

Poster Session II

Classification and Molecular Phylogeny, and Ecology of Acetic Acid Bacteria

PII-4: Characterization of Bacterial Population During Spirit Vinegar Production

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Acetic acid bacteria (AAB) are mainly characterized by their capacity to produce acetic acid from ethanol, which comes from different raw materials such as: wine, cider, spirit, fruits or flowers. This reaction is accomplished by two enzymatic complexes: alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). Depending on the bacterial species and the raw material used, the submerged semi-continuous fermentation method allows the production of high-acid vinegar, with a final acetic acid concentration that may reach up to 15%. Resistance of AAB to acetic acid and ethanol varies among species. It has been related to different enzymatic responses, but it can also be linked to the capsular polysaccharides that surround these Gram-negative bacteria.

The aim of this study was to characterize the AAB species involved in submerged spirit vinegar production and to determine their evolution at the strain level during the process. Molecular identification at the species level was performed using the PCR-RFLP of 16S rRNA gene, and the sequencing of 16S rRNA gene, ITS1 region and partial *adhA* gene. Identification at the strain level was performed using the (GTG)₅-PCR fingerprinting. Additionally, cytochemical analyses were used to investigate the polysaccharide layer and immunogold labelling technique to localize the ADH enzyme.

The fingerprinting technique showed the presence of 4 different strains in the culture broth. One of them was predominant during the whole fermentation process. At the species level, these AAB strains were classified into a group formed by two phylogenetically very close species: *Gluconacetobacter intermedius*/*Gluconacetobacter oboediens*. Among the three synthetic media evaluated (GYP, modified BME, and YPM), AAB growth was observed on GYP and modified BME only when they were plated with low-acid (<10%) vinegar samples. No growth was observed on any culture media when they were plated with high-acid (>10%) vinegar samples. Growth on synthetic media leads us to think that AAB from spirit vinegar becomes unviable or non-cultivable at high acetic acid concentrations, which were reached during the vinegar production process (10-14%).

Scanning and transmission electron microscopy results have shown a thin and smooth layer of polysaccharides around the bacteria. Our previous observations of cells harvested from wine vinegar¹ showed that AAB are surrounded by a thick and rough layer. A difference was also observed by polysaccharide PAGE profiles of both vinegar samples. These results suggest that the synthesis of the membrane polysaccharides could be influenced not only by the acetic acid or ethanol content of the fermentation broth, but also by the nature of the raw material.

The immunogold labelling demonstrates that ADH enzymatic complex is localized in the outer membrane of the bacteria. This result is consistent with previous cellular fragmentation observations².

1. Barja, F. et al., *International Symposium*. **OL04-11**. Reggio Emilia, Italy (2005)

2. Matsushita, K. et al., *Biosc. Biotechnol. Biochem.*, **56**, 304-310 (1992)

P11-5: Diversity of Thermotolerant Acetic Acid Bacteria from Fermented Foods Isolated in Thailand

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Acetic acid bacteria play an important role in food and pharmaceutical industries by producing various valuable oxidation products. Nowadays, taxonomic study of acetic acid bacteria can be divided into eleven genera; *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Asaia*, *Acidomonas*, *Kozakia*, *Neoasaia*, *Swaminathania*, *Saccharibacter*, *Granulibacter*, and *Tanticharoenia*. Some species and genera among these acetic acid bacteria were isolated from the various sources in Thailand. We are interested in thermotolerant acetic acid bacteria and the previous report showed that thermotolerant acetic acid bacteria from fruits and flowers were isolated in Thailand¹. Most of isolated thermotolerant acetic acid bacteria were *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter*.

In this work we isolated thermotolerant acetic acid bacteria from fermented foods in Thailand and screened for their oxidation products. As the results of isolation, twenty-eight isolates of thermotolerant acetic acid bacteria grown at 37°C were obtained. All of isolates were short rod or oval Gram-negative bacteria. They produced acid from various sugars. They had catalase test positive and grew at 37°C. Among all the isolates, there were ten isolates able to grow at 40°C. Identification of thermotolerant acetic acid bacteria were carried out based on amplified ribosomal DNA restriction analysis of the 16S-23S rDNA internal transcribed spacer region with *Hpa*II and *Hae*III restriction endonucleases. Three distinct groups of restriction pattern were found. PCR amplification of 16S rRNA gene was performed and amplified PCR products were sequenced and analyzed. The results revealed that group 1 was closely related to the type strain of *Gluconobacter frateurii*. They contained Q-10 as ubiquinone type. Group 2 was closely related to *Acetobacter tropicalis* type strain which contained Q-9 in ubiquinone system. Group 3 was closely related to the type strain of *A. pasteurianus* which contained Q-9. In this work we reported that *G. frateurii* was found in Thai fermented foods for the first time.

The ability of the isolated thermotolerant acetic acid bacteria from Thai fermented foods on production of oxidation products such as dihydroxyacetone (DHA), acetic acid, D-fructose and L-sorbose was also observed. It was found that *G. frateurii* produced DHA from glycerol with the highest yield of 34.44 % at 37°C but not at 40°C. The other isolates produced small amount of acetic acid, D-fructose, and L-sorbose at both temperatures. The results suggested that thermotolerant acetic acid bacteria isolated from fermented foods had production activity lower than that of thermotolerant acetic acid bacteria isolated from fruits and flowers. This may be due to the nature of each habitat that contains high or low concentration of sugars or alcohols.

1. Moonmangmee, D. et al., *Biosci. Biotechnol. Biochem.*, **64**, 2306-2315 (2000)

PII-6: Identification of strains isolated in Thailand and assigned to the genera *Kozakia* and *Swaminathania*

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Among the eleven genera described in the acetic acid bacteria,¹ the genera *Kozakia* Lisdiyanti et al. 2002 and *Swaminathania* Loganathan and Nair 2004 are monotypic and include a single species, respectively, *K. baliensis* and *S. salitolerans*. The occurrence of strains assigned to the two genera is very rare, since there are no reports concerning either isolation of additional strains or description of additional species of the genera.

Four isolates designated as CT8-1 and CT8-2, which were isolated from fruit of sapodilla collected at Chantaburi on July 6, 2006, and as SI15-1 and SI15-2, which were isolated from seeds of ixora ('khem' in Thai, *Ixora* species) collected at Rayong on July 3, 2006,² were used in this study. The four isolates were selected from a total of 181 isolated acetic acid bacteria.

Isolates CT8-1 and CT8-2 were non motile and produced a levan-like mucous polysaccharide from sucrose or D-fructose, but did not produce a water-soluble brown pigment from D-glucose on CaCO₃-containing agar slants. The isolates produced acetic acid from ethanol and oxidized acetate and lactate to carbon dioxide and water, but the intensity of the acetate and lactate oxidation was weak. Their growth was not inhibited by 0.35% acetic acid (v/v) at pH 3.5. The isolates did not grow on 30% D-glucose (w/v), and utilization of methanol was not found.

Isolates SI15-1 and SI15-2 had peritrichous flagella and grew in the presence of either 0.35% acetic acid (v/v) at pH 3.5, 3% NaCl (w/v), or 1% KNO₃ (w/v). Acetate and lactate were oxidized to carbon dioxide and water, but the intensity was weak. The isolates grew on mannitol agar and glutamate agar as well as on 30% D-glucose (w/v), but did not utilize methanol.

The 16S rRNA gene sequence analysis and DNA-DNA hybridization indicated that isolates CT8-1 and CT8-2 and isolates SI15-1 and SI15-2 were unequivocally identified respectively as *K. baliensis* and *S. salitolerans*.

1. Yukphan, P. et al., *Biosci. Biotechnol. Biochem.*, **72**, 672-676 (2008)

2. Kommanee, J. et al., *Annals Microbiol.*, **58**, 319-324 (2008)

P11-7: *Gluconobacter kanchanaburiensis* sp. nov., for strains isolated at Thong Pha Phum, Kanchanaburi, Thailand

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For Thai isolates, two new genera and six new species were described: the genera *Neoasaia*¹ and *Tanticharoenia*² and *Asaia siamensis*³, *Asaia krungthepensis*⁴, *Gluconobacter thailandicus*⁵, *Neoasaia Chiangmaiensis*¹, *Asaia lannensis*⁶ and *Tanticharoenia sakaeratensis*².

This paper proposes *Gluconobacter kanchanaburiensis* sp. nov., the tenth species of the genus *Gluconobacter*, for two isolates, which were isolated from fermented fruits of *Artocarpus heterophyllus* (jackfruit) collected at Thong Pha Phum, Kanchanaburi, Thailand.

In phylogenetic trees based on 16S rRNA gene sequences, the two isolates were included in the lineage of *G. oxydans* and formed an independent cluster along with the type strains of *G. cerinus*, *G. frateurii*, *G. thailandicus* and *G. japonicus*. The calculated pair-wise sequence similarities of isolate AD92^T were 97.4-99.5% to the type strains of nine *Gluconobacter* species. DNA base composition was 59.4-59.5 mol% G+C with a range of 0.1 mol%. A labeled DNA of isolate AD92^T represented levels of DNA-DNA hybridization of 100, 32, 10, 16, 27, 20, 39, 47, 19, 44 and 7% to DNAs respectively from isolates AD93 and the type strains of *G. oxydans*, *G. cerinus*, *G. frateurii*, *G. albidus*, *G. thailandicus*, *G. kondonii*, *G. roseus*, *G. japonicus*, *G. sphaericus* and *Acetobacter aceti*. The two isolates can be phenotypically discriminated from *G. oxydans*, *G. albidus*, *G. kondonii*, *G. roseus* and *G. sphaericus* by growth without nicotinic acid. Q-10 was major. The unique phylogenetic, genetic and phenotypic characteristics obtained indicate that the two isolates are adequate to be classified into a separate species, and *Gluconobacter kanchanaburiensis* sp. nov. is proposed. The type strain is isolate AD92^T (= BCC 15889^T = NBRC 103587^T), which has a DNA G+C of 59.5 mol%.

1. Yukphan, P. et al., *J. Gen. Appl. Microbiol.*, **51**, 301-311 (2005). 2. Yukphan, P. et al., *Biosci. Biotechnol. Biochem.*, **72**, 672-676 (2008). 3. Katsura, K. et al., *Int. J. Syst. Evol. Microbiol.*, **51**, 559-563 (2001). 4. Yukphan, P. et al., *Int. J. Syst. Evol. Microbiol.*, **54**, 313-316 (2004). 5. Tanasupawat, S. et al., *J. Gen. Appl. Microbiol.*, **50**, 159-167 (2004). 6. Malimas, T. et al., *Biosci. Biotechnol. Biochem.*, **72**, 666-671 (2008).

PII-8: Genera and Species in Acetic Acid Bacteria (2)

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In a previous paper,¹ we described a historical survey of taxonomic studies of acetic acid bacteria. This paper deals with subsequent and recent progress in the taxonomic studies.

Recently, a new genus and a new species were reported, viz., *Tanticharoenia* Yukphan et al. 2008 with a single species, *Tanticharoenia sakaeratensis* Yukphan et al. 2008.² So a total of eleven genera were described in the family *Acetobacteraceae*: *Acetobacter*, *Gluconobacter*, *Acidomonas*, *Gluconacetobacter*, *Asaia*, *Kozakia*, *Swaminathania*, *Saccharibacter*, *Neoasaia*, *Granulibacter* and *Tanticharoenia*. Of the eleven genera, the genus *Granulibacter* interestingly has a major quinone, Q-8,³ which was never found in the members of the family *Acetobacteraceae*, *Alphaproteobacteria*. The major quinone Q-8 is quite unique chemotaxonomically with Q-9 of the genus *Acetobacter*.

In the genus *Acetobacter*, two new species were described: *Acetobacter senegalensis* Ndoye et al. 2007,⁴ *Acetobacter ghanensis* Cleenwerck et al. 2007.⁵ In the genus *Gluconobacter*, two new species were described: *Gluconobacter kondonii* Malimas et al. 2008⁶ and *Gluconobacter roseus* Malimas et al. 2008.⁷ In the genus *Gluconacetobacter*, one new species was described: *Glucon-acetobacter kombuchae* Dutta and Gachhui 2007.⁸ This species interestingly has polar flagellation, along with *Acetobacter nitrogenifigens* Dutta and Gachhui 2006. Generally, the genera *Acetobacter* and *Gluconacetobacter* are characterized by peritrichous flagellation when motile. Accordingly, the two species were quite unique morphologically. In the genus *Asaia*, the fifth species, *Asaia lannensis* Malimas et al. was described.⁹ Polyamine composition, cellular fatty acid composition and cellular lipid composition will be also discussed taxonomically.

1. Yamada, Y. and Yukphan, P., *Int. J. Food Microbiol.*, **125**, 15-24 (2008). 2. Yukphan, P. et al., *Int. J. Syst. Evol. Microbiol.*, **58**, 1511-1512 (2008). 3. Taweesak, M. et al., *J. Gen. Appl. Microbiol.*, (2008), in preparation. 4. Ndoye, B. et al., *Int. J. Syst. Evol. Microbiol.*, **57**, 1576-1581 (2007). 5. Cleenwerck, I. et al., *Int. J. Syst. Evol. Microbiol.*, **57**, 1647 - 1652 (2007). 6. Malimas, T. et al., *Int. J. Syst. Evol. Microbiol.*, **58**, 529-530 (2008). 7. Malimas, T. et al., *Int. J. Syst. Evol. Microbiol.*, **58**, 1511-1512 (2008). 8. Datta, D. and Gachhui, R., *Int. J. Syst. Evol. Microbiol.*, **57**, 353-357 (2007). 9. Malimas, T. et al., *Int. J. Syst. Evol. Microbiol.*, **58**, 1511-1512 (2008).

P11-9: Diversity of Acetic Acid Bacteria in Japan

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Acetic acid bacteria (AAB) are widely distributed in foods, flowers, and fruits in the tropical zone in Southeast Asia (Lisdiyanti et al.¹). However, relationships are still unknown among sources, isolation media, and climatic zones on AAB.

From this point of view, we collected 776 samples including fermented foods, flowers, and fruits at 40 prefectures from Okinawa in the subtropical zone, Honshu, Kyusyu, and Shikoku in the temperate zone to Hokkaido in the subarctic zone in Japan from 2004 to 2007. This study deals with the identification and distribution of AAB in Japan.

Enrichment media (EM) I, II, and VI were used for isolation of AAB. EM I contained 1.0 % D-glucose, 1.5 % peptone, 0.8 % yeast extract, 0.5 % ethanol, 0.3 % acetic acid, and 100 ppm cycloheximide. EM II was composed of 2.0 % D-sorbitol, 0.5% peptone, 0.3% yeast extract, and 100 ppm cycloheximide. EM VI consisted of 1.0 % D-sorbitol, 1.0 % dulcitol, 0.5 % peptone, 0.3 % yeast extract, and 100 ppm cycloheximide. The isolates were identified on the basis of phenotypic characteristics, analysis of 16S rRNA gene sequences, and DNA-DNA hybridization.

Of 345 isolates, 96 were identified as *Acetobacter* spp., 46 as *Gluconobacter* spp., 41 as *Gluconacetobacter* spp., 157 as *Asaia* spp., one as *Saccharibacter* sp., and four as *Frateuria* sp. Further, the strains identified were included in 6 genera and 39 species including 8 new species of AAB. *Acetobacter* and *Gluconacetobacter* strains were mainly isolated fermented foods using EM I. While, *Asaia* strains were isolated from flowers using EM VI, and hardly found in fermented foods. This indicates that EM VI is appropriate for isolation of *Asaia* strains and those were found specifically in flowers. AAB were widely distributed from the subtropical zone to the subarctic zone in Japan, and the zone specificity was not found.

This study would indicate the rich diversity of AAB in Japan, and further modification of enrichment media is useful for isolation of targeted AAB in nature.

1. Lisdiyanti, P. et al., *Microbiol. Cult. Coll.* **19**, 91-99 (2003)

PII-10: Identification of *Acetobacter*, *Gluconobacter* and *Asaia* Strains Isolated in Thailand Based on 16S-23S rDNA ITS Restriction and 16S rDNA Sequence Analyses

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Acetic acid bacteria are Gram-negative, strictly aerobic bacteria and are commonly found in nature on various plants (flowers, herbs, fruits etc.). They have an ability to oxidize different kinds of alcohols and sugars into commercially important foods and chemical products (vinegar, kombucha, tea, sorbose, gluconic acid, etc.).¹⁻⁴

Twenty-six strains of acetic acid bacteria were isolated from fruits, flowers and related materials collected in Bangkok and some provinces, Thailand by an enrichment culture approach. The isolates were divided into three genera, *Acetobacter*, *Gluconobacter* and *Asaia* by phenotypic characterization. On the basis of 16S-23S rDNA ITS restriction and 16S rDNA sequence analyses, the fourteen strains that were assigned to the genus *Acetobacter* were divided into five groups, which corresponded to 1) *Acetobacter pasteurianus* of Group 1A, including five strains; 2) *Acetobacter orientalis* of Group 2A, including four strains; 3) *Acetobacter ghanaensis* of Group 3A, including three strains; 4) *Acetobacter syzygii* of Group 4A, including one strain; 5) *Acetobacter tropicalis* of Group 5A, including one strain. The eleven strains that were assigned to the genus *Gluconobacter* were divided into five groups, which corresponded to 6) *Gluconobacter frateurii* of Group 1B, including three strains; 7) *Gluconobacter japonicus* of Group 2B, including five strains; 8) an unidentified of Group 3B, including two strains; 9) an unidentified of Group 4B, including one strain. 10) The remaining one strain of Group C that was assigned to the genus *Asaia* was unidentified and supposed to constitute a new taxon on the basis of 16S rDNA sequence analysis.

1) Kommanee, J. et al., *Annals Microbiol.*, **58**, 319-324 (2008)

2) Yukphan, P. et al., *Int. J. Syst. Evol. Microbiol.*, **54**, 313-316 (2004)

3) Malimas, T. et al., *Biosci. Biotechnol. Biochem.*, **72**, 666-671 (2008)

4) Malimas, T. et al., *Int. J. Syst. Evol. Microbiol.*, (2008), in press

PII-11: *Gluconobacter sphaericus* (Ameyama 1975) comb. nov., a brown pigment-producing acetic acid bacterium in the *Alphaproteobacteria*

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In the studies on 16S-23S rRNA gene ITS restriction and sequence analyses of strains assigned to the genus *Gluconobacter*, several new species have been described: *Gluconobacter albidus* (ex Kondo and Ameyama 1958) Yukphan et al. 2005, *Gluconobacter thailandicus* Tanasupawat et al. 2005, *Gluconobacter kondonii* Malimas et al. 2008, *Gluconobacter roseus* (ex Asai 1935) Malimas et al. 2008 and *Gluconobacter japonicus* Mlimas et al. 2009.

This paper describes *Gluconobacter sphaericus*, the new combination for strain NBRC 12467^T, which was once classified as *Gluconobacter oxydans* subsp. *sphaericus* Ameyama 1975.¹⁾ The DNA G+C content of the strain was 59.5 mol%. The strain showed low levels of DNA-DNA hybridization of 49-9% to the type strains of eight *Gluconobacter* species. The strain formed a cluster along with the type strains of *G. albidus* and *G. kondonii* in phylogenetic trees based on 16S rRNA gene sequences. In a phylogenetic tree based on 16S-23S rRNA gene ITS sequences, however, the strain formed an independent cluster from the type strains of the eight *Gluconobacter* species. Phenotypically, the strain produced brown pigment from D-glucose, differing from the type strains of the eight *Gluconobacter* species. The strain was discriminated by digestion with *Bsa*JI, showing the *G. sphaericus* type of restriction patterns. Q-10 was major. Accordingly, strain NBRC 12467^T was distinguished phenotypically, genetically and phylogenetically from the type strains of the eight *Gluconobacter* species, viz., *G. oxydans*, *G. albidus*, *G. kondonii*, *G. roseus*, *G. cerinus*, *G. frateurii*, *G. thailandicus* and *G. japonicus*. *Gluconobacter oxydans* subsp. *sphaericus* is therefore elevated to the species level, and *Gluconobacter sphaericus* (Ameyama 1975) comb. nov. is proposed for the strain in the higher DNA G+C content group, the sublineage of *G. oxydans* or Phenon B. The type strain is NBRC 12467^T (= BCC 14448^T), whose DNA G+C is 59.5 mol%.²⁾

1. Ameyama, M., *Int. J. Syst. Bacteriol.*, **25**, 365-370 (1975).

2. Malimas, T. et al., *J. Gen. Appl. Microbiol.*, **54** (2008), in press.

P11-12: Phylogenetic diversity of acetic acid bacteria isolated from Thailand

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The NITE Biological Resource Center (NBRC) of Japan and the BIOTEC culture collection (BCC) of Thailand have started joint research projects on bacteria, yeasts and fungi since 2005 to enrich microbiological resources in both collections and to enhance their utilizations. Accordingly, we performed taxonomic studies on the acetic acid bacteria isolated from various sources in Thailand in the last three years.

We determined 16S rRNA gene sequences for the three hundreds and two isolates. A phylogenetic tree based on the sequences showed that five strains should be classified into new genera in the family *Acetobacteraceae*.¹ The other two hundreds and ninety-seven strains were assigned to thirty-three sequence groups of the four known genera, *Acetobacter*, *Asaia*, *Gluconacetobacter* and *Gluconobacter* based on the 16S rRNA gene sequences. Seventeen strains were classified into eight groups (AB1-AB8) in the genus *Acetobacter*. Seven groups except for the AB2 were closely related to the known *Acetobacter* species. The AB2 was remote from known *Acetobacter* species with the highest similarity of 98.0% to *Acetobacter orientalis* 21F-2^T. One hundred and fifty strains were separated into eleven sequence groups (AS1-AS11) of the genus *Asaia*. The AS4 was distantly related to known *Asaia* species and we proposed to classify it in *Asaia lannensis* sp. nov.² Nine strains assigned to the genus *Gluconacetobacter* showed a 100% similarity to *Gluconacetobacter liquefaciens* NBRC 12388^T. One hundred and twenty one strains were classified in the genus *Gluconobacter* and divided into thirteen sequence groups (GB1-GB13). Two of the thirteen groups (GB1 and GB6) seemed to constitute distinct lineages in the genus. The highest similarities to the other species of GB1 and GB6 were 99.5% to *Gluconobacter albidus* NBRC 3250^T and 99.1 % to *G. frateurii* NBRC 3264^T, respectively. Acetic acid bacteria living in Thailand were so diverse that we found several new sequence groups to be classified in new species or genera. Most of these strains examined in this study are now available from both the NBRC and the BCC collections.

1. Yukphan P. et al., *Biosci. Biotechnol. Biochem.*, **73**, 672-676 (2008).

2. Malimas T. et al., *Biosci. Biotechnol. Biochem.*, **73**, 666-671 (2008).

P11-13: Differentiation of species of the family *Acetobacteraceae* by AFLP DNA fingerprinting and reclassification of *Gluconacetobacter kombuchae* as *Gluconacetobacter hansenii*

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Amplified fragment length polymorphism (AFLP) DNA fingerprinting was investigated as a tool for fast and accurate identification of acetic acid bacteria (AAB) to the species level. One hundred and thirty five reference strains and 15 additional strains, representing the 50 recognized species of the family *Acetobacteraceae*, were subjected to AFLP analysis using the restriction enzyme combination *ApaI/TaqI* and the primer combination A03/T03. The reference strains were previously subjected to either DNA-DNA hybridizations or 16S-23S rDNA spacer region sequence analysis¹ and were regarded as being accurately classified to the species level.

The present study revealed that six of these strains have to be reclassified, namely *Gluconacetobacter europaeus* LMG 1518 and *Gluconacetobacter xylinus* LMG 1510 as *Gluconacetobacter xylinus* and *Gluconacetobacter europaeus*, respectively; *Gluconacetobacter kombuchae* LMG 23726^T as *Gluconacetobacter hansenii*; and *Acetobacter orleanensis* LMG 1545, LMG 1592 and LMG 1608 as *Acetobacter cerevisiae*. Cluster analysis of the AFLP DNA fingerprints of the reference strains, using UPGMA linkage of DICE correlation coefficients, revealed one cluster per species, showing a linkage level below 50 % with other clusters, except for *Acetobacter pasteurianus*, *Acetobacter indonesiensis*, and *Acetobacter cerevisiae*. The latter species were separated into two, two, and three clusters, respectively. The AFLP data further supported to classify *Gluconacetobacter oboediens* and *Gluconacetobacter intermedius*, for which at present confusion exists concerning their taxonomic status^{2,3}, as different species. The 15 additional strains could all be identified to the species level. AFLP analysis further revealed that some species harbour genetically diverse strains, whereas other species consist of strains showing similar banding patterns, indicating a more limited genetic diversity.

It can be concluded that AFLP DNA fingerprinting is suitable for accurate identification and classification of a broad range of AAB, as well as for determination of intraspecific genetic diversity.

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PII-14: (GTG)₅-PCR fingerprinting, a promising genotypic tool for rapid and reliable classification and identification of acetic acid bacteria

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Acetic acid bacteria (AAB) are involved in the production and/or spoilage of several foods and beverages, such as vinegar, beer, wine, and cocoa-based products. Therefore, species identification of AAB is of high interest. Several studies have shown that accurate identification of AAB is often difficult. In the past, AAB have been classified on the basis of their physiological and chemotaxonomic properties. However, identification methods based on the phenotypic characteristics of AAB are not reliable and very time-consuming. In the last decade, research has been focused on molecular, DNA- and RNA-based methods for genotyping of AAB, which seems to complement or even replace classical phenotypic tests.

PCR amplification of repetitive bacterial DNA elements (rep-PCR) has been recognized as a powerful low-cost tool for reliable classification and identification of several Gram-negative and Gram-positive bacteria. To test the validity of this method for classification of AAB, a set of 159 reference strains, including 48 type strains, was subjected to rep-PCR fingerprinting, using the single oligonucleotide primer (GTG)₅. Almost all AAB strains tested clustered according to their respective taxonomic designations. As shown before, *Acetobacter aceti* LMG 1531 and *A. peroxydans* (LMG 1633, LMG 1635^T) strains did not cluster with their respective taxa; *A. indonesiensis* (LMG 1571, LMG 1588, LMG 19824^T) strains, *Gluconacetobacter liquefaciens* LMG 1509, and *Ga. xylinus* subsp. *sucrofermentans* LMG 18788^T were dispersed¹. The present study indicated that *A. pasteurianus* (LMG 1552, LMG 1553, LMG 1805) strains, *Asaia bogorensis* (LMG 21650^T, LMG 23141) strains, *Gluconobacter cerinus* (LMG 1368^T, LMG 1417) strains, *G. frateurii* LMG 1369 t1, and *G. oxydans* LMG 1424 were dispersed, while *A. pasteurianus* (LMG 1587, LMG 1604) strains, *A. senegalensis* LMG 23690^T, *Acidomonas methanolica* LMG 1668^T, *As. siamensis* (LMG 23144, LMG 23146) strains, and *G. frateurii* (LMG 1365^T, LMG 1371) strains did not cluster with their respective taxa. Former misclassifications, the nature of the banding patterns (species- and/or strain-specific), and/or the number of strains from diverse origins included may be responsible for this. For a few strains, such as all *A. indonesiensis* strains, (GTG)₅-PCR fingerprinting may not be adequate enough for species identification.

The (GTG)₅-PCR fingerprinting method allowed the classification and identification of more than 500 isolates from spontaneous cocoa bean heap fermentations performed in Ghana.^{2,3}

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