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# Biodiversity of Bacteria Isolated from Home-Made Wine and Vinegar

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**Abstract:** Wine is an alcoholic beverage made grapes fermented without the addition of sugars, acids, enzymes, water. It has been consumed by human beings in religious ceremonies since ancient times. Vinegar is sour juice that is used as a sweetener in meals, in salads, or as a preservative such as brine. It has a great variety of industrial, medical, and domestic uses are still commonly practiced today. The aim of this study was to determine the bacterial biodiversity of home-made wine and vinegar using classic and molecular methods. Morphological, cultural and biochemical identifications were made according to the Bergey's Manual of Systematic Bacteriology. For molecular identification 16S rDNA-PCR method was used. PCR results of these samples were send to the sequencing. BLASTn software was used to match our sequences with the ones in GenBank. In this study, bacteria colonies were isolated from home-made wine and vinegar. According to molecular results acetic acid and lactic acid bacteria were found.

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## 1. Introduction

Biodiversity is the foundation of ecosystem to which well-being of all living things is dependent variety of the living beings that exist known as biodiversity. It is one of the basic components of nature and it ensures the survival of earth by all means. Biodiversity relies upon the climatic conditions and areal parts of the district [1].

Vinegar is expended worldwide as a sustenance sauce and additive. It is the oldest preservative of vegetables, meat and fish [2]. Fermentation of the wine and vinegar is a spontaneous microbiological process [3]. There are different techniques to produce. The customary advances depend at first glance microbiota that is immobilized on various help materials, (for example, beech-wood shavings) or it is drifting on the surface of ethanol containing substrates [4]. Winemaking is older than the recorded history and the development of this technology begins nearly 7000 years ago. Different organisms found on the surface of grape skins and the indigenous microbiota related with winery surfaces take an interest in these regular wine maturation [5].

The conversion rate of liquid-state fermentation, the speed of acid production, and the flavor of vinegar are all dependent on the quality of the acetic acid bacterial strains, the selection

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and culture of excellent acetic acid bacteria have attracted much attention from scholars [6]. The aim of this study was to determine the bacterial biodiversity of home-made wine and vinegar using molecular methods.

## 2. Material and Methods

### 2.1. Sample collection and bacterial isolation

Home-made wine and vinegar samples were collected aseptically from the villages of Aydın. Bacterial growth was realized on HS (Hestrin-Schramm) Agar at 30ºC for 72 h. After incubation, each different colony were isolated and stocked in skim milk [7].

### 2.2. Identification of microorganisms

Morphological, cultural and biochemical identifications were made according to the Bergey's Manual of Systematic Bacteriology [8]. For molecular identification, DNA isolation of the samples were made according to De Boer and Ward (1995) [9]. After isolations DNA concentration and purity was measured with nanodrop spectrometer (Thermo Scientific). Their purity were between the values of 1.73 and 2.20. For PCR 16S universal rDNA primers were used (27F: 5'- AGA GTT TGA TCM TGG CTC AG-3', 1492R: 5'- CGG TTA CCT TGT TAC GAC TT-3'). 16S rRNA PCR reactions were carried out at initial denaturation 95ºC 5 min, denaturation 94ºC 40 sec, annealing 50ºC 40 sec, extension 72ºC 40 sec with 35 cycles and a final extension at 72ºC 10 mins. Reagents concentrations were 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM $MgCl_2$ and 1U Taq polymerase with the final volume of 25 µl. PCR products were sent to the sequencing (GATC BioTech, Germany) after electrophoresis at 1.4% agarose gel at 90 V 40 min.

## 3. Results and Discussion

### 3.1. Morphological and Biochemical Identification

Morphological and biochemical tests were done according to Bergey's Manual of Systematic Bacteriology [8]. Results were shown in Table 1.

**Table 1.** Classical identification of bacteria from wine and vinegar samples.

| Sample Number | Gram Staining | Cell Shape | G | L | S | M | NR | SH | $H_2S$ | C | GH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 2 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 3 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 4 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 5 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 6 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 7 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 8 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 9 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 10 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 11 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 12 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 13 | _ | Rod | _ | _ | _ | _ | + | _ | + | + | + |
| 14 | + | Rod | + | + | + | _ | + | _ | + | _ | + |
| 15 | + | Coc | _ | + | + | + | + | _ | + | _ | + |
| 16 | + | Rod | + | + | + | _ | + | _ | + | _ | + |

G: Glycerol, L: Lactose, S: Sucrose, M: Mannitol C: Citrate, NR: Nitrate Reduction, SH: Starch Hydrolyse, GH: Gelatine Hydrolyse

### 3.2. Molecular identification

PCR results of these samples (Figure 1) were send to the sequencing (GATC BioTech, Germany). Molecular identification was made by comparing sequence results with Genebank using BLASTn software. Our analysis showed that there 26 different strains and 16 different species (Table 2). MEGA 6 software was used for evolutionary analysis. Maximum likelihood method was used to infer evolutionary history. Maximum likelihood tree was shown in Figure 2.

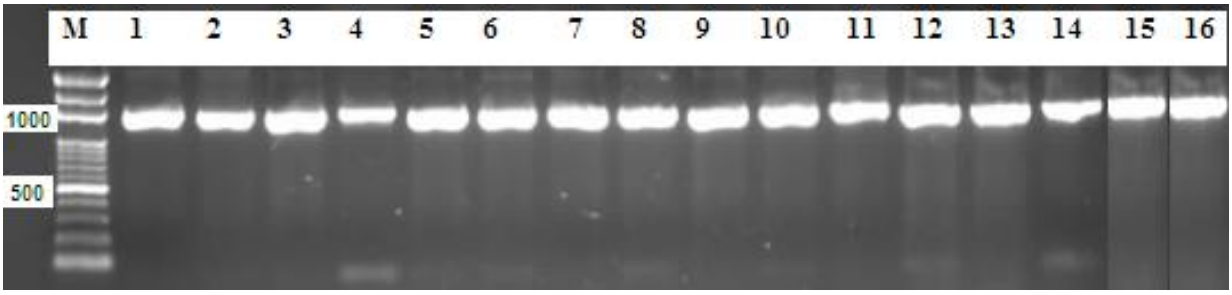


**Figure 1.** 16S rDNA PCR results of the samples (M: Marker (100bp), 1-16: Samples

**Table 2.** Molecular identification of the species from wine and vinegar samples.

| No | Name | Number of Strains | Accession No |
|---|---|---|---|
| 1 | *Komagataeibacter saccharivorans* strain MI5SAII | 1 | KY287776.1 |
| 2 | *Gluconacetobacter europaeus* | 1 | FN429075.1 |
| 3 | *Komagataeibacter saccharivorans* strain JCM 25121 | 6 | NR_113398.1 |
| 4 | *Gluconacetobacter saccharivorans* | 2 | AB759966.1 |
| 5 | *Gluconacetobacter xylinus* strain 1-5 | 4 | KF030727.1 |
| 6 | *Gluconobacter oxydans* strain A292 | 1 | DQ523497.1 |
| 7 | *Gluconacetobacter europaeus* | 2 | FN429075.1 |
| 8 | *Acetobacteraceae bacterium* J2 | 1 | GU213109.1 |
| 9 | *Komagataeibacter xylinus* strain ATCC 53524 | 1 | KX216689.1 |
| 10 | *Acetobacter pasteurianus* strain NH6 | 1 | KR150441.1 |
| 11 | *Gluconacetobacter hansenii* | 1 | KF155166.1 |
| 12 | *Acetobacter ghanensis* strain CIFT MFB 15295 HSA15 | 1 | KP240986.1 |
| 13 | *Acetobacter pasteurianus* strain L1 | 1 | MF179549.1 |
| 14 | *Leuconostoc mesenteroides* strain 10-7 | 1 | KJ477420.1 |
| 15 | *Lactococcus lactis strain* RCB462 | 1 | KT260674.1 |
| 16 | *Lactobacillus brevis strain* JNB23 | 1 | JQ741972.1 |

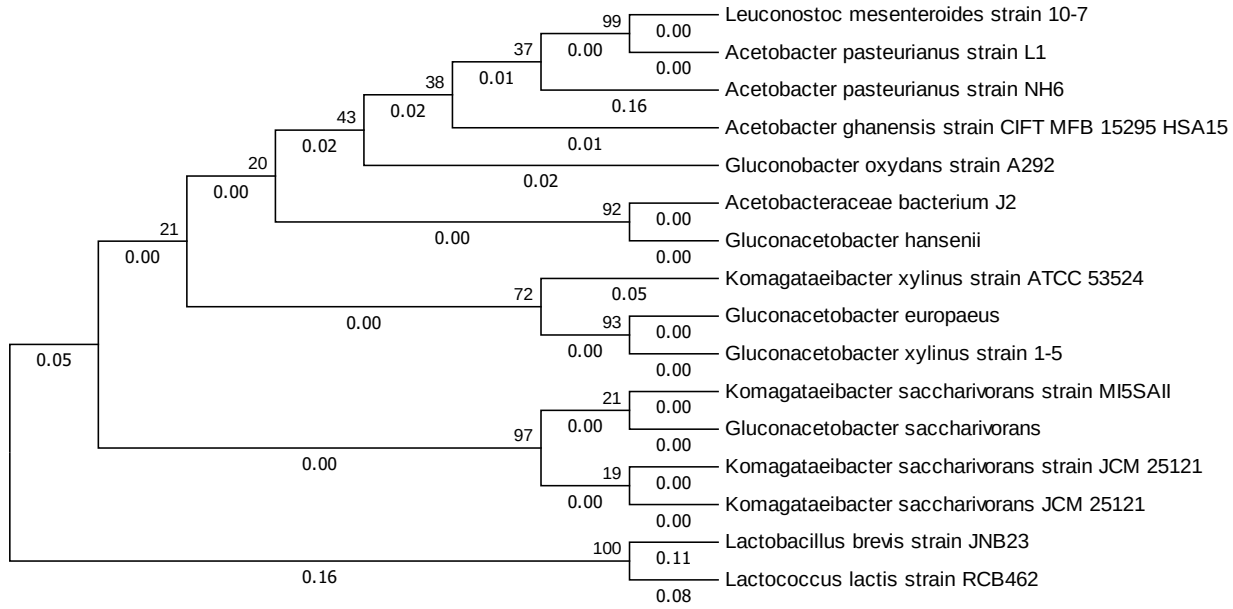


**Figure 2.** Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log-likelihood value. Evolutionary analyses were conducted in MEGA6.

## 4. Discussion

It can be seen that different strains of same species grouped together except one *A. pasterianus* strain. This can be caused because of a polymorphism earlier in the branch. *Gluconobacter* and *Acetobacter* genuses grouped separetly while each of their species grouped together as expected. Ruiz et al (2010) studied bacterial biodiversity of Tempranillo wines and found *Oenococcus oeni* was dominant species. Besides *Oenococcus oeni; Gluconobacter oxydans*, *Asaia siamensis*, *Serratia* sp., and *Enterobacter* sp. was also observed [10]. Torija et al. (2010) studied to develop a Real-Time PCR assay for acetic acid bacteria in wine and vinegar using Taqman minor grove binder probes [11]. Sharafi et al. (2010) studied characterization and optimisation indigenous acetic acid bacteria and isolated thirty-seven acetic acid bacteria from *Acetobacter* and *Gluconobacter* members and observed *Acetobacter pasteurianus* was dominant species [12].

Wu et al. (2010) studied diversity of Acetobacter pasteurianus strains from cereal vinegars and isolated 21 strains with 16S-PCR method [13]. Kommanee et al. (2012) studied the restriction analysis of 16S-23S rRNA gene internal transcribed spacer regions (ITS) using TaqI, AluI, HpaII, and AvaII revealed that forty-seven bacterial isolates found in fruits and flowers collected in Thailand belong to the genus Acetobacter [14]. Wu et al. (2012) studied biodiversity of yeast and lactic acid bacteria of traditional Chinese vinegar and found 47 yeast isolates, 28 lactic acid bacteria isolates and 58 acetic acid bacteria isolates [15]. Trček et al. (1997) and Hidalgo et al. (2012) suggested in a vinegar with high percentage of acetic acid (>6%) the dominant species are *Komagataeibacter europaeus*, *Komagataeibacter oboediens* and/or *Komagataeibacter intermedius*, whereas in vinegar with low percentage of acetic acid (<6%), the dominant species are *Acetobacter aceti*, *Acetobacter pasteurianus* and/or *Acetobacter pomorum* [16, 17]. Petri et al. (2013) studied wine related lactic acid bacteria with

multiplex PCR method and found 13 different species [18]. Wang et al. (2015) investigated Acetobacter bacteria in Zhenjiang vinegar and found six significant acetic acid bacteria strains which two of them was dominant, *Acetobacter aceti* and *A. pasteurianus* [19]. Štornik et al. (2016) studied cultivable acetic acid bacteria from apple cider vinegar and observed 96 bacteria from organic and 72 bacteria from conventional apple cider vinegar using 16S and 23S rRNA restriction analysis [20]. Treck et al. (2016) collected unfiltered vinegar samples and microbial analyses carried out by Illumina MiSeq sequencing of 16S rRNA gene variable regions. They showed that in all wine vinegar samples *Komagataeibacter oboediens* (formerly *Gluconacetobacter oboediens*) was a predominating species and the acetic acid and lactic acid bacteria were two major groups of bacteria in apple cider vinegar [21]. It is possible to find both acetic acid and lactic acid bacteria in fermentative products. Lactic acid bacteria include the genera of *Leuconostoc* sp., *Lactococcus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Pediococcus* sp., *Bifidobacterium* sp., *Aerococcus* sp. [22]. The acetic acid bacteria and lactic acid bacteria can easily be mixed in terms of their morphological and biochemical properties. Therefore, the diversity of these bacteria can be demonstrated more safely using molecular techniques including inter-delta/PCR, PCR-RFLP, ERIC/PCR analysis, as well as 16S rRNA and 26S rRNA partial gene sequencing [23].

## 5. Conclusion

In this study, bacteria were isolated from home-made wine and vinegar. We determined *Komagataeibacter saccharivorans*, *Gluconacetobacter europaeus*, *Gluconacetobacter saccharivorans*, *Gluconacetobacter xylinus*, *Gluconobacter oxydans*, *Acetobacteraceae bacterium*, *Acetobacter pasteurianus*, *Komagataeibacter xylinus*, *Gluconacetobacter hansenii* and *Acetobacter ghanensis* as acetic acid bacteria. In additional *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Lactobacillus brevis* as lactic acid bacteria were identified. The acetic acid bacteria and lactic acid bacteria have important roles in food and beverage production, in the bioproduction of industrial chemicals, to improve the preservation, nutritional value, and sensorial characteristics of a variety of fermented foods and products derived from animal and vegetable origins.

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## 6. References

[1] Hainzelin, É., Nouaille, C. (2013). *The Diversity of Living Organisms: The Engine for Ecological Functioning*, É. Hainzelin (Eds.), Cultivating Biodiversity to Transform Agriculture, Montpellier, France, pp. 11-35.

[2] Johnston, C.S., Gaas, C.A. (2006). Vinegar: Medicinal Uses and Antiglycemic Effect**.** *Medscape General Medicine*, 8(2): 61-73.

[3] Liu, D.R., Zhu, Y., Beeftink, R., Ooijkaas, L., Rinzema, A., Chen, J. (2004). Chinese vinegar and its solid-state fermentation process. *Food Reviews International*, 20(4): 407-424.

[4] Kersters, K., Lisdiyanti, P., Komagata, K., Swings, J. (2006). The family Acetobacteraceae: the genera *Acetobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, and *Kozakia*. Dworkin M., Falkow S., Rosenberg E., Schleifer K.H., Stackebrandt E. (Eds.), The Prokaryotes, Springer, New York, pp. 163-200.

[5] Pretorius, I.S., Van der Westhuizen, T.J., Augustyn, O.P.H. (1999). Yeast Biodiversity in vineyards and wineries and its importance to the South African Wine Industry. A Review. *South African Journal for Enology and Viticulture*, 20(2): 61-70.

[6] Wang, C.Y., Zhang, J., Gui, Z.Z. (2015). *Acetobacter* bacteria are found in Zhenjiang vinegar grains. *Genetics and Molecular Research*, 14(2): 5054-5064.

[7] Sharafi, S.M., Rasooli, I., Beheshti-Maal, K. (2010). Isolation, characterization and optimization of indigenous acetic acid bacteria and evaluation of their preservation methods. *Iranian Journal of Microbiology*, 2(1): 38-41.

[8] Krieg, N.R., Holt, J.G., (1984). *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, Baltimore, MD.

[9] De Boer, S.H., Ward, L.J. (1995). PCR detection of *Erwinia carotovora subsp atroseptica* associated with potato tissue. *Phytopathology*, 85(8): 854-858.

[10] Ruiz, P., Seseña, S., Izquierdo, P.M., Palop, M.L. (2010). Bacterial biodiversity and dynamics during malolactic fermentation of Tempranillo wines as determined by a culture-independent method (PCR-DGGE). *Applied Microbiology and Biotechnology*, 86(5): 1555-1562.

[11] Torija, M.J., Mateo, E., Guillamón, J.M., Mas, A. (2010). Identification and quantification of acetic acid bacteria in wine and vinegar by TaqMan–MGB probes. Food Microbiology, 27(2): 257-265.

[12] Sharafi, S.M., Rasooli, I., Beheshti-Maal, K. (2010). Isolation, characterization and optimization of indigenous acetic acid bacteria and evaluation of their preservation methods. Iranian Journal of Microbiology, 2(1): 38-45.

[13] Wu, J., Gullo, M., Chen, F., Giudici, P. (2010). Diversity of *Acetobacter pasteurianus* strains isolated from solid-state fermentation of cereal vinegars. *Current Microbiology*, 60(4): 280-286.

[14] Kommanee, J., Tanasupawat, S., Yukphan, P., Thongchul, N., Moonmangmee, D., Yamada, Y. (2012). Identification of *Acetobacter* strains isolated in Thailand based on the phenotypic, chemotaxonomic, and molecular characterizations. *Science Asia*, 38(1): 44-53.

[15] Wu, J.J., Ma, Y.K., Zhang, F.F., Chen, F.S. (2012). Biodiversity of yeasts, lactic acid bacteria and acetic acid bacteria in the fermentation of "Shanxi aged vinegar", a traditional Chinese vinegar. *Food Microbiology*, 30(1): 289-297.

[16] Trček, J., Ramuš, J., Raspor, P. (1997). Phenotypic characterization and RAPD-PCR profiling of *Acetobacter* sp. isolated from spirit vinegar production. *Food Technology and Biotechnology*, 35(1): 63-67.

[17] Hidalgo, C., Mateo, E., Mas, A., Torija, M.J. (2012). Identification of yeast and acetic acid bacteria isolated from the fermentation and acetification of persimmon (Diospyros kaki). *Food Microbiology*, 30(1): 98-104.

[18] Petri, A., Pfannebecker, J., Fröhlich, J., König, H. (2013). Fast identification of wine related lactic acid bacteria by multiplex PCR. *Food Microbiology*, 33(1): 48-54.

[19] Wang, Z., Ning, Z., Shi, J., Feng, W., Liu, Y., Liang, X. (2015). Comparative proteome of *Acetobacter pasteurianus* Ab3 during the high acidity rice vinegar fermentation. *Applied Biochemistry and Biotechnology*, 177(8): 1573-1588.

[20] Štornik, A., Skok, B., Trček, J. (2016). Comparison of cultivable acetic acid bacterial microbiota in organic and conventional apple cider vinegar. *Food Technology and Biotechnology*, 54(1): 113-119.

[21] Trček, J., Mahnič, A., Rupnik, M. (2016). Diversity of the microbiota involved in wine and organic apple cider submerged vinegar production as revealed by DHPLC analysis and next-generation sequencing. *International Journal of Food Microbiology*, 16(223): 57-62.

[22] Liu, W., Pang, H., Zhang, H., Cai, Y. (2014). *Biodiversity of Lactic Acid Bacteria*. H. Zhang and Y. Cai (eds.), Lactic Acid Bacteria. Springer, New York, pp. 103-203.

[23] Wu, J.J., Ma, Y.K., Zhang, F.F., Chen, F.S. (2012). Biodiversity of yeasts, lactic acid bacteria and acetic acid bacteria in the fermentation of Shanxi aged vinegar, a traditional Chinese vinegar. *Food Microbiology*, 30:289-297.