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The production of wine vinegar using different types of acetic acid bacteria

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ABSTRACT

This work aimed to study the properties of acetic fermentation bacteria during the acetic fermentation of wine. Attention was focused on the ability of the bacteria to metabolize selected organic substances and their suitability for wine vinegar production. For the production of wine vinegar, white wine of the variety Veltliner Green was used. Three variants were established for this experiment. The first variant was fermented with *Gluconobacter oxydans*, the second with *Acetobacter aceti*, and the third variant of vinegar production was carried out by spontaneous fermentation. During the vinegar fermentation, samples were taken at regular 24-hour intervals and subsequently analyzed. The alcohol, acetic, malic, and tartaric acid contents were monitored. The results showed that all variants showed a strong acetic and malic acid increase. Bacteria *Acetobacter aceti* produced the most acetic acid within nine days (25 g.L⁻¹). This bacterium also produced the most lactic acid (18 g.L⁻¹). Tartaric acid was also produced in all three variants, but not to the same extent as the previous two organic acids. *Acetobacter aceti* was found to metabolize ethanol more rapidly than *Gluconobacter oxydans*.

Keywords: vinegar fermentation, wine vinegar, *Acetobacter aceti*, *Gluconobacter oxydans*, spontaneous fermentation

INTRODUCTION

The earliest mentions of vinegar can be found in the Old and New Testaments. It is believed that since people have known spirits, they have also known vinegar. As early as the fourteenth century, there are references to artisanal vinegar production and the establishment of vinegar makers' guilds. Many types of fruit were used, and they were left to ripen on the tree. The harvested fruit was transferred to uncovered pots, which were left to ferment spontaneously. The resulting fermented liquid was placed in barrels left to ferment spontaneously [1]. Vinegar fermentation bacteria are widely used in the biotechnology and food industries. Most strains of vinegar bacteria are used in the food industry to produce important organic acids. In addition to acetic acid, these include gluconic, glucuronic, and propionic acids [2]. In biotechnology, vinegar bacteria are mainly used to produce ascorbic acid (vitamin C) or cellulose. The primary acetic acid producers are strictly aerobic bacteria of the genus *Acetobacter*. In addition to ethanol oxidation, these bacteria can oxidize aliphatic alcohols, and some species oxidize carbohydrates. This is a two-stage process of substrate oxidation. First, ethyl alcohol is oxidized to acetaldehyde by the enzyme alcohol dehydrogenase, and in the second stage, acetaldehyde is further oxidized to acetic acid by the enzyme acetaldehyde dehydrogenase [3]. The genus *Acetobacter* of the family *Acetobacteraceae* is an aerobic bacterium with a pronounced ability to oxidize ethanol to acetic acid. For this reason, this genus is exclusively used in vinegar fermentation. Adversely, these bacteria are found in the acetation of wine or beer or as an undesirable contaminant in yeast production. Bacteria of the genus *Acetobacter* have a strictly aerobic metabolism so that even a short interruption of the oxygen supply leads to their death in the presence of ethanol. When the ethanol concentration drops, the bacteria oxidize the resulting acetic acid to carbon dioxide and water (so-called over-fermentation) [4]. Researchers [5] determined values of 0.26 to 0.41 mg.L⁻¹ of volatile acids expressed as acetic acid in white Slovak wines of the 2015 – 2017 vintages. These were faultless wines. The legal limit for the content of volatile acids in wine within the EU is above 1 mg.L⁻¹ per wine category. Wine with a volatile acid content above 1.4 mg.L⁻¹ is already considered diseased. However, it can be used for

the production of wine vinegar. Some strains can grow in solutions of up to 24% vol. ethanol. These microorganisms can also grow at low pH values. These agents are also capable of spoiling must and can cause strong acidification, and 'must disease' in sweet musts, characterized by an unpleasant smell and taste and producing acetaldehyde with polyphenols that cause a milky, colloidal precipitate [6]. The genus *Gluconobacter* is characterized by its ability to incompletely oxidize a wide range of carbohydrates and alcohols, with the resulting metabolites, such as aldehydes, ketones, and organic acids, almost entirely excreted into the environment. The enzyme dehydrogenase catalyzes these reactions. These organisms can grow at low pH values and in environments with high concentrations of sugars. Members of this genus are used in modern fermentation processes such as L-sorbose (synthesis of vitamin C) and 6-amino-L-sorbose (synthesis of the antidiabetic drug Miglitol). These species produce other important metabolites: dihydroxyacetone, gluconates, and ketogluconates [7]. The most widely used method for vinegar production is the so-called submerged method, which can use either a fed, a continuous, or a discontinuous process. In this method, the bacteria are dispersed throughout the dilution volume. This method produces vinegar in a device known as an acetator. This equipment depends on the correct oxygen content since a short interruption in aeration will kill the bacteria. These vessels are equipped with an agitator and with refrigeration and aeration equipment. As a rule, the dilution contains ethanol at a concentration of 11 – 12% vol. and is enriched with additional nutrients such as glucose, urea, glycerol, yeast, casein, phosphates, magnesium salts, potassium salts, and many trace elements. The process is completed by reducing the ethanol to 0.3% vol. The production cycle time is 48 to 72 hours. This method has a high yield, with daily increments of acetic acid up to 4%. The resulting product must be subsequently purified and filtered due to the high turbidity of the bacterial origin [8]. Other methods of producing vinegar from wine by bacteria are the traditional surface Orleans method and the generator method using current and carriers [9]. Wine vinegar is one of the most used vinegars in our country. It is formed by natural transformation from wine when exposed to air. Its quality depends on the quality of the wine. The wine used to make vinegar may be of poor quality or even poorly oaked, but it must not be defective or diseased. Bitter wines, wines with a mousy taste, and wines affected by lactic fermentation are unsuitable. The bitter or musty flavour is not removed during vinegar-making but is accentuated. The lactic acid is usually converted into butyric acid, rendering the vinegar inedible. Vinegar from red and white wines can be made separately or in mixtures. However, white wine vinegar must never be mixed with red wine vinegar. The wine used to make vinegar should contain at least 8% alcohol. The wine is usually between 10 and 12% alcohol [10]. For higher alcohol contents, it is recommended that the wine be diluted with water before vinegar-making to give an alcohol content of 8% to 9%. In the original recipes, the vinegar should be aged for a long time in barrels. Modern methods try to speed up the process, but this is done at the expense of quality, as the vinegar loses its characteristic aroma. Wine vinegar can be the basis for other vinegar types [11]. A necessary condition for the successful course of vinegar fermentation in the production of wine vinegar from the wine of the genus *Acetobacter* is a temperature above 18 °C, a low value of free SO₂ below 10 mg.L⁻¹ and access of oxygen to the wine, e.g. in the form of air (20%) [12]. Each genus of vinegar fermentation bacteria has its specific metabolism. Differences in metabolism are even found in different strains of these bacteria. The most significant differences in the resulting metabolites between species are found in the production of higher fatty acids, furan compounds, enol derivatives, and some esters. The significant changes in acetic fermentation products also depend on the bioconversion of acetic acid from ethanol. This is primarily a function of the composition of the wine itself and the fermentation temperatures, aeration intensities, etc. [13].

Scientific Hypothesis

Although several genera of acetic fermentation bacteria are capable of complete acetic fermentation, some of these genera are more suitable for this fermentation because they produce more organic acids and, in some cases, more rapidly. This study looks at which strain is more suitable for wine vinegar production.

MATERIALS AND METHODOLOGY

Samples

A total of three variants were investigated. The first variant was fermented with *Gluconobacter oxydans*, the second with *Acetobacter aceti* and the third variant of vinegar production was carried out by spontaneous fermentation.

Chemicals

Wine: For this experiment, a specific wine had already been produced in 2018. Spontaneous fermentation was used in producing this wine by the reductive method. No malolactic fermentation took place in this wine, of which 50 litres were produced. After the alcoholic fermentation, the must, which was no longer fermenting, was statically racked off, and filtration was not used. Only minimal amounts of sulphur preparations were applied to the wine

during production. Therefore, the wine was a 2018 vintage of the Green Veltliner variety. This wine was characterized by low free and bound sulphur (total sulphur: 24 mg.L⁻¹).

Distilled water

Animals and Biological Material

Gluconobacter oxydans CCM 3618.

Acetobacter aceti CCM 3620.

Instruments

WineScan analyser (Foss, Denmark), microbiological incubators (Liebherr, Germany), compressors (Tetra, Germany).

Laboratory Methods

Spectroscopy: To measure the basic analytical parameters of wine and subsequent vinegar, a WineScan analyzer (Foss, Denmark) was used. This method is based on Fourier transform infrared spectroscopy combined with the partial least squares method. Sampling was carried out with an autosampler, using approximately 50 mL of sample for duplicate measurements, including a pre-wash system.

Description of the Experiment

Sample preparation: The wine was sterilized, and the alcohol level was adjusted before the individual bacteria were inoculated.

Number of samples analyzed: The total number of samples analysed was 81.

Number of repeated analyses: Each sample was analyzed three times, and the result is the average of these measurements.

Number of experiment replications: Each of the three variants was performed in three repetitions. Thus, nine mini-acetators were produced.

Design of the experiment: In vinegar production, three variants were chosen. Among the acetic fermentation bacteria, one representative of acetic fermentation bacteria from the genus *Gluconobacter* and one from the genus *Acetobacter* were selected for this research experiment. The first species was *Gluconobacter oxydans* CCM 3618, and the second was *Acetobacter aceti* CCM 3620. These bacterial species came from the Czech Collection of Microorganisms of the Faculty of Science of Masaryk University in Brno. In the third variant, vinegar fermentation was spontaneous. The wine of the variants inoculated with specific strains of acetic fermentation bacteria was sterilised at 70 °C for 120 minutes before inoculation. Subsequently, it was diluted to 8% alcohol to ensure a smooth vinegar fermentation [11]. Each of the different variants was run in three repetitions. Thus, nine 5L mini-acetators were produced by us and oxygenated by a compressor (Tetra, Germany). The acetators were placed in special microbiological incubators (Liebherr, Germany), and each variant had its incubator to avoid cross-contamination. Fermentation was carried out over nine days, with samples taken from each acetator every 24 hours during fermentation. Samples were collected with a glass pipette into 50 mL Eppendorf® mini centrifuge tubes (Sigma–Aldrich, Germany). After collection, all samples were placed in a freezer at -25 °C. After fermentation, all samples were thawed and subsequently analyzed on a WineScan analyzer (Foss, Denmark).

Statistical Analysis

The experiment used statistical analysis of variance (one-way ANOVA), which showed statistically significant differences between the variants. Statistical results are significant at $p = 0.05$. Subsequently, a post-hoc test (Tukey HSD test) was performed for each variant. All statistical evaluation is available from the author.

RESULTS AND DISCUSSION

Monitoring the Acetic Acid Content

According to Table 1, we can observe that *Acetobacter aceti* could produce the most acetic acid of all the variants during fermentation. This bacterium produced up to 25 g.L⁻¹ of acetic acid in nine days. The other variants did not achieve such high values. *Gluconobacter oxydans* was able to produce only 15.14 g.L⁻¹. In the variant with spontaneous fermentation, we measured 16.96 g.L⁻¹ acetic acid on the last day of fermentation. In Figure 1, we can see that *Acetobacter aceti* produced the most acetic acid and that this bacterium started fermentation earlier than the other variants. The acetic acid production of *Gluconobacter oxydans* was not as intense compared to the previous representative of acetic fermentation. The same applies to the variant with spontaneous fermentation.

Table 1 Changing values of acetic acid (expressed in g.L⁻¹) during acetic fermentation.

Acetic acid	<i>Acetobacter aceti</i>	<i>Gluconobacter oxydans</i>	Spontaneous fermentation
Day No. 1	0.21 ±0.01	0.20 ±0.00	0.20 ±0.00
Day No. 2	4.13 ±0.44	1.64 ±1.22	0.67 ±0.13
Day No. 3	8.35 ±1.03	2.73 ±1.84	2.84 ±1.97
Day No. 4	10.67 ±1.50	3.54 ±2.00	3.43 ±2.26
Day No. 5	13.36 ±2.67	5.88 ±2.29	4.74 ±3.08
Day No. 6	16.06 ±1.53	9.49 ±2.18	6.76 ±3.75
Day No. 7	17.91 ±2.19	12.08 ±1.77	8.68 ±4.22
Day No. 8	18.65 ±1.38	12.98 ±0.64	10.79 ±3.09
Day No. 9	24.75 ±3.53	15.14 ±0.70	16.96 ±5.07

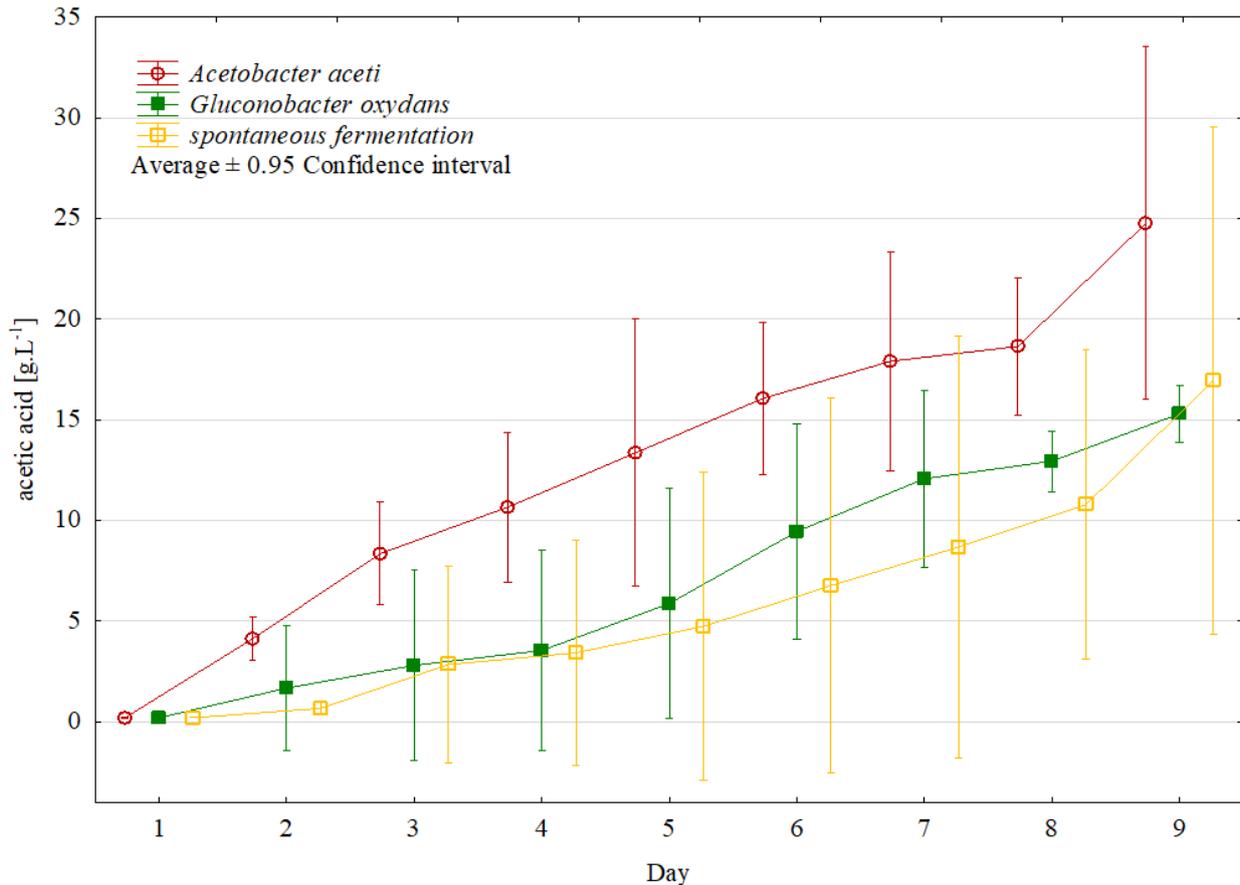


Figure 1 Statistical expression of changing acetic acid values (expressed in g.L⁻¹) during acetic fermentation.

Monitoring Malic Acid Content

Table 2 shows the changing values of malic acid during acetic fermentation. The bacterium *Acetobacter aceti* was capable of the highest malic acid production. Similarly to the previous result, *Acetobacter aceti* produced most of this organic acid of all the variants during acetic fermentation. This bacterium produced up to 18.53 g.L⁻¹ of acetic acid over nine days. The other variants did not reach such high values. Similar values were measured for the other two variants. *Gluconobacter oxydans* was able to produce only 13.70 g.L⁻¹. For the spontaneous fermentation variant, we measured only a slightly lower malic acid value of 13.47 g.L⁻¹ on the last day of fermentation. According to Figure 2, *Acetobacter aceti* again produced the most malic acid, and this bacterium started fermentation at the earliest of the three variants. Compared with the other variants, the production of this organic acid was already much more intense from the first day of fermentation, and higher values were measured.

Table 2 Changing values of malic acid (expressed in g.L⁻¹) during acetic fermentation.

Malic acid	<i>Acetobacter aceti</i>	<i>Gluconobacter oxydans</i>	Spontaneous fermentation
Day No. 1	1.57 ±0.6	1.53 ±0.06	1.50 ±0.00
Day No. 2	2.77 ±0.91	1.60 ±0.20	2.00 ±0.44
Day No. 3	4.93 ±1.55	2.17 ±0.32	3.13 ±1.18
Day No. 4	6.40 ±0.96	2.97 ±0.85	3.53 ±1.27
Day No. 5	7.73 ±1.25	4.20 ±0.82	4.63 ±1.54
Day No. 6	9.93 ±1.98	6.03 ±0.40	5.90 ±1.93
Day No. 7	12.54 ±2.17	7.97 ±0.35	7.10 ±2.09
Day No. 8	14.67 ±3.48	10.97 ±2.91	8.30 ±0.85
Day No. 9	18.53 ±2.22	13.70 ±1.25	13.47 ±1.31

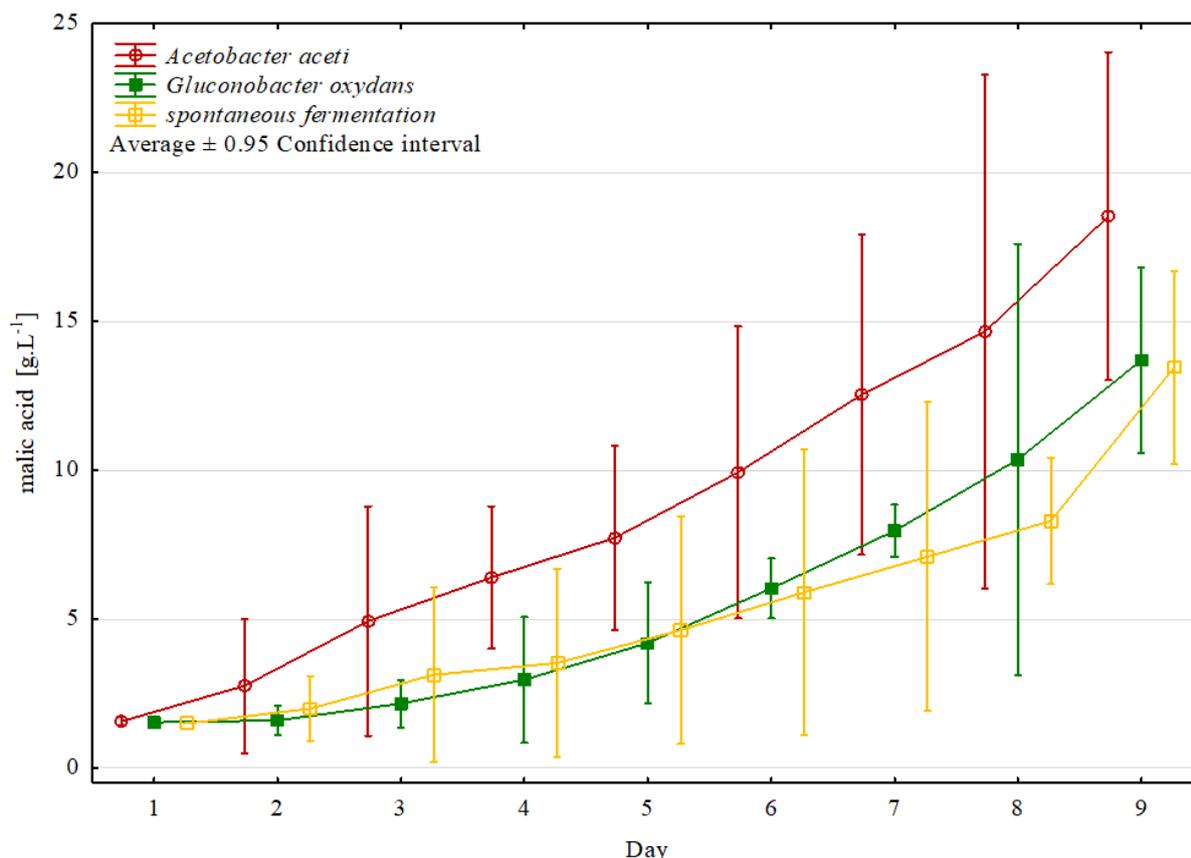


Figure 2 Statistical expression of changing malic acid values (expressed in kg.m⁻³) during acetic fermentation.

Monitoring Tartaric Acid Content

Table 3 summarises the levels of tartaric acid that the acetic acid fermentation plants can also produce. However, compared with the previous organic acids' production, this acid's production is considerably less. Acetic acid bacteria only produces this organic acid to a small extent. In our experiment, after nine days of fermentation, the greatest increase in tartaric acid was observed in the variant fermented by *Acetobacter aceti*. This was an increase from 1.20 g.L⁻¹ to only 2.80 g.L⁻¹. In Figure 3, the spontaneously fermented variant and the variant fermented by *Gluconobacter oxydans* also showed an increase in tartaric acid. Still, again, the production of this organic acid was lower than in the variant fermented by *Acetobacter aceti*, which showed the greatest increase in measured tartaric acid values.

Table 3 Changing values of tartaric acid (expressed in g.L⁻¹) during acetic fermentation.

Tartaric acid	<i>Acetobacter aceti</i>	<i>Gluconobacter oxydans</i>	Spontaneous fermentation
Day No. 1	1.20 ±0.00	1.27 ±0.12	1.20 ±0.00
Day No. 2	1.40 ±0.10	1.33 ±0.06	1.37 ±0.12
Day No. 3	1.60 ±0.10	1.37 ±0.06	1.50 ±0.10
Day No. 4	1.70 ±0.17	1.43 ±0.06	1.57 ±0.06
Day No. 5	1.67 ±0.21	1.47 ±0.12	1.80 ±0.10
Day No. 6	2.00 ±0.26	1.73 ±0.12	1.73 ±0.15
Day No. 7	2.20 ±0.20	1.87 ±0.06	1.77 ±0.21
Day No. 8	2.27 ±0.15	1.80 ±0.17	1.90 ±0.17
Day No. 9	2.80 ±0.10	2.00 ±0.10	2.13 ±0.06

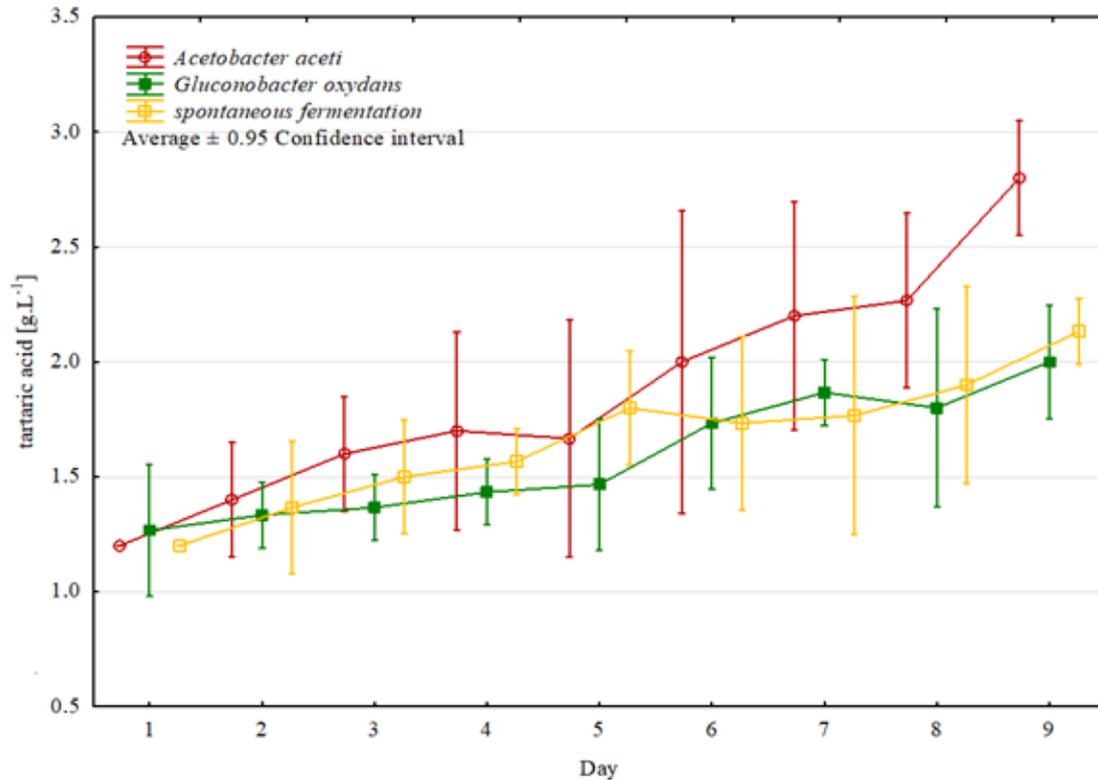


Figure 3 Statistical expression of changing tartaric acid values (expressed in g.L⁻¹) during acetic fermentation.

Monitoring Alcohol Content

Table 4 shows the decreasing values of alcohol content during the vinegar fermentation over the nine days. The biggest difference in the measured values between the first and last day can be seen in the variant fermented with *Acetobacter aceti*. In this variant, the alcohol content changed from 7.82% vol. to 2.14% vol. Figure 4 clearly shows that *Acetobacter aceti* can metabolize alcohol much more rapidly than *Gluconobacter oxydans*. The measured data show that the variant fermented spontaneously did not consume alcohol to the same extent as the variants inoculated with the different species of acetic acid bacteria.

Table 4 Changing values of alcohol (expressed in % vol.) during acetic fermentation.

Ethyl alcohol	<i>Acetobacter aceti</i>	<i>Gluconobacter oxydans</i>	Spontaneous fermentation
Day No. 1	7.82 ±0.18	7.71 ±0.18	7.70 ±0.14
Day No. 2	6.72 ±0.24	7.02 ±0.48	7.43 ±0.28
Day No. 3	4.90 ±0.75	6.42 ±0.49	6.66 ±0.74
Day No. 4	4.29 ±0.35	6.08 ±0.19	6.31 ±0.73
Day No. 5	3.98 ±0.07	5.80 ±0.32	6.56 ±0.78
Day No. 6	3.57 ±0.15	5.13 ±0.31	6.17 ±0.86
Day No. 7	3.15 ±0.08	3.80 ±0.93	5.82 ±0.95
Day No. 8	2.31 ±0.22	3.30 ±0.12	4.63 ±1.20
Day No. 9	2.14 ±0.19	3.19 ±0.21	4.42 ±1.12

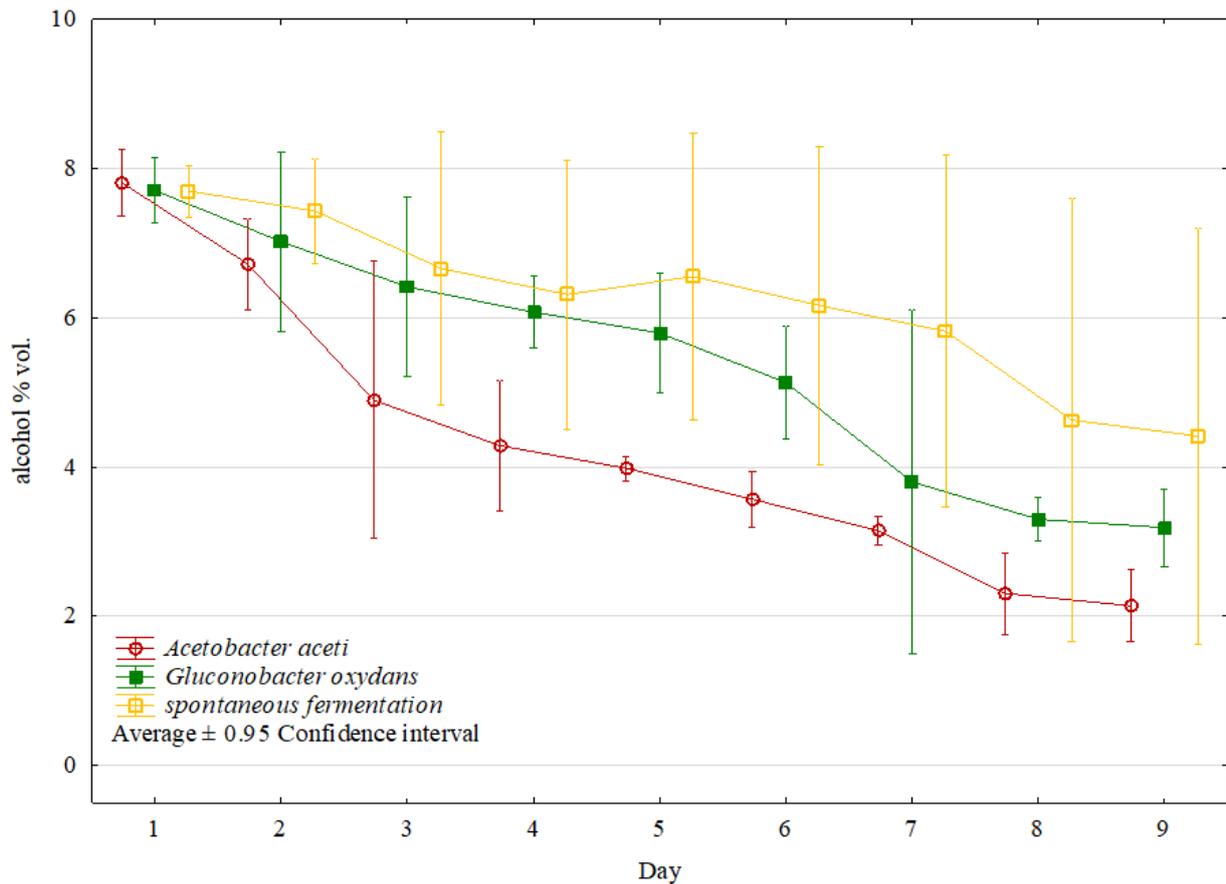


Figure 4 Statistical expression of the changing values of alcohol (expressed in % vol.) during acetic fermentation.

Acetic acid fermentation is a biochemical process in which acetic acid bacteria oxidize ethanol to acetic acid under strict aerobic conditions. Our research concluded that although both genera of *Acetobacter* and *Gluconobacter* are suitable for acetic acid fermentation, the former is much more suitable. This is also due to the fact that bacteria of the genus *Acetobacter* oxidize ethanol more strongly than glucose, while *Gluconobacter* oxidizes glucose more strongly than ethanol. Our original substrate was a white wine fermented completely dry with a low sugar content. The *Acetobacter aceti* strain was able to produce more organic acids and consumed ethanol faster than the other variants [14]. Our research focuses on the ability of acetic acid bacteria to produce various organic acids. These bacteria are capable of producing many organic biomolecules. Since there is a recent need to try to limit chemical synthesis, acetic acid bacteria are an ideal way to produce many organic substances. These properties can be used in the food, chemical, pharmaceutical, and medical industries. This work mainly focused on monitoring the increasing acid content during acetic fermentation. The ability of these bacteria to produce acetic, lactic and tartaric acid was also confirmed by this research, and the examined representatives produced different amounts of the aforementioned organic acids. These microorganisms can produce other organic acids such as malic, formic, citric, succinic, gluconic, and glucuronic acids. The production of organic acids also depends on the fermentation temperature since, at lower temperatures, these bacteria produce only limited amounts of the above compounds. This was confirmed in the optimization of this research. Proper acetic fermentation was achieved only when the appropriate fermentation medium temperature was maintained [15], [16], [17]. The importance of knowing the bacteria involved in vinegar fermentation is described in a 2006 study. Pure cultures of acetic acid bacteria are rarely used for vinegar fermentation. And when this does happen, it is very likely that other unwanted acetic acid bacteria subsequently contaminate precisely selected strains during fermentation, and these undesirable microorganisms can produce vinegar of inferior quality. In the industry, a mixed culture of *Acetobacter* is mainly used to produce acetic acid, but no attention is paid to its proper maintenance [18, 19]. Following the experiment presented, our assertion that *Gluconobacter* bacteria are not suitable for total vinegar fermentation is well supported, as research from 2010 also shows that *Acetobacter* bacteria are predominant in traditional vinegar fermentation. In this research, the population dynamics of acetic fermentation bacteria were determined in two independent *Acetobacter* plants at both the species and strain levels. The effect of four different wood species of fermentation barrels on the diversity of acetic fermentation bacteria was also investigated. Vinegar fermentation bacteria were isolated on solid media. RFLP-PCR of 16S rRNA genes

then identified individual species, and confirmation was performed by 16S rRNA gene sequencing, while the strains were typed by ERIC-PCR and (GTG)₅-rep-PCR. *Acetobacter pasteurianus* was the most frequently isolated species, accounting for almost 100% of all isolates detected during the whole vinegar fermentation. Representatives of the genus *Gluconacetobacter* appeared only at the end of the process and only in oak barrels from one of the vinegar plants investigated. The different *A. pasteurianus* showed a precise sequence with increasing acetic acid concentration. In all the vinegar houses, the dominance of the other strains changed with increasing acetic acid concentration, and the diversity of strains tended to decrease at the end of the process [20]. Surprisingly, a 2010 study showed that a representative from *Acetobacter* (*A. pasteurianus*) was not as successful as expected for total vinegar fermentation. On the contrary, higher oxygen and acetic acid concentrations seem to have promoted the development of *Gluconacetobacter* species (*G. europaeus* and *G. Intermedius*). This study indicated that mixed inoculum of *A. pasteurianus* and selected *Gluconacetobacter* species are the most likely candidates for use as initial cultures for suitable vinegar fermentation of wine. *Gluconacetobacter* species have the advantage of better tolerance to high concentrations of acetic acid [21]. The claim that *Gluconacetobacter* genera are partly involved in vinegar fermentation can also be found in a 2008 study. This study investigated the identification of the dominant genera of acetic fermentation bacteria in coconut wine, the so-called mnazi. First, the bacteria were isolated on GYP agar, and physiological and biochemical tests followed. Both *Acetobacter* and *Gluconobacter* strains detected were oxidase negative and catalase positive. *Acetobacter* strains could oxidize lactate and acetate, while *Gluconobacter* strains oxidized only lactate. The research shows that both of these genera are responsible for the spoilage of coconut wine by mnazi [22]. The suitability of strains of the genus *Acetobacter* for vinegar fermentation can also be found in a study from 1999 when the possibility of producing a new type of vinegar from onions that did not meet the quality standards required for marketing was investigated. Various kinds of onion were first tested as raw material for vinegar production, and vinegar was successfully produced from the juice of red onion, the Kurenai cultivar, using culture with yeast and *A. aceti* [23]. Another suitable strain for acetic fermentation is *Acetobacter europaeus*, isolated and described by molecular methods in 1992. This strain was isolated from an inoculum from a large vinegar factory in Hamburg [24]. In 1998, two new strains were described that carry out acetic fermentation. This time the strains were isolated directly from red wine in which acetic fermentation was already taking place. These were the acetic fermentation bacteria *Acetobacter oboediens* sp. and *Acetobacter pomorum* sp. Molecular techniques again carried out the identification of these strains. The closest relatives of these strains are *Acetobacter europaeus*, *Acetobacter xylinus*, and *Acetobacter pasteurianus* [25]. Another strain suitable for acetic fermentation is the strain described in 1985. This strain was named *Acetobacter polyoxogenes* sp. It is a strain capable of producing high amounts of acetic acid and was isolated from fermented vinegar broth with a high acidity [26]. In a 2013 study, blueberry wine was acetified using naturally occurring microorganisms in the first instance, and a second variant was fermented using an inoculated strain of *A. cerevisiae*. The acetification was carried out in three repetitions using the so-called Schützenbach method. It was found that the spontaneous fermentation processes lasted up to 66% longer than those of the variants involving inoculation with the *A. cerevisiae* strain. The isolation of the acetic fermentation bacteria and the subsequent analysis of these microorganisms by molecular methods made it possible to identify the main genotypes responsible for the acetification of blueberry wine. Although *A. cerevisiae* was the predominant strain isolated from samples from the inoculated processes, *A. pasteurianus* was isolated from samples for both processes and was the only species present for the spontaneous acetification variant. This study shows that the isolated strains of *A. pasteurianus* appear to be the most suitable representatives of acetic fermentation bacteria for the large-scale production of wine vinegar [27]. In a 2010 study, the volatile components of fruit vinegar fermented with two acetic acid bacteria were quantitatively analyzed. These were strains of *Acetobacter pasteurianus* AC2005 and *A. rancens* (AS1.41) by headspace solid-phase microextraction combined with gas chromatography and mass spectrometry. As a result, 45 species of volatile compounds were detected in the hawthorn vinegar produced by AC2005, including nine esters, 12 alcohols, three ketones, 12 acids, and nine other compounds. In vinegar fermented by AS1.41, 39 species were found, including 13 esters, nine alcohols, two ketones, 10 acids, and five other compounds. Of these, 26 species of volatile compounds detected in the two kinds of vinegar were the same. The characteristics of each *Acetobacter* strain were responsible for the variety of vinegars in taste [9]. In the 2016 study, *Acetobacter aceti* CCT 0190 and *Gluconobacter oxydans* CCMA 0350 were used simultaneously to produce jaboticaba vinegar. This combination of two species of acetic fermentation bacteria showed good results. In particular, these bacteria were able to produce high concentrations of citric acid (6.67 g.L⁻¹), malic acid (7.02 g.L⁻¹), and succinic acid (5.60 g.L⁻¹). Fruit wine produced from *Myrciaria jaboticaba* was used as starting material. The yeast species *Saccharomyces cerevisiae* CCMA 0200 was used to ferment the must for the production of jaboticaba wine [28]. In our research, spontaneous fermentation was used to produce the wine used for the subsequent acetic fermentation. Research from 1998 suggests that not every yeast always produces a suitable substrate for subsequent fermentation by acetic acid bacteria. It is also possible that *G. oxydans*

was not as suitable for wine vinegar production because of the unsuitable fermentate produced by *S. cerevisiae*, since, according to this research, the fermentate produced by *Candida stellata* positively affected the growth of acetic acid bacteria and the quality of the vinegar. In contrast, the wine produced by fermentation of *Kloeckera apiculata* was a good substrate for the growth of acetic acid bacteria and acetic acid production and could be used for 'ordinary' vinegar production [29]. A Slovenian industrial vinegar production plant was used for samples of unfiltered vinegar from three oxidation cycles of red wine and organic apple cider vinegar. *Gluconacetobacter oboediens* was the predominant species in all wine vinegar samples. At the beginning of fermentation, the acetic acid bacterial consortium was dominated by *Acetobacter*, with the genus *Gluconacetobacter* predominating over *Acetobacter* at the end of the oxidation cycle in all cider vinegar samples. Two dominant genera, *Lactobacillus* and *Oenococcus*, were identified among the lactic acid bacterial consortium, with *Oenococcus* predominating with increasing acetic acid concentration in the vinegar. Unexpectedly, the minor genus of the acetic acid bacterial consortium in organic apple cider vinegar was *Gluconobacter*, suggesting the possible evolution of a *Gluconobacter* population with tolerance to ethanol and acetic acid. The genus *Rhodococcus* was detected among the companion bacteria of wine vinegar but declined significantly towards the end of the oxidation cycles [30]. Our contention that the genus *Acetobacter* is a suitable genus for acetic fermentation is suggested by research from Garg, who produced vinegar from mango pulp by a dual fermentation and oxidation process using *Saccharomyces cerevisiae* and *Acetobacter aceti*. *S. cerevisiae* was recycled to increase the fermentation rate, while *A. aceti* was immobilized on wood shavings for semi-continuous vinegar production. The vinegar produced had 5.3% acidity as acetic acid. Conversion efficiency of 60% was achieved [31]. Acetic acid is an important basic chemical. It is mainly produced synthetically, and only 10% of the world's production is produced by bacterial fermentation for vinegar production. As reported in a 2018 study, several microorganisms can produce acetic acid, and some may incorporate CO₂ during production. To ensure proper acetic fermentation, choosing the right kind of acetic fermentation bacteria is important. Still, it is also essential to choose the right fermentation environment and original substrate [32].

CONCLUSION

This work aimed mainly to determine a suitable acetic fermentation bacterium for the production of wine vinegar. For our experiment, we chose one bacterium of the genus *Acetobacter* (*Acetobacter aceti*) and one bacterium of the genus *Gluconobacter* (*Gluconobacter oxydans*). For the production of wine vinegar, white wine of the Veltliner Green variety was used. The vinegar fermentation was carried out in 5 L mini-acetators for nine days. Samples were taken at regular 24-hour intervals and analyzed. This experiment was further enriched by one variant, fermented by the so-called fermenter spontaneously. The alcohol, acetic, malic, and tartaric acid contents were monitored. The results revealed that all variants showed a strong acetic and malic acid increase. Tartaric acid was also produced in all three variants, but not to the same extent as the previous two organic acids. The results showed that *Acetobacter aceti* produced higher levels of all the organic acids studied than the other acetic acid bacteria mentioned. It was also found that *Acetobacter aceti* could metabolise ethanol more rapidly than *Gluconobacter oxydans*. Because of the above results, we can recommend *Acetobacter aceti* as a more suitable bacterium for wine vinegar production, as this bacterium produced up to 25 g.L⁻¹ acetic acid and up to 18 g.L⁻¹ lactic acid within nine days.

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