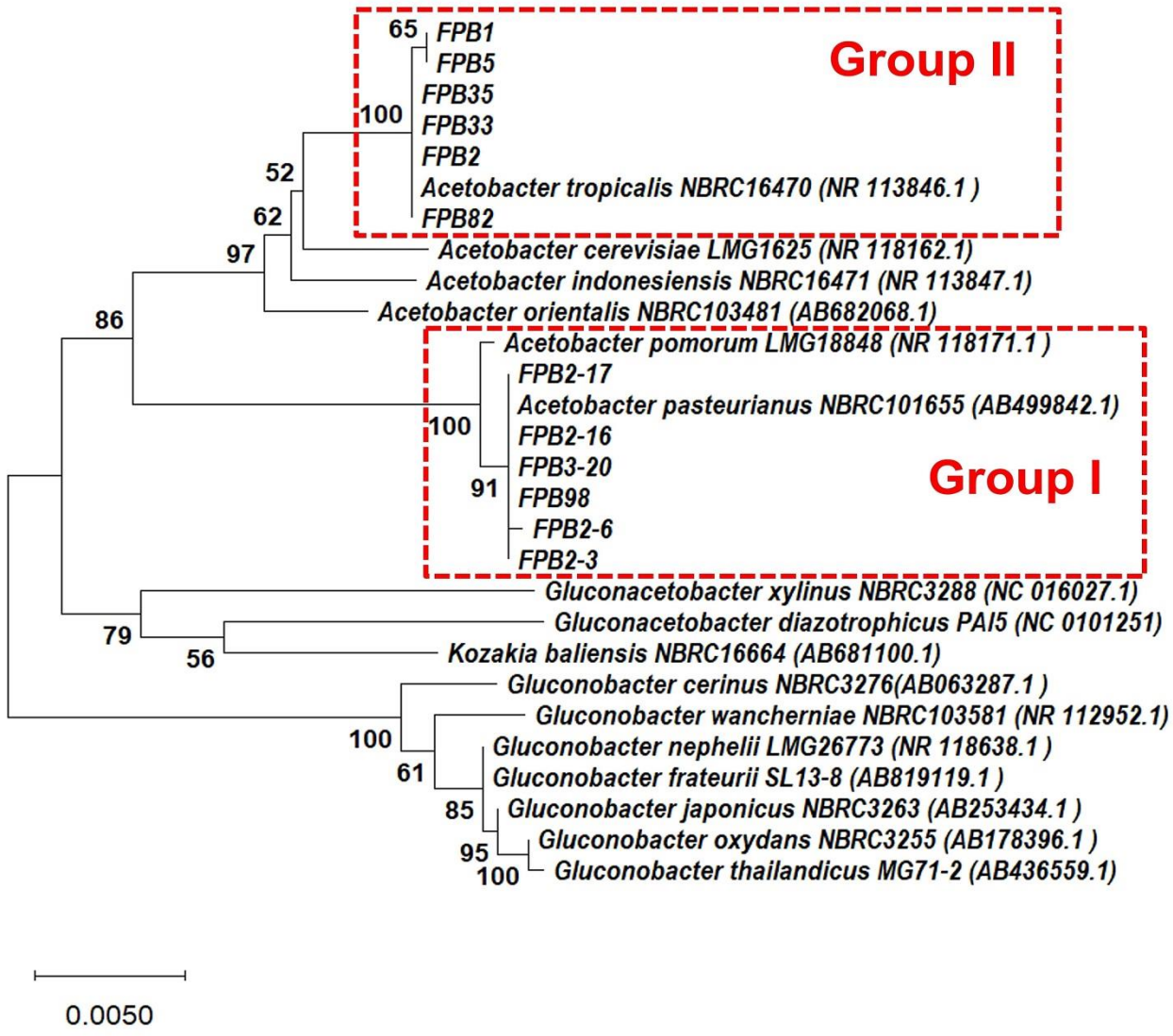

Figure 1. Phylogenetic relationship of TH-AAB with various AAB based on 16S rRNA sequences. The neighbour-joining phylogenetic tree was constructed with 1500 bootstrap replicates

3-2 Comparison of growth and acetic acid production characteristics of TH-AAB

The TH-AAB isolates in Group 1, which seemed to be the most effective isolates for high temperature fermentation, were selected for further studies. Growth and acetic acid accumulation were assessed on media containing 5% of ethanol at 39ºC (Figure 2). In fact, FPB2-3 showed a lower growth rate with no lags. The highest acetic acid yield was detected after two days of cultivation with no over oxidation, which might suppress growth rates and result in lower biomasses. However, it could be beneficial for vinegar fermentation. The FPB2-16 included a shorter lag time but could accumulate a higher rate of acetic acid and start to grow rapidly again. Thus, these two isolates were selected for further studies. When acetic acid reached more than 4%, the over-oxidation phase could not be seen even in SKU1108, as previously reported in other studies [3]. The acetic acid production with an initial ethanol concentration of 6% was suppressed at 39°C, showing a long lag time for acetic acid production, especially in SKU1108 (Figure 3). However, FPB2-3 could produce up to 4% of acetic acid within 120 h of cultivation under similar conditions. Fermentation at 39°C obviously affected the acetic acid production. This was due to the combination of heat stress and high ethanol concentration, which suppressed bacterial growth and acetic acid production, as previously reported. When thermotolerant *A. pasteurianus* isolated from Chinese vinegar Pei was cultured at 37°C with 6% of ethanol, acetic acid production decreased from 50 to 40 g l^{-1} [25].

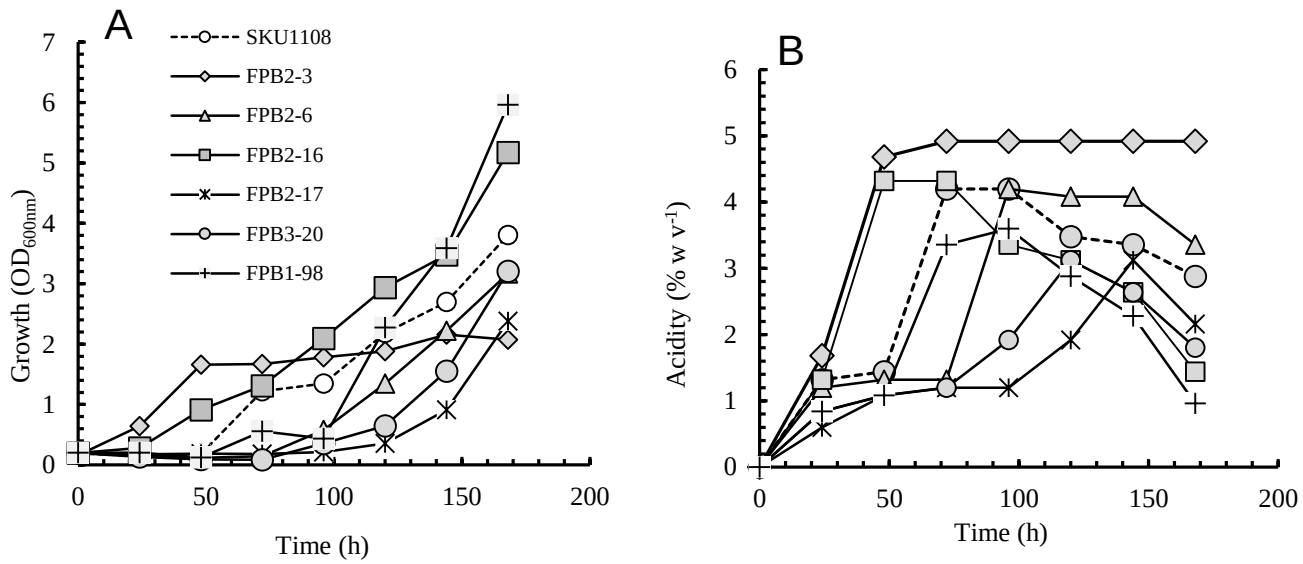

Figure 2. Growth (A) and acetic acid production profile (B) of TH-AAB Group I cultured in YPGD media containing 5% ethanol at 39°C and 200 rpm

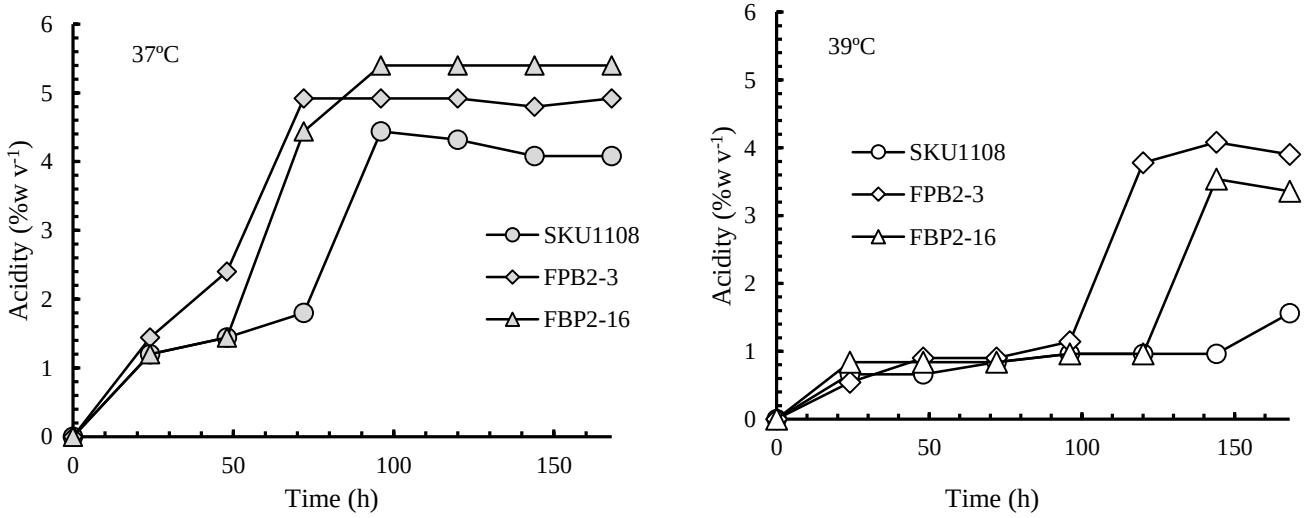

Figure 3. Capability of acetic acid production within the selected TH-AAB on media containing 6% of ethanol at 37°C (left panel) and 39°C (right panel)

3-3 Acetic acid resistance at various temperatures

To examine the effect of acetic acid on growth at high temperatures, dot plate analysis was carried out (Figure 4). Cells were spotted and grown on YPGD agar plates containing different concentrations of acetic acid. Increasing temperatures led to slower cell growth, especially at higher acetic acid concentrations. At 39°C, the strains could still grow with 1.5% acetic acid, and only FPB2-3 showed growth with 2.0% acetic acid. This result has indicated that FPB2-3 shows more resistance to acetic acid than that the other two strains do. Similarly, cells were grown in YPGD in liquid media supplemented with various acetic acid concentrations at 39°C (Figure 5). Addition of acetic acid at higher concentrations led to slower cell growth as well as delayed production of acetic acid. However, FPB2-3 strain showed a gradual increase in acetic acid accumulation of almost 6%, indicating complete oxidation of 4% of ethanol after 72 h of cultivation. Two other strains of TH-AAB could accumulate acetic acid at lower levels. Mechanisms of acetic acid resistance are linked to flux and ion transporters, which control pH homeostasis and intracellular pH synthesis [27,28].

It has been reported that several proteins in TCA cycle metabolism and ethanol oxidation were drastically upregulated under acetic acid stress conditions, while energy production increased by 150% through glucose consumption induced by initial acetic acid [29]. In addition to heat shock proteins (Hsp), which play key roles in transient thermotolerance, several enzymes involved in amino acid metabolism and plasma membrane biosynthesis are associated to acetic acid resistance [15].

3-4 Isolation and characterization of capsular polysaccharides of TH-AAB

Acetobacter spp. produce hetero-polysaccharides, which can be classified as polysaccharides attached to CPS of the bacterial cells. The CPSs can be secreted into culture media as extra polysaccharides. The CPSs allow cells to form pellicles that float on surface of the culture media under static conditions and to form rough colonies (R strain) on agar media. These polysaccharides have been reported to play crucial roles in acetic acid resistance [30]. The CPSs have been purified and characterized as hetero-polysaccharides, composing of various monosaccharides and ratios in strains of *Acetobacter* [23,26].

In the present study, CPSs of TH-AAB were partially purified and identified for their sugar compositions. Results showed that the CPSs of three strains included three monosaccharides of glucose, galactose and rhamnose at a ratio of 2:1:1 (Figure 6). This composition was similar to compositions reported in Acetobacter strains [23, 26]. However, CPSs purified from *A. pasteurianus* SL13E4 included xylose instead of galactose [26].

Generally, addition of ethanol could induce pellicle formation in TH-AAB isolated from Sri Lanka coconut water vinegar [23]. Pellicle formation in R strains of *A. pasteurianus* resulted in cells with a higher acetic acid resistance. This was shown by a lower acetate uptake (influx), compared to S strains [3].

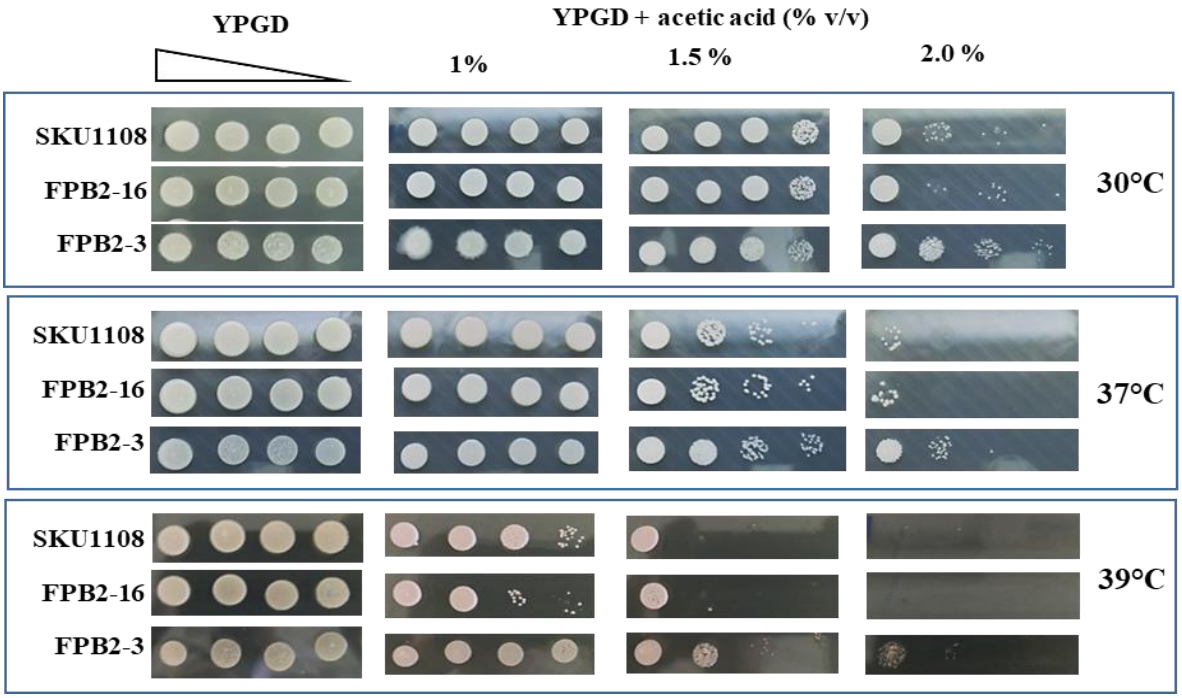

Figure 4. Comparison between acetic acid resistances using dot plate test. A total of 5 µl of serially diluted cells were spotted on YPGD agar plates containing various concentrations of acetic acid and incubated at various temperatures for 48 h.

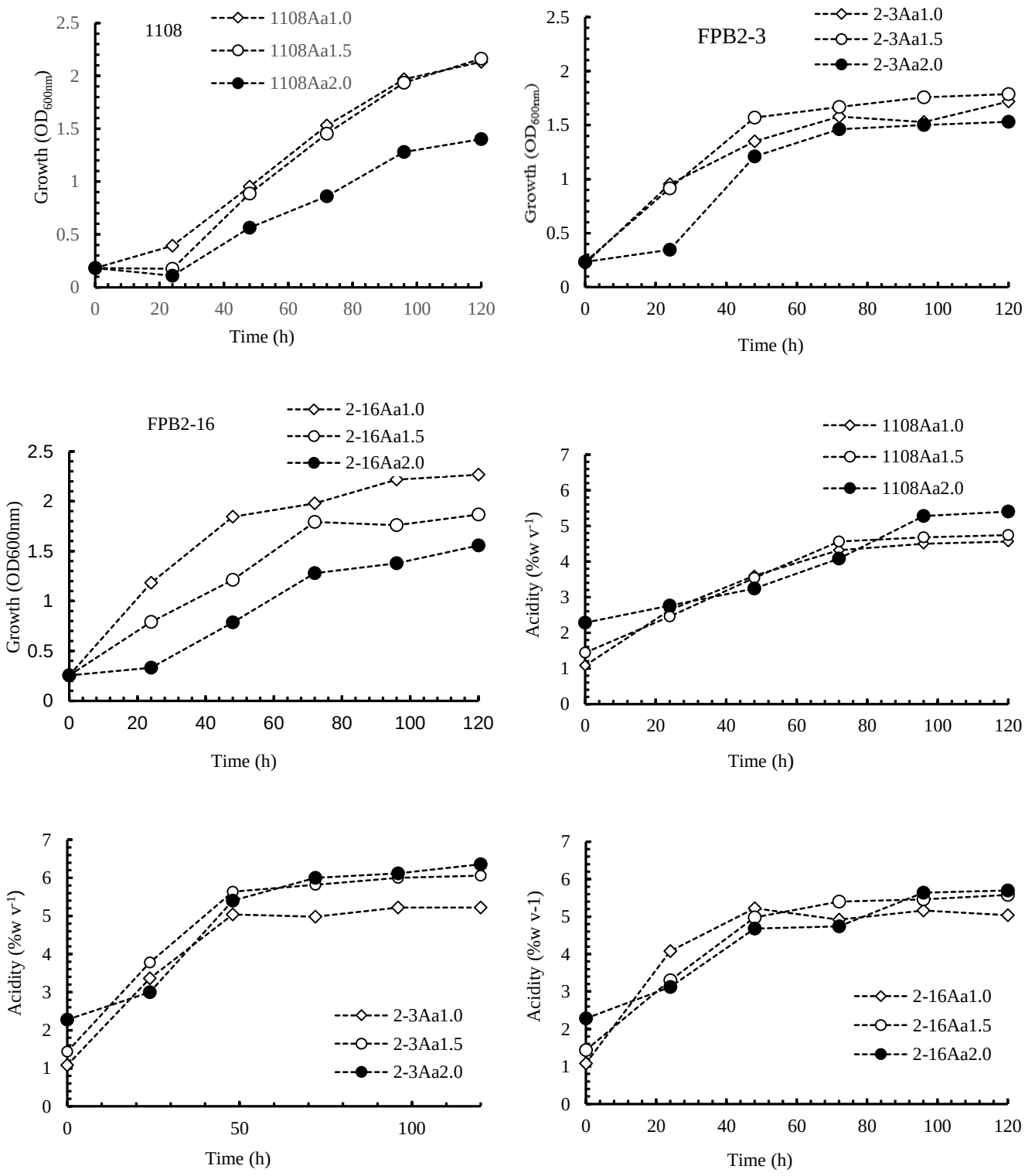

Figure 5. Growth and acetic acid accumulation under acetic acid stress at high temperatures.
Cells were grown on YPGD media containing 4% of ethanol and supplemented with various initial acetic acid concentrations of 1, 1.5 and 2.0%, incubating at 39°C with shaking at 200 rpm.

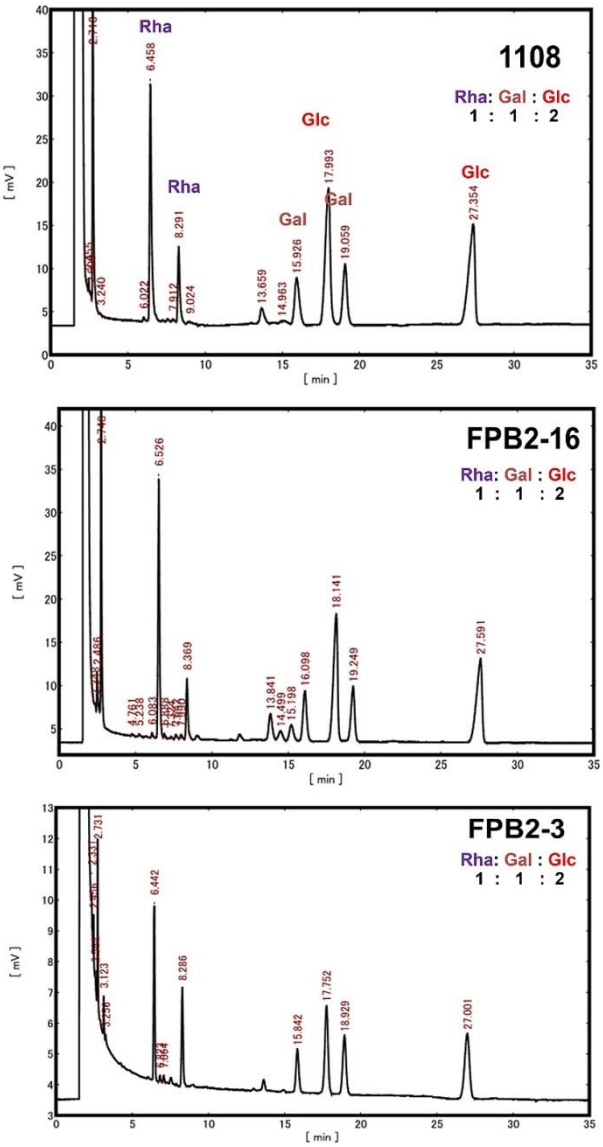

Figure 6. Sugar composition of the crude CPS of TH-AAB. After acid hydrolysis, samples were analysed using GLC

The FT-IR analysis of freeze-dried crude CPSs of TH-AAB was carried out as demonstrated in Figure 7. Strong -OH group and ether signals (C-O-C) of the sugar units were absorbed at strong intensities of approximately 3,420 (1) and 1,070 cm^{-1} (6), respectively. Prominent C-H stretching vibration was observed at 2920 cm^{-1} (2). Moreover, several well-defined peaks in the region of 1,800–600 cm^{-1} were seen. Peaks were assigned as unconjugated C=O stretching at 1,740 cm^{-1} (3) and carboxyl groups at approximately 1,640 cm^{-1} (4) (asymmetrical COO− stretching vibration). Specifically, peaks at 1,740 developed *O*-acetyl ester, as clearly shown for SKU1108 and FBP2-16 but not for FPB2-3. As previously reported, presence of peaks at 1250 and 1750 cm^{-1} in crude skin polysaccharides of aloe vera indicated the presence of *O*-acetyl ester (31). However, bands at 1,740 and 1,250 cm^{-1} disappeared, which might indicate either coverage by a strong peak at 1,640 cm^{-1} (4) or absence of *O*-acetyl group [31].

This might demonstrate that *O*-acetyl moieties for *O*-acetylation of the CPSs of SKU1108 and FPB2-16 were higher than those of FBP2-3. However, the intensity peak at 1219 cm^{-1} (5), which shifted slightly to close wavenumbers (1,236-1, 226 cm^{-1}) and linked to the stretching of C-O and C=O deformations in ester bonds formed during acetylation [32], was clearly shown in FBP2-3. Distinct characteristics of CPSs using FT-IR might be involved in acetylation or other relevant mechanisms; possibly linked to thermotolerance and acetic acid resistance. Based on the authors' best knowledge, no reports have described mechanisms or analyses of the acetyl moieties including CPS of AAB. The *O*-acetylation is a post-modification mechanism found in bacterial extra polysaccharides-enhanced biofilm formation.

This mechanism affects bacterial pathogenicity, virulence and environmental adaptability [32]. Alginates lacking *O*-acetyl groups in *P. aeruginosa* FRD1 mutant showed decreased viscosity of extracellular materials and defective attachment [33] and aggregation when adhering to steel surfaces [34]. This revealed that loss of *O*-acetyl groups resulted in weakening of inter and intra-polymer interactions within the biofilm matrices. Degrees of *O*-acetylation often vary in various microorganisms, depending on growth conditions [35,36]. Lee and Day (1998) reported that the availability of acetyl-coA, a suggested acetyl donor, affects *O*-acetyl contents in alginates [37].

4. Conclusion

This study has demonstrated that the potential TH-AAB from FPBs includes high effective growth and acetic acid production at high temperatures. Furthermore, *A. pasteurianus* FPB2-3 do not show over-oxidation, which can be advantageous for acetic acid fermentation. The distinct characteristics of CPS or pellicle polysaccharides in TH-AAB may contribute to their capacity for acetic acid resistance and thermotolerance. Further studies on the structure of CPS in this strain are recommended. Moreover, in vitro adaptation to vital stress conditions can improve this strain to an efficient TH-AAB for high-temperature acetic acid fermentation.

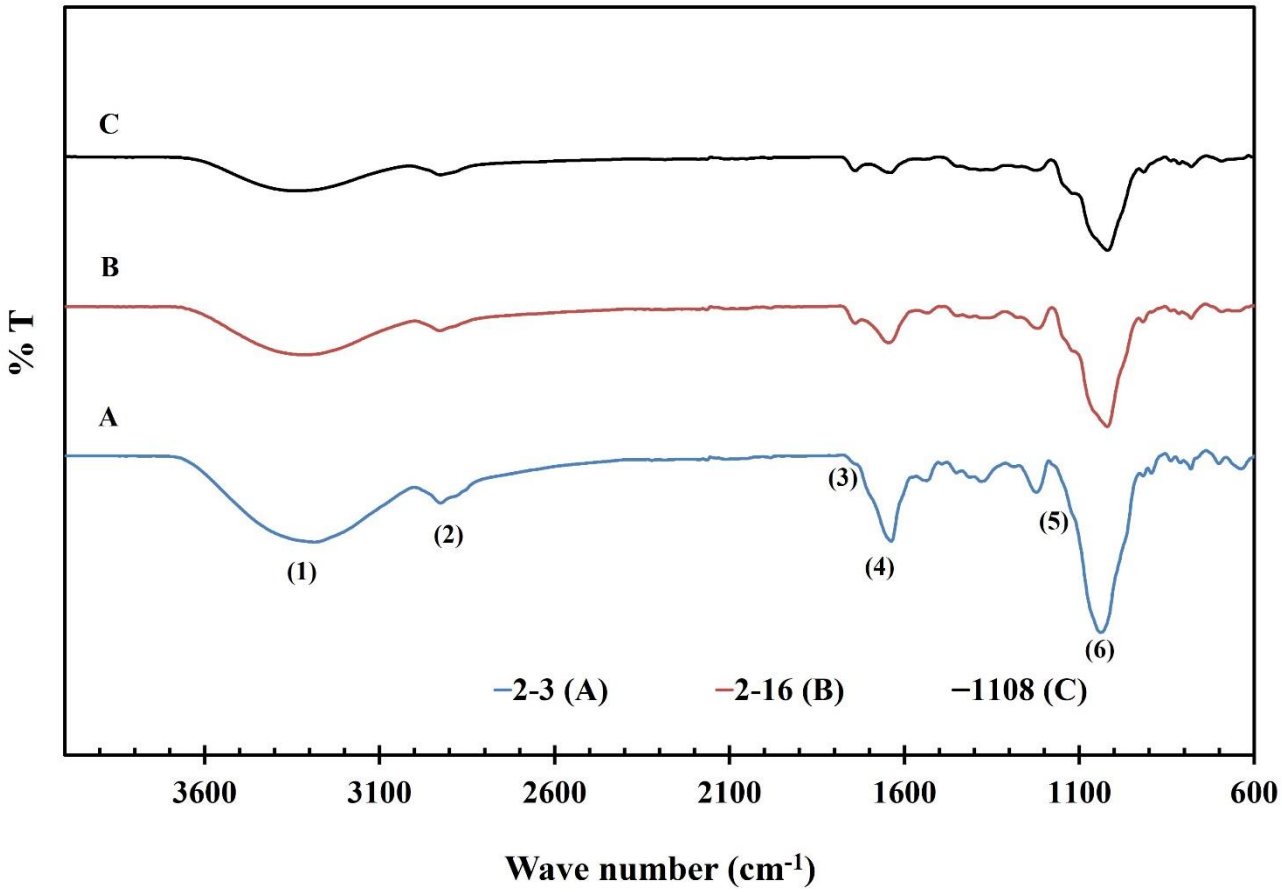

Figure 7. FT-IR analysis of partially purified CPS. Spectrum analysis of the partially purified CPS of FPB2-3 (A) and FPB2-16 (B), compared to partially purified CPS of *A. pasteurianus* SKU1108 (C)

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6. Conflict of interest

The authors declare no conflict of interest.

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تعیین ویژگی های باکتری‌های استیک اسید گرماتاب جداشده از نوشیدنی‌های گیاهی گوناگون در تایلند

پانادا چاری[1]، واراوات تانامول[2]، هیروهید تویاما[3]، ویچای سوام فول[1و4*]

۱- دانشکده علوم و مهندسی کاربردی، دانشگاه خوآن کائن، خوابگاه نونگ خای، نونگ خای، تایلند ۴۳۰۰۰

۲- برنامه شیمی، دانشکده علوم و م هندسی کاربردی، دانشگاه ناخون راتچاسیما رجب هات، ناخون راتچاسیما، تایلند ۳۰۰۰۰

۳- گروه علوم زیستی و زیست فناوری، دانشکده کشاورزی، دانشگاه رای کیتس، اوکیناوا، ژاپن ۰۲۱۳-۰۹۳

۴- مرکز تحقیقات تخمیر فرآورده های کشاورزی با ارزش افزوده (FerVAAP)، گروه زیست فناوری، دانشکده تکنولوژی، دانشگاه خوآن کائن، نونگ خای، تایلند ۴۰۰۰۲

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***نویسنده مسئول**

ویچای سوام فول

مرکز تحقیقات تخمیر فرآورده های کشاورزی با ارزش افزوده (FerVAAP)، گروه زیست فناوری، دانشکده تکنولوژی، دانشگاه خوآن کائن، نونگ خای، تایلند ۴۰۰۰۲

پست الکترونیک:
wichso@kku.ac.th

چکیده

سابقه و هدف: باکتری‌های ا ستیک ا سید گرماتاب بی شتر از سایر باکتری‌ها به درجه حرارت‌های بالا مقاومند. این تفاوت‌ها شامل ۵ تا ۱۰ درجه سل سیوس می‌با شد، که هزینه بالای سی ستم‌های خنک کننده مورد نیاز برای ر شد چنین باکتری هایی را کاهش می‌دهد، در عین حال بهره‌وری بالای آنها حفظ می شود. در مطالعه حا ضر، ویژگی‌های باکتری‌های ا ستیک ا سید گرماتاب[1] جدا شده از نو شیدنی های گیاهی تخمیری گوناگون از نظر توانایی تولید ا ستیک اسید تعیین شد و مورد بررسی قرار گرفت.

مواد و روش ها: توانایی باکتری‌های ا ستیک ا سید گرماتاب گوناگونی از نمونه های نو شیدنی‌های گیاهی تخمیری در تولید ا ستیک ا سید در درجه حرارت‌های بالا مورد برر سی قرار گرفت. دو ایزوله *ا ستوباکتر پا ستوریانس* انتخاب و رشد و توانایی تولید استیک اسید تحت شرایط گوناگون مورد بررسی قرار گرفت. سپس ویژگی های پلی‌ساکاریدهای پوشـــینه‌ای[2] این گونه‌ها اســـتخراج و با اســـتفاده از کروماتوگرافی گازی-مایع (GLC) و طیف بینی مادون قرمز تبدیل فوریه[3] مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: در این مطالعه، *ا ستوباکتر پا ستوریانس* و *ا ستوباکتر تروپیکالیس* باکتری‌های ا ستیک ا سید گرماتاب غالب در نمونه‌های نو شیدنی‌های گیاهی تخمیری بودند. در مقایـ سه با *ا ستوباکتر پا ستوریانس* SKU1108، به‌عنوان شاهد برتر باکتری‌های ا ستیک ا سید گرماتاب ، دو *ا ستوباکتر پا ستوریانس*، جدا شده نو شیدنی‌های گیاهی تخمیری ۲-۳ و ۲-۱۶، در درجه حرارت ۳۹ درجه سـلسـیوس به‌طور مؤثری اسـتیک اسـید تولید کردند؛ نوشـیدنی گیاهی تخمیری ۲-۳ قادر بود در درجه حرارت ۳۹ درجه سل سیوس به طور کامل اتانول ۵ در صد حجمی حجمی را به ا ستیک ا سید اک سید کند، در حالی‌که دوره طولانی تاخیر ر شد در غلظت ۶ در صدی اتانول م شاهده شد. با این حال، میزان تولید اسـتیک اسـید تا ۴ درصـد وزنی حجمی بود. علاوه بر این، این ایزوله در مقایسـه با دو گونه دیگر مقاومت عالی به اســـتیک اســـید در درجه حرارت‌های بالا در محیط های مایع و جامد نشـــان داد. با این حال، پلی ساکاریدهای پو شینه‌ای جدا شده از سه گونه م شابه ترکیب شکر بود (گلوکز، رامنوز و گالاکتوز) بود، در طیف بینی مادون قرمز تبدیل فوریه پلی ساکارید پو شینه‌ای نو شیدنی گیاهی تخمیری ۲-۳ در $1740\ cm^{-1}$ ا ستر *O*-ا ستیل به و ضوح شنا سایی شد. این می‌تواند به مقادیر متفاوت ا ستر *O*-ا ستیل پلی ساکاریدهای پو شینه ای و نیز مقاومت به استیک اسید و درجه حرارت بالا نسبت داده شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] Thermotolerant acetic acid bacteria
[2] Capsular polysaccharides