Committees

Third International Conference on

Acetic Acid Bacteria

Vinegar and other products

Cordoba, Spain

17-20 April 2012

Honour Committee
José Manuel Roldán Nogueras
Ana María Troncoso González
Francisco Javier de las Nieves López

Sr. Rector Mgco. University of Córdoba, Spain
Executive Director of AESAN, Spain
President of IFAPA, Spain

Conference Chair
Isidoro García-García

University of Córdoba, Spain

Organizing Committee
M. Carmen Millán-Pérez
José L. Bonilla-Venceslada
Jorge E. Jiménez-Hornero
Carmen Álvarez-Cáliz
Teresa García-Martínez
Ana M. Cañete-Rodríguez
Isabel Arcos Gallardo (Secretariat)

University of Córdoba, Spain

Scientific Committee
Albert Mas
Carlos J. González-Navarro
Colín Webb
Domingo Cantero-Moreno
Edgardo Escamilla
Eveline Bartowsky
Francisco López-Bonillo
Frank Emde
Fusheng Chen
Gunjana Theeragool
Hiroyuki Togana
Inés María Santos-Dueñas
Isabel López-Infante
Jaime Romero
José Manuel Guillamón-Navarro
José María González-Sáiz
Juan Carlos García-Mauricio
Kazunobu Matsushita
Luc De Vuyst
Mª Carmen García-Parrilla
María Gullo
Paolo Giudici
Ramón Natera-Marín
Raul O. Pedraza
Uwe Deppenmeier

Rovira i Virgili University, Spain
CNTA, Spain
The University of Manchester, UK
University of Cádiz, Spain
National University of Mexico, Mexico
The Australian Wine Research Institute, Australia
Rovira i Virgili University, Spain
Heinrich Frings GmbH & Co. KG, Germany
Huazhong Agricultural University, China
Kasetsart University, Thailand
University of the Ryukyus, Japan
University of Cordoba, Spain
IFAPA, Spain
University of Chile, Chile
IATA, CSIC, Spain
University of La Rioja, Spain
University of Cordoba, Spain
Yamaguchi University, Japan
Vrije University Brussel, Belgium
University of Sevilla, Spain
University of Modena and Reggio Emilia, Italy
University of Modena and Reggio Emilia, Italy
University of Cádiz, Spain
National University of Tucumán, Argentina
University of Bonn, Germany
# Table of Contents

**ACETIC ACID BACTERIA 2012, VOLUME 1, SUPPLEMENT 1**

Third International Conference on

**Acetic Acid Bacteria**

**Vinegar and other products**

**Cordoba, Spain**

**17-20 April 2012**

*Guest Editor: Isidoro García-García, Spain*

## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Session</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening Session</td>
<td>1</td>
</tr>
<tr>
<td><strong>SESSION I.</strong> Why do we care about Acetic Acid bacteria? History. Traditional vinegar. Food and Health</td>
<td>1</td>
</tr>
<tr>
<td><strong>SESSION II.</strong> What are they? Taxonomy and Phylogenesis. Ecological aspects</td>
<td>6</td>
</tr>
<tr>
<td>(natural occurrence of AAB; Biodiversity)</td>
<td></td>
</tr>
<tr>
<td><strong>SESSION III.</strong> How do they work?</td>
<td>13</td>
</tr>
<tr>
<td>Session III.A. Genetics and Molecular Biology</td>
<td>13</td>
</tr>
<tr>
<td>Session III.B. Biochemistry and Physiology</td>
<td>16</td>
</tr>
<tr>
<td><strong>SESSION IV.</strong> What can we do with them? Biotechnological applications. Bioengineering aspects. New products</td>
<td>22</td>
</tr>
<tr>
<td><strong>SESSION V.</strong> How do we know what they are up to? Measurement techniques. Biosensors. Quality control. Other aspects</td>
<td>32</td>
</tr>
<tr>
<td>Closing Session</td>
<td>36</td>
</tr>
<tr>
<td>Index of Authors</td>
<td>37</td>
</tr>
</tbody>
</table>
Opening Session

FOOD SAFETY, A TOOL FOR SUCCESSFUL INNOVATION
A.M. Troncoso-González1*
1Nutrition and Food Science. Faculty of Pharmacy, University de Sevilla, Spain.
*Corresponding author: amtroncoso@us.es Phone: +34 954556759.

The globalisation of the food chain is continually throwing up new challenges and risks to the health and interests of consumers. The central goal of the European Union’s food safety policy is to achieve the highest possible levels of protection for human health and consumers’ interests in relation to food. This is accomplished by ensuring that food is safe and appropriately labelled - taking into account diversity, including traditional products - while at the same time ensuring the effective functioning of the internal market. General Food Law of 2002 sets out the basic principles and recognises the shared responsibility of all actors in ensuring the safety of our food.

Safety is the essential precursor on which the food industry is built – the absolute bottom line. Without safety, issues such as quality and innovation have no grounding. The EU has developed a comprehensive body of food safety legislation, which is being continually monitored and adapted as new developments arise. This legislation is based on risk analysis. The establishment of the European Food Safety Authority (EFSA) was a key step to support the work of the EU institutions in protecting EU consumers in this field, providing independent scientific advice on existing and emerging risks. Safety issues have to be taken into account since the beginning and play an important role for successful innovation. Several examples will be discussed in this presentation.

Keywords: food safety.

SESSON I. Why do we care about Acetic Acid bacteria? History, Traditional vinegar. Food and Health.

S1-KN
PROTECTED GEOGRAPHICAL STATUS: LIMITS AND OPPORTUNITIES FOR VINEGARS

P. Giudici1*
1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.
*Corresponding author: paolo.giudici@unimore.it Phone: +39 0522522034.

Protected Geographical Status (PGS) is a legal structure defined by European Union to protect regional food and specialties. It groups three distinctive systems: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG). The geographical appellation to distinguish food and beverages, especially wine, is very old, but it was applied systematically, starting from 1935 for the French wines with the constitution of the Institut National des Appellations d’Origine. Then, legally enforced with the Appellation d’Origine Contrôlée. Nowadays, geographical appellation or PGS is quite spread and many countries has adopted systems that remind the PGS structure of EU, were the number of regional foods with a PGS is huge. E.g. France claims 1578 PGS plus 640 wines with appellation, Italy has 238 PDO/PGI products, plus 467 wines. The other EU countries follow with relatively less products. This huge number of PGS products clearly shows how much is considered this policy in the EU. The reason is due to the broad idea that geographical appellation policy is a key to improve social and economical life of local agricultural area. In particular the PGS has been accredited of five main features: 1) to protect the reputation of regional food; 2) to promote rural and agricultural activity; 3) to help producers obtaining better price; 4) to eliminate unfair competition; 5) to help consumers to discriminate among authentic and non-genuine products.

Now, after few decades since the implementation of the PGS structure, it is time to make a balance cost/benefit of the system. The first question to answer is: does the PGS reach the initial purposes? In particular, does it help and promote rural area? Does it help consumers to obtain better price for authentic products? If we consider that many PDO products have cyclic commercial difficulties and prices paid to farmers are often lower than the production cost, the answer is no. Furthermore, the price of PDO products paid by consumers on the retail centers is higher than the price paid for analogous products, it means that something wrong it happen in the distribution chain. The vinegars with a PGS are a restricted number: Vinagre de Jerez; Vinagre del Condado de Huelva; Aceto Balsamico Tradizionale di Modena; Aceto Balsamico Tradizionale di Reggio Emilia. Then, PDO attribution is in progress for two other vinegars, Vinagre de Montilla.
Moriles and the Chinese Zhenjiang Xiang Cu (镇江香). The concerns about GPS policy are particularly relevant for PDO/PGI vinegars.

**Keywords: protected geographical status, vinegars, PGI, POD.**

**S1-O-01** THIRD JUBILEE OF THE DISCOVERY OF THE MECHANISM OF THE ACETOUS FERMENTATION BY LOUIS PASTEUR IN 1861

J.F. Bourgeois,1* I. García-García2

1Consultant for H. Frings, D Bonn, 19 route de Chevrier, CH 1244 Choudex, Switzerland; 2Department of Chemical Engineering, Faculty of Sciences, University of Córdoba, Campus Universitario de Rabanales, Ctra(a) de Madrid km 396. 14071 Córdoba, Spain.

*Corresponding author: jacquesfbourgeois@gmail.com Phone: +41 227501267.

In July 26th 1861 Pasteur addressed a first memorandum to the “Société Chimique de Paris” regarding his studies about the acetous fermentation. That same year 1861, Pasteur obtained a patent called “For the fabrication of acetic acid”, dated July 9th, sent by the Institut Pasteur in Paris. Pasteur wrote also: “I am determined from today to restitute this patent to the public domain”. A mark of his generosity. It is interesting that Pasteur used the new nomenclature Mycoderma aceti in the patent. The genus Mycoderma was described for the first time by Persoon in 1822, the year Pasteur was born. In “Etudes sur le vin” (1873), Pasteur wrote: “Wine and vinegar are very similar with respect to the causes of their susceptibility to diseases”. The style of Pasteur is very clear and explicatory. He is full of enthusiasm and modesty when he writes about his own artistic drawings: “The reality is much superior to a drawing... and, I may say, superior to the beauty of these charming little strings”.

Pasteur is often remembered for his extraordinary discoveries in Microbiology. For instant, Prof. Pasteur Vallery-Radot, grandson of Louis Pasteur, wrote in the Introduction of Volume III of his book “Œuvres de Pasteur, Etudes sur le vinaigre et sur le vin”, in 1924: “Pasteur Jubilee, 100 years of vinegar bacteriology”, the great microbiologist J.L. Shimwell wrote: “It seems that it was not until the next year (1862) that he definitely named it Mycoderma aceti. That would constitute a round figure of exactly 150 years, to celebrate the Jubilee of the naming of the species aceti. The occasion of the celebration of the Pasteur Jubilee of his discovery of the mechanism of acetous fermentation occurred in 1961, in Dole (France), the birth place of Pasteur. It coincided with the annual celebration of his birth on December 27th 1822. A local newspaper “Les Dépêches” published on December 27th 1961 an article to commemorate the event making reference to Mr. Bourgeois as “a vinegar brewer, instigator of the Pasteur Jubilee”. So, the same “instigator” suggests this Conference to be an excellent occasion for the “Third Jubilee of the discovery of the acetous fermentation”.

**Keywords: mycoderma aceti, acetous fermentation, acetic acid bacteria, jubilee.**

**S1-O-02** CHINESE CEREAL VINEGARS IN SOLID-STATE FERMENTATION

F.S. Chen,1 L. Li,1 J. Qn,1 C.X. Chen1*

1College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070 P.R. China.

*Corresponding author: chenfs@mail.hzau.edu.cn Phone: +86 2787282927.

In China there is a proverb saying that in a daily life seven indispensable substances are firewood, rice, edible oil, salt, sauce, vinegar and tea. From the proverb, we can see the vinegar has a very important position in Chinese life. In the historical literature, we can find that vinegar originated more than three thousands years ago in China. It is reported that the first mention about vinegar was in 1058 B.C. in the book named Zhou Li about rites of the Zhou Dynasty, and the professional workshop for vinegar appeared in Chunqiu Dynasty (770 B.C. ~ 476 B.C.). At that time, vinegar was so costly that only the noble could afford it. Vinegar became popular in folk in Donghan Dynasty (25A.D.-220 A.D.). Up to Northern and Southern Dynasties (420A.D.-581 A.D.), the book named Qi Ming Yao Shu about the essential techniques of farming, written by Sixie Jia, recorded 23 kinds of methods about how to brew vinegars in detail. Besides seasoning, vinegar is regarded as a herbal medicine in China. In the book of Compendium of Materia Medica written by Shizhen Li in 1857, medicinal functions of vinegar were described as dissipating the blood stasis, treating the diseases of jaundice and yellow sweat, improving appetite and nourishing the liver. In Dictionary of Chinese Medicine, it was also recorded that the vinegar had the medicine’s curative effect for acute and chronic hepatitis. Nowadays, in Chinese markets, there are more than 20 types of homemade cereal vinegars, most of which are brewed with starchy materials such as rice, sorghum, corn, barley and wheat. Among them, the most famous Chinese vinegars are Shanxi aged vinegar, Zhenjiang aromatic vinegar, Sichuan bran vinegar and Fujian Monascus vinegar, respec-
tively using sorghum, sticky rice, wheat bran and red yeast rice as the main raw materials or the starter. These vinegars are also honored as Four China-style Famous Vinegars due to their unique favors, long production histories, massive yields and characteristic fermentation processes.

Besides the raw materials, according to the colors, the special favors and the production processes, Chinese vinegars can be classified into different groups, such as black (brown) vinegar, red vinegar, white vinegar, smoky vinegar, herbal vinegar, solid-state fermentation (SSF) vinegar, liquid-state fermentation (LSF) vinegar and so on.

In this paper, we will take Shanxi aged vinegar as an example to make a description about the history, the general SSF process, the main microorganisms and functional materials of Chinese cereal vinegars.

Keywords: Chinese cereal vinegars, solid-state fermentation, microorganisms functional materials.

S1-O-03 SIMPLE PROCEDURE TO DISCRIMINATE AMONG BALSAMIC VINEGARS

F. Lemmetti,1* P. Giudici1

1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.

*Corresponding author: federico.lemmetti@unimore.it Phone: +39 0522522034.

A lot of sweet and sour vinegars and dressing, derived from grape must and appealed as “balsamic”, are currently available in the market all over the world. We have grouped the different products on the basis of their Protected Geographical Status, ingredients and additives used, as well as the production technology.

The consumer perception of the attribute “balsamic” is associated with a series of common features that clearly discriminate them from other types of vinegars. They are dark, opalescent, shiny, thick and viscous, and the acidic taste is modulated by a variable amount of sugars. The features described are usually achieved in different ways and thus this make the difference among the balsamic vinegars and sauces.

The key point refers to the fact that different methods, used for the same purpose, can have dramatically different costs, which then affect the price and quality of the finished product; i.e. the dark colour can be obtained with addition of caramel (E150d) or by long aging. The differences on production cost is huge and obvious.

The most expensive step in industrial production is aging, and of course this is particularly important for traditional balsamic vinegars. Aging plays a key role in determining many valuable characteristics of balsamic, for example, the dark brown colour belongs from the initial process of must cooking, which triggers a series of non-enzymatic browning reactions that slowly continue over the years. The process of aging in wood barrels increases the specific gravity and viscosity due to the progressive loss of water and the formation of high molecular weight compounds (melanoidins) that modify the rheological properties of the product.

We set up a simple procedure able to discriminate among different balsamic products, with evident convenience for producers and for consumers protection. The procedure is based on few descriptors like the difference from Brix value and reducing sugars, pH, total and fixed acidity. The samples of Traditional Balsamic Vinegar are clearly separated from all the other balsamic vinegars and dressings. This could be explained with the higher concentration of melanoidins and other sugar degradation compounds that are formed during the long aging.

Keywords: traditional balsamic vinegar, aging, melanoidins.

S1-P-01 AGING OF WINE VINEGARS WITH DIFFERENT WOODS: INFLUENCE IN THE POLIFENOLS AND COLOUR

V. González,1* I. López,1 J.M. León,1 J. Lasheras,1 F. Heredia,2 P. Ramírez1

1IFAPA Centro de Cabrera. Carretera Cabrera-Doña Mencia, km 2.5. 14940 Cabrera (Córdoba), Spain. 2Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.

*Corresponding author: virginia.gonzalez.c@juntadeandalucia.es Phone: +34 957596644.

Colour is an important factor related to the sensorial properties of the products, and it is one of the quality parameters of wine vinegar that is monitored, because of its relevance in sensory analysis and as indicator of processes, such as oxidation and aging.

The aging during 7 months of two kinds of vinegars, with 2 % (v/v) of ethanol (vinegar 1) and with 0.5% (v/v) of ethanol (vinegar 2) have been studied. The barrels were made from two types of oak wood, American oak and French oak, and two kinds of barrels new used. This study aims to determine how wood barrels affect vinegar quality. Due to the importance that slight changes in phenolic composition have on organoleptic properties analysis of samples included colorimetric parameters and total phenol.

All of the colorimetric parameters evolve constantly in a positive way during the whole period of aging indicating a positive effect. Specifically, the lightness, L*, of the initial must decreased slightly in the vinegar 1 by 2 % and 5%, in the vinegar 2 has remained practically constant. The chroma, C*ab, in the vinegars aged in French oak increased by 28% doubling the values of the American oak vinegar. h*ab of the initial must decreased by 1 and 3% in all the vinegars except in the old French vinegar, that decreased of the initial by 7% at 8% in the two tipes of vinegars studied. The total phenol increased by 65% in the vinegar 2 aged on new barrel French oak, it doubling the content respect the American oak vinegar, in the vinegar 1 occurs something similar but with slightly lower values.

Sensory analysis of wine vinegars were described on the basis of the attributes previously selected. The general
impression of vinegars aged in new French barrel vinegars were those who scored higher. These results prove that wood used in vinegar aging influences in a positive way in the polyphenol content and color of the final vinegar.

**Keywords:** quality, american oak, french oak, new barrels, used barrels.

**S1-P-02**
**ANALYSIS OF VARIATION OF MAIN COMPONENTS AND FUNCTIONAL FACTORS DURING AGING PROCESS OF SHANXI AGED VINEGAR**
T. Chen,¹ J.J. Shi,¹ F.S. Chen†
¹College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070 P.R. China.
†Corresponding author: chenfs@mail.hzau.edu.cn Phone: +86 2787282927.

Shanxi aged vinegar is one of the most famous traditional vinegars in China. It is produced from several kinds of cereal including sorghum as main raw material, a large dosage of starter Daqu (about 60% of the raw material and made from barley and pea), wheat bran and millet chaff as supplementary and filling material with traditional solid state fermentation techniques. Shanxi aged vinegar was found to be beneficial to human health, and large numbers of researches focused on nutrition, flavor and functional components of the vinegar. However, there are no reports on the changes of main components and functional factors during aging process in Shanxi aged vinegar.

In this study, the volatile flavor compounds, amino acids, organic acids, trace elements, other conventional ingredients and functional factors in Shanxi aged vinegar were detected by instrument technology. The results showed that most conventional ingredients and functional factors (polyphenols, flavones, GABA, tetramethyl pyrazine and ferulic acid) were increased during aging process. There were abundant nutritional and functional components in 8-year-old vinegar. Total polyphenols and total flavonoids were 321.20 μg/mL and 54.52 mg/100 mL respectively in the 8-year-old vinegar.

**Key words:** shanxi aged vinegar, components analysis, common components, volatile components, functional components.

**S1-P-03**
**EVOLUTION OF WINE VINEGAR COMPOSITION DURING AGING WITH OAK BARRELS**
V. González,¹ P. Ramírez,¹ J.M. León,¹ J. Lasheras,¹ I. López¹
¹IFAPA Centro de Cabra. Carretera Cabra-Doña Mencía, km 2.5. 14940 Cabra (Córdoba), Spain.

A crucial step in the production of high-quality wine vinegars is aging in wood. This study aims to determine how wood barrels affect vinegar quality. For this purpose, two kinds of barrels new and used were evaluated, vinegars were aged for 7 months in barrels made from two types of oak wood, american oak and french oak. To test the effect of alcohol content on the extraction from wood, two kinds of vinegars were studied, one of them with 2 % (v/v) of ethanol (vinegar 1) and the other one with 0.5% (v/v) of ethanol (vinegar 2).

Analysis of samples included dry extract, ashes, alcohlic degree, total acidity fixed acidity, volatile acidity, pH and color intensity.

Barrels aging increased the concentration of dry extract specially in the vinegar 1 in french oak (new and used) and in the vinegar 2 of new american oak, total acidity increased in all the vinegars mostly on the vinegar 2 of french oak, fixed acidity increased in all the vinegars specially in the vinegar 2 of french oak and of american old oak, ashes increased a greater proportion in vinegar 2 of new american barrels and color intensity increased considerably in vinegar 1 french oak barrels.

Sensorial analysis of wine vinegars were described on the basis of the attributes previously selected and from these results their spider charts were drawn. The intensity of ethyl acetate odor is more evident in vinegar 1 in new french barrels and old american barrels. Woody odor in french barrels (new and used) and new american barrel in vinegar 1 were the greatest. Vainilla odor in french barrels new and used in vinegar 1 were the greatest.

The remaining alcoholic degree of vinegars plays an important role in the ethyl acetate and woody aroma components. Overall impression, aroma intensity and quality clearly increase with aging.

These results prove that wood for aging in vinegar determines both the chemical composition and sensorial properties.

**Keywords:** high-quality, american oak, french oak, new barrels, used barrels.

**S1-P-04**
**ORAL ADMINISTRATION OF LIPOPOLYSACCHARIDE OF ACETIC ACID BACTERIA PROTECTS POLLEN ALLERGY IN MICE MODEL**
H. Inagawa,¹,²,³ S. Amano,² T. Nagashima,²,⁴ C. Kohchi,¹,²,³ G.I. Soma¹,²,³,⁵
¹Faculty of Medicine, Kagawa University, Kagawa, 761-0793; ²Control of Innate Immunity, Technology Research Association, Kagawa, 761-0301; ³Macrophi Inc., Kagawa, 761-0301; ⁴Toyohakko Co., Ltd. Aichi, 474-0046; ⁵Institute for Health Sciences, Tokushima Bunri University, Tokushima, 770-8514, Japan.
*Corresponding author: pxs07205@nifty.ne.jp Phone: +81 878677712.

Abstracts
Acetic acid bacteria are one of Gram-negative bacteria produce many fermented foods such as vinegar and Kefir (Caspian yogurt) which contribute to health, but the substances contributing to health in these foods are not clarified yet. Lipopolysaccharide (LPS), a major component of cell wall of Gram-negative bacteria, is known to have strong immune-regulatory activity. We have found and reported the existence of biologically active LPS in acetic acid bacteria. The LPS shows limulus positive activity and activation of macrophage to produce nitric oxide and tumor necrosis factor. In this study, to estimate anti-allergic effect by oral administration of acetic acid bacteria extract containing with LPS were investigated, the cedar polinosis model was used. Establishment of the protection of pollen allergy with food derived materials are quite important matter in Japan.

In this study, we isolated acetic acid bacteria from various fruits by GYC medium. The bacteria were cultured with modified Nodai medium and the collected cells were suspended with distilled water and heated (120°C, 20 min) to prepare LPS containing extract. The extract contained about 0.001-0.01 mg/mL of Limulus positive substances. The extract of acetic acid bacteria induced higher level of IL-10 and FOXP3 mRNA expression in macrophage (RAW246.7 cell) by DNA microarray analysis. Then the anti-allergic effect of acetic acid bacteria extracts was investigated. BALB/c mice were immunized with mixture of cedar pollen and alum into peritoneal cavity, and additional immunizations of pollen to nasal cavity. After immunization, the mouse were administered the pollen into nasal cavity for trigger allergy reaction and the number of scratch their nose were counted during 5 minutes. By the oral administration of acetic acid bacteria extract demonstrated significantly less the scratch number than control water group with pollen immunization. These results showed that LPS in acetic acid bacteria have a possibility to protect of allergic reaction, especially cedar polinosis. Further analysis should be needed to clarify the mechanism for anti-pollen effect of LPS of acetic acid bacteria.

**Keywords:** lipopolysaccharide, oral administration, anti-allergy, macrophage.

**S1-P-05**

**THE AGE OF BALSAMIC VINEGAR: RESIDENCE TIME AND CHEMICAL DESCRIPTORS**

F. Lemmetti,1* P. Giudici1

1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.

*Corresponding author: federico.lemmetti@unimore.it
Phone: +39 0522522034.

Traditional balsamic vinegar (TBV) production involves some distinct steps: the cooking of the must is followed by its alcoholic and acetic fermentation; then, during the aging in a barrel set, the volume loss due to evaporation is restored according to the technique of “rincalzo” (topping up) that remind the Solera system. This procedure leads to a set of barrel containing a mixture of vinegars of different ages, and the TBV age is defined as “residence time” (RT), that is the weighted average among each aliquots of vinegar mixed through the years. From an algebraic point of view, the problem has been solved and the result is a mathematical model in which the TBV age is easily estimated from the volumes involved in the maintenance of the barrel set (Giudici and Rinaldi, 2007). While it is intuitive and commonly accepted that losses affect the yield of the barrel set, it is more interesting to clarify the relationship between yield, evaporation rate and age of TBV. The lower is the yield, due to losses by evaporation and other causes, the lower is the RT and thus the effective aging. The intensity of aging of TBV is indeed inversely proportional to the flow of vinegar through the barrel set. This simulation system has been modified by excluding the water and the calculation is based on the actual dry matter that undergoes aging, i.e. the solutes dissolved in the vinegar. To this end, the solute losses, due to chemical and physical phenomena and biological activities, have been quantified for every stages of TBV production. Based on the mass balance performed, a rigorous calculations can be set, in order to simulate the trend of the RT and concentration profile for the product. Yields calculated in this manner are consistent with data collected from different barrel sets during a few years and it is clear that the yield significantly influences the RT of TBV: older TBVs correspond to high yields. The calculation of the RT on the basis of dry mass, besides being more correct because water is excluded from the calculation, provides higher values than those calculated by the volume, and these differences are greater for the most aged barrels of vinegar. In short, increasing the yield during the aging process correspond to the increase of TBV quality. From a chemical point of view, melanoids are potential indicator of TBV age and quality, since these compounds vary in terms of quality and quantity during the residence time of TBV in the barrels.

**Keywords:** traditional balsamic vinegar, aging, residence time, yield.

**References:**

SESSION II. What are they? Taxonomy and Phylogenesis. Ecological aspects (natural occurrence of AAB; Biodiversity).

S2-KN-01

TAXONOMY OF ACETIC ACID BACTERIA AND MOLECULAR TECHNIQUES SUITABLE FOR THEIR IDENTIFICATION AT THE SPECIES LEVEL

I. Cleenwerck,1* P. De Vos1,2
1BCCM/LMG Bacteria Collection, Laboratory of Microbiology, Faculty of Sciences, Ghent University, Belgium; 2LM-UGent, Laboratory of Microbiology, Faculty of Sciences, Ghent University, Belgium.

*Corresponding author: ilse.cleenwerck@ugent.be Phone: +32 92645105.

The taxonomy of acetic acid bacteria (AAB) has undergone many changes during the last 30 years in accordance with the development and application of new methodologies. The genera Acetobacter and Gluconobacter were introduced in 1898 and 1935, respectively, and the early classifications systems of AAB were based mainly on morphological and biochemical characteristics. Gradually, molecular and chemotaxonomic techniques were introduced, and the family Acetobacteraceae was proposed by Gillis & De Ley in 1980 on the basis of DNA-rRNA hybridization data supported by phenotypic data. In the same period, Gosselé and co-workers examined a wide variety of Acetobacter and Gluconobacter strains by numerical analyses of 177 phenotypic features, since it was expected that objective comparison of large sets of characteristics for a large number of strains would yield superior classifications. Based on their work, several taxonomic changes were proposed. Mid eighties, the first DNA-DNA relatedness studies with AAB strains were published by Yamada et al. (1984) and Micales et al. (1985). Both authors studied independently the genus Gluconobacter by DNA-DNA hybridizations and revealed the existence of at least three genospecies. At the end of the eighties, Gillis and co-workers were the first to describe a novel species of the family Acetobacteraceae based on a polyphasic approach, A. diazotrophicus. Shortly thereafter, the first phylogenetic studies of AAB based on 16S rRNA gene sequences were published. In 1997, Yamada and co-workers introduced a major change in the classification of AAB by proposing the genus Gluconacetobacter for the Q-10 containing Acetobacter species (A. xilinus, A. liquefaciens, A. hansenii, A. diazotrophicus and A. europaicus) on the basis of partial 16S rRNA sequences and chemotaxonomic comparisons of ubiquinone systems. Finally, since 1998, polyphasic taxonomic studies of AAB from novel niches and from culture collections, led to reclassifications in the family Acetobacteraceae, as well as to the introduction of new taxa at the generic and species levels.

Although the classification scheme for AAB became more robust, accurate identification of AAB at the species level remained time-consuming. During the last decade several molecular techniques, such as sequence analysis of 16S-23S rDNA spacer regions, MLSA of the housekeeping genes dnaK, groEL and rpoB, and DNA fingerprinting techniques such as rep-PCR and AFLP™ have been proven useful for rapid and more reliable identification of AAB.

Keywords: taxonomy, fast and accurate identification of AAB.

S2-KN-02

ECOLOGY OF ACETIC ACID BACTERIA AND THEIR ROLE AS PLANT GROWTH-PROMOTERS

R.O. Pedraza1*
1National University of Tucumán. Faculty of Agronomy and Zootechnics. Av. Kirchner 1900. (4000) Tucumán, Argentina.

*Corresponding author: rpedraza@herrera.unt.edu.ar Phone: +54 3814390003.

Aerobic bacteria able to oxidize ethanol to acetic acid in neutral or acid media are candidates of belonging to the family Acetobacteraceae that includes several genera. They are widespread in nature and most species have been isolated from vinegar, wine, fruits and flowers. However, some species were found also associated to different plants, colonizing inner tissues and roots. Several of these species are capable of promoting plant growth through different mechanisms such as the biological nitrogen fixing process, phytohormones production, phosphate solubilization and siderophores production, among others. Some of the species showing these features belong to the genera Gluconacetobacter, Swaminathania and Acetobacter. The first N2-fixing acetic acid bacterium (AAB) (Gluconacetobacter diazotrophicus) was described in Brazil, colonizing inner tissues of sugarcane plants. Then, two new species associated to coffee plants were described in Mexico: G. johannae and G. azotocaptans. In 2004, a salt-tolerant bacterium named Swaminathania salitolerans was found associated to wild rice plants. And later, N2-fixing Acetobacter peroxydans and Acetobacter nitrogenifigens, associated with rice plants and Kombucha tea, respectively, were described in India. Gluconacetobacter diazotrophicus, the most studied AAB as plant growth-promoter, is an endophytic bacterium, able to colonize several plant species (e.g., sugarcane, pineapple, coffee, sweet potato, Pennisetum pupureum, etc.). Besides being able to fix nitrogen it has shown to possess some other characteristics potentially valuable in the area of agriculture such as the production of substances responsible for plant growth-promotion (phytohormones), action against pathogens and mineral nutrient solubilization.

In the last two decades it was observed that plant growth-promoting bacteria are valuable for agriculture as a tool for improving crop performance and environmental conditions, as they may reduce and avoid the use of chemical fertilizers and pesticides. Hence, at present N2-fixing acetic acid bacteria are also considered to be used as biofertilizers in different crops to exploit their benefits derived
from an efficient association, mainly related to the biotechnological potential for a sustainable agriculture.

Keywords: Gluconacetobacter diazotrophicus, nitrogen-fixing AAB, plant growth-promoting bacteria.

S2-0-01 MALDI-TOF MASS SPECTROMETRY FINGERPRINTING TOOL FOR RAPID IDENTIFICATION AND CLASSIFICATION OF ACETIC ACID BACTERIA

C. Andrés-Barrao,1 M.L. Chappuis,1 C. Benaglia,2 M. Tonolla,2,3 F. Barja1*

1Microbiology Unit, Botany and Plant Biology Dept., University of Geneva, Ch. Des Embrouiches 10, CH-1254 Jussys, Geneva, Switzerland; 2Cantonal Institute of Microbiology, Via Mirasole 22A, CH-6500 Bellinzona, Switzerland; 3Microbiology Unit, Botany and Plant Biology Dept., University of Geneva, Quai Ernest-Anserment 30, CH-1211 Geneva, Switzerland.

*Corresponding author: francois.barja@unige.ch
Phone: +41 223793750.

Acetic acid bacteria (AAB) are involved in multiple natural processes leading to high valued food and beverages products, such as vinegar. The taxonomy of these microorganisms has always been very tedious, overall when using classical phenotypic traits. Molecular techniques have been shown to overcome in some extent this constraint, but the unequivocal identification of several AAB species remains unresolved. The most extensively used technique for the identification of microorganisms is the analysis of the 16S rRNA gene sequence, but the analysis of this solely gene fails to discriminate between bacterial species that are very closely related. Additionally, the necessity of culturing to isolate bacteria, and all subsequent manipulations until the obtaining of the 16S rRNA gene sequence, makes this approach still time-consuming.

The analysis of whole cells by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been revealed as a methodology of great interest for the identification and typing of bacteria and other microorganisms due to the really short time that is required for the complete analysis. The aim of this study was to evaluate the suitability of MALDI-TOF MS for the identification of cultured AAB that are related with the industrial production of vinegar. Reference and isolated strains belonging to the genera Acetobacter, Gluconobacter and Gluconacetobacter were used. The analysis of the reference strains was consistent with their phylogenetic classification. Most isolates were clustered in accordance with their molecular identification. Some isolates that were not clustered within a group in the AAB MALDI-TOF MS dendogram were revealed as non AAB or mixed cultures. Our results showed that MALDI-TOF MS analysis is a fast and reliable method for the clustering and identification of AAB species.

Keywords: acetic acid bacteria, phylogeny, MALDI-TOF MS, whole cell identification.

S2-0-02 CULTURE-DEPENDENT AND CULTURE-INDEPENDENT COMMUNITY DYNAMICS ANALYSIS OF ACETIC ACID BACTERIA DURING COCOA BEAN FERMENTATION

Z. Papalexandratou,1 K. Illeghems,1 L. De Vuyst1*

1Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.

*Corresponding author: ldvuyst@vub.ac.be
Phone: +32 26293245.

Acetic acid bacteria (AAB) play an important role during cocoa bean fermentation, as they oxidize ethanol produced by yeasts and lactic acid produced by lactobacilli into acetic acid and further oxidize acetic acid and lactic acid into carbon dioxide and water, thereby generating heat that, together with volatile acetic acid, kills the seed embryo. Alternatively, AAB are strictly aerobic microorganisms that oxidize their substrates (alcohols, sugar alcohols, organic acids) under certain temperature and pH conditions. Therefore, isolation of AAB from and monitoring AAB community dynamics during fermentation processes is not straightforward. The present study aimed at developing culture-dependent and culture-independent techniques to follow up the identity and size of AAB communities during cocoa bean fermentation. Selective isolation and enumeration of AAB colonies was performed on different agar media (DMS, BME, AAM, GY) at 37°C aerobically. Colony identification was performed through (GTG)5-PCR fingerprinting making use of an extended reference database. Culture-independent AAB community dynamics were monitored by denaturing gradient gel electrophoresis (DGGE) of PCR amplicons targeting certain regions of 16S rRNA genes, making use of group-specific (WBAC) primers targeting both LAB and AAB) and bacterial universal primers. The results indicated that selective isolation of AAB species depends on the agar media used. For instance, DMS/GY and AAM agar selected for A. pasteurianus and Acetobacter spp., respectively. BME agar was not selectively enough for AAB. In addition, enterobacterial and lactic acid bacterial species could easily grow on BME and AAM agar, respectively, while DMS/AAM agars allowed the isolation of pimaricin-resistant yeasts. Whereas Gluconacetobacter could be detected culture-independently and isolated from AAM agar only occasionally, several new Acetobacter species were found among the isolates from fermenting cocoa pulp-bean mass. Further, the culture-dependent community dynamic results indicated that AAB survived during the initial anaerobic phase of a cocoa bean fermentation process, followed by slow growth up to high densities in the mid-phase of the fermentation. PCR-DGGE analysis with universal primers allowed the detection of AAB at genus level, while WBAC primers could reveal some of the Acetobacter species as well. However, the development of a new primer set targeting AAB solely was necessary.
COMMUNITY OF ACETIC ACID BACTERIA AND YEASTS IN CONSUMER-FRIENDLY KOMBUCHA BEVERAGES

E. Mateo,1 M.J. Torija,1 A. Mas,1 E. Bartowsky2
1Departamento de Bioquímica y Biotecnología.
Facultad de Enología. Universidad Rovira i Virgili. C/ Marcel·li Domingo s/n. E.-43007 Tarragona, Spain; 2Australian Wine Research Institute, Urrbrae, SA, Australia.

*Corresponding author: estibaliz.mateo@urv.cat
Phone: +34 977558688.

Grapes have a complex microbiota ecology which plays central roles in the quality of this fruit and wine. It is well known that if oxygen conditions are appropriate, Acetic acid bacteria (AAB) are the main family of bacteria that can proliferate and cause wine spoilage. The aim of this study was to analyse the diversity of AAB population isolated from grape bunches of vineyards in the Adelaide Hills region of Australia. Grapes were classified as healthy, mouldy-infected and dry. Up to 62% AAB colonies were directly recovered from grapes in glucose medium supplemented with piramycin (200 mg/L) to suppress fungal and yeast growth. Some of the species detected have been described in previous studies of grapes and wine as Gluconobacter oxydans, Acetobacter malorum and Asaia stiannensis. However, other species never before described either in wine or on grapes as Gluconobacter cerinus, Gluconobacter frateurii, Asaia lannensis or Asaia bogorensis have also been recovered and identified in this study. The first two are very closely related to Gluconobacter oxydans, widely described on grapes and wine. As some recently described species have also been reported in ecological studies of wine and grapes, it is necessary to conduct an in-depth revision of AAB diversity using appropriate molecular tools in order to determine the real AAB microbiota present on grapes.

Keywords: wine, spoilage, vinegar, Asaia, Gluconobacter.

NO-CULTURE STRATEGY FOR TRACKING AAB AND YEASTS IN LOW-ACIDIC AND HIGH POLYPHENOLIC Niches

D. Mamlouk,1 L. Solieri,1 M. Gullo1
1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.

*Corresponding author: dhouhamamlouk@yahoo.fr
Phone: +39 3467373449.

Gluconacetobacter xylinus forms a pellicle at the liquid-air interface of statically grown broth cultures. A major structural component of this interfacial biofilm is cellulose which serves to protect the organism from unfavorable environmental fluctuations. In addition, cellulose formation enhances surface colonization to provide a competitive advantage for resource acquisition.

Keywords: AAB, yeasts, no-culture method, kombucha, dynamics, PCR/DGGE.
Exogenously supplied phytohormones are known to affect plant growth and development. Some microorganisms can also synthesize phytohormones and many have been investigated for their potential as plant growth promoters; however, there have been few studies investigating the effect of phytohormones on bacteria. The aim of this study was to investigate the effect of the phytohormones indole-3-acetic acid (IAA), gibberellic acid (GA), abscisic acid (AA) and zeatin on growth and pellicle formation in G. xylinus.

The growth, cellulose yield and pellicle properties of G. xylinus were significantly affected by exogenous phytohormones. Each of the phytohormones tested stimulated the growth rate of G. xylinus between 1.1 to 1.7 times that observed for untreated controls. The addition of phytohormone caused a significant reduction in pellicle thickness for most concentrations tested. Exogenous AA caused G. xylinus to produce pellicles with greater wet weight than the other three hormones tested. While pellicles produced by control cultures were thicker, the yield of bacterial cellulose was greater in the presence of AA and zeatin suggesting that these pellicles were denser. Bacterial cellulose yield decreased in the presence of IAA. GA increased the cellulose yield at higher concentrations with the greatest effect seen at 10 μM. The crystallinity index (CI(IR)) of cellulose produced in each treatment was determined by Fourier transform infrared spectroscopy. The observed effect on cellulose crystallinity was concentration and hormone dependent. GA affected the cellulose crystallinity the most with the highest CI(IR) of 1.01 observed at 5 μM. Zeatin treated cultures produced the least crystalline cellulose of any treatment with CI(IR) of 0.56 at 0.5 μM.

In conclusion, these results underscore the importance of studying the broader ecological effect of agricultural practices in which exogenous growth promoters are applied. In addition, phytohormones can be used as a tool to manipulate the properties of bacterially produced cellulose for industrial application.

Keywords: cellulose, phytohormones, crystallinity, Gluconacetobacter xylinus.

S2-P-01
ACETIC ACID BACTERIA CULTURE COLLECTION: SIGNIFICANCE AND MANAGEMENT
L. De Vero1*  
1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.
*Corresponding author: luciana.devero@unimore.it  
Phone: +39 0522522057.

Acetic acid bacteria (AAB) occur in very specialized habitats such as plants, fruits and flowers, as well as several kinds of sugary or alcoholic substrates. In the last decades, some AAB species have been extensively investigated not only for foods and beverages but also others industrial biotechnology applications as the development of processes for high-value products or biosensors. The potential exploitation of AAB strain diversity requires the existence of culture collections which represent important biological resources for selection and genetic improvement of microbial cultures useful for biotechnological applications. The greatest hurdles to acetic acid bacteria study are related to their difficult isolation, thus several media and incubation conditions are necessary for successful cultivation and preservation of pure cultures. Furthermore culture collections have to evaluate microbial changes over time as well as guarantee appropriate tools in preserving “authentic” strains.

These are also the tasks of the Food and Industrial Microbial Collection (FIMC) of the Department of Agricultural and Food Sciences (DipSAA) that, currently, holds about 300 AAB strains, above yeast and lactic acid bacteria strains, isolated from several matrices. Specifically, the AAB strains maintained in the collection have been isolated from musts, wines, various vinegars, solid-state fermentation matrix and kombucha tea. Different preservation techniques are used, ranging from continuous growth methods, for short-term maintenance, to cryopreservation methods at -80°C, for long-term maintenance and for avoiding genetic drift or instability. Full understanding of metabolic potential of the AAB strains collected is achieved by the integration of sequence data with transcriptional and functional studies. A “Strain Database” (www.renewx.org) and a Bioinformatics Software (GelCompare II - Applied Math) support all the information related to the strains starting from isolating source until the full characterization, as well as the collection management.

Keywords: acetic acid bacteria, culture collections, preservation methods.

S2-P-02
CHARACTERISATION OF ACETIC ACID BACTERIA PRODUCING HIGH-ACID VINEGAR
C. Andrés-Barrao,1 D. Bravo,2 M.L. Chappuis,1 R. Ortega Pérez,1 M. Ojha,3 P. Junier,2 F. Barja1*  
1Microbiology Unit, Department of Botany and Plant Biology, University of Geneva, Chemin des Embrouichis 10, CH-1254 Jussy-Geneva, Switzerland; 2Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Emile-Argand 11, PO Box 158, CH-2009 Neuchâtel, Switzerland; 3Département de Biochimie, University of Geneva, 30, Quai Ernest-Ansermet, CH-1211 Genève 4, Switzerland.
*Corresponding author: francois.barja@unige.ch  
Phone: +41 223793750.

The modern submerged fermentation for the production of vinegar is currently the main method used in industrial vinegar factories. Rapid aceticification rate as well as high concentration of acetic acid (up to 20%) can be obtained. Several strains of the genus Gluconacetobacter are involved in the production of high-acid vinegars produced by this methodology. These strains are characterised by their higher ADH stability and activity, and also by a higher acetic acid resistance, than other acetic acid bacteria.

In this study, two spirit vinegar fermentations were...
performed by using the submerged method. Both processes reached a maximum acidity of 14%. The molecular characterisation was performed using culture-dependent: restriction fragment length polymorphism (RFLP) and multilocus sequence alignment (MLSA) and culture-independent: denaturant gradient gel electrophoresis (DGGE) methodologies. The characterisation showed a major abundance of one strain in both processes, confirmed the monoculture nature of industrial processes. The phylogenetic analysis identified the species present in each fermentation as *Ga. oboediens* and a species related to *Ga. hansenii/entanii* respectively.

In the other hand, scanning and transmission electron microscope observations showed that high-acid vinegar bacteria lacked the characteristic polysaccharide layer surrounding the outer membrane, which has been previously observed in low-acid Acetobacter strains.

**Keywords**: submerged high-acid vinegar, *Gluconacetobacter*, direct identification, RFLP, MLSA, DGGE, polysaccharides.

---

**S2-P-03**

**DIVERSITY AND DYNAMICS OF CULTIVABLE POPULATION OF ACETIC ACID BACTERIA AND YEASTS IN KOMBUCHA**

L. Solieri, 1 D. Mamlouk, 1 L. Kallel, 2 M. Gullo 1*

1Dept. of Agricultural and Food Science, University of Modena and Reggio Emilia, Italy; 2National Institute of Applied Sciences and Technology, LETMI, Tunisia.

*Corresponding author: maria.gullo@unimore.it Phone: +39 0522520057/63.

In recent years functional foods promoted with health claims have attracted increasing attention on the market. Among them *kombucha* is a fermented beverage widely consumed in Eastern Asian countries, but little is known about its constituent microbial communities. In this study two 12 days benchmark *kombucha* fermentations from green and black tea were carried out. A culture-dependent approach was applied both on exopolysaccharidic and liquid phases to monitor dynamics and diversity of acetic acid bacteria (AAB) and yeasts community. Among AAB one main profile was observed (86% of strains); remaining strains were grouped in 4 profiles by 16S/RFLP-based analysis. Whereas by (GTG)5/PCR typing sixteen clusters were obtained. 16S rRNA gene sequencing confirmed the occurrence of *Gluconacetobacter xylinus* as predominant both in green and black samples at 0, 6 and 12 days of fermentation. Mainly on ACB medium minor bacterial groups often colonizing tea leaves (*Paenibacillus spp.*), plants (*Plantibacter spp.*) and moisturizing environments (*Williamsia spp.*), were detected starting from 6th fermentation day. Yeast population consisted of a restricted number of dominant species: *Dekkera sp.*, *Schizosaccharomyces sp.*, *Zygossaccharomyces sp.*, *Dekkera sp.* and *Pichia sp.* *D. anomala* was prevailing in both phases though all black and green *kombucha* fermentation times. *Sc. pombe* was detected only within 6 days of both green and black tea and it was not isolated after 9 days, when the high ethanol-producing species *D. bruxellensis* was detected. *Z. bailii* was isolated from exopolysaccharidic of black tea from 6 to 12 days. Finally *P. membranifaciens* was detected occasionally at the end of the fermentative process. For each species the degree of diversity was determined by combined M13 and OPA20-based RAPD method. Cluster analysis showed that one to two prevailing biotypes occurred through all the process.

**Keywords**: AAB, yeasts, *kombucha* population, culture-dependent approach.

---

**S2-P-04**

**ISOLATION, CLASSIFICATION AND IDENTIFICATION OF YEAST FROM SHANXI AGED VINEGAR**

J. J. Wu, 1 X. Y. Zhang, 1 F. S. Chen 1*

1College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070 P.R. China.

*Corresponding author: chenfs@mail.hzau.edu.cn Phone: +86 2787282927.

Shanxi aged vinegar (SAV) is one of famous traditional Chinese vinegars made from several kinds of cereal by spontaneous solid-state fermentation techniques, in which *Daqu* (Chinese pronunciation) is used as the initial starter. Because *Daqu* is prepared in an/a “open” or “half-open” workplace, many kinds of microorganisms from the raw materials and production environments are enriched in it. Yeast, as one of the main microbial populations in *Daqu*, plays important role in alcoholic fermentation and flavor formation of SAV. Here, the yeast were isolated from different fermentation period and identified by phenotypic methods combined with molecular tools including ITS-5.8S rDNA RFLP, inter-delta/PCR and 26S rRNA D1/D2/PCR. 47 yeast strains were isolated during SAV alcoholic fermentation. Among them, 29, 9 and 9 strains were isolated from earlier stage (the 1st-3rd day of aerobic fermentation), middle stage (the 9th day of anaerobic fermentation) and later stage (the 18th day of anaerobic fermentation), respectively. According to their morphological characters, 36 strains were classified as *S. cerevisiae* and 2 strains were classified as *P. anomala*. The rest 9 strains had the same morphological characters with *S. cerevisiae*, but they had pseudohyphae on the culture medium containing corn powder and couldn’t form ascospore on MacConkey medium. According to the physiological and biochemical characteristics, the 47 yeast strains were also classified as the same 3 groups. The results of ITS-5.8S rDNA RFLP (restriction fragment length polymorphisms) analysis with HeaIII and Hif I indicated that 45 strains had the same profile with *S. cerevisiae* CBS11711, while the other 2 strains had the same profile as *P. anomala* CBS5759. And 45 presumptive *S. cerevisiae* strains identified by ITS-5.8S rDNA RFLP could be grouped into 4 clusters according to the results of inter-delta PCR analysis, the first cluster included 20 strains and had 5 amplified fragments (1000bp, 680 bp, 580 bp, 500 bp and 350 bp), the second cluster included 19 strains and had 2 amplified fragments (1000bp and 680 bp), the third cluster included 4 strains and had 3 amplified fragments (1000bp, 680 bp and 580 bp), and the fourth cluster included 2 strains and had 1 amplified fragment (1000bp).
fragments (750 bp and 460 bp), the third cluster included 4 strains and had 2 amplified fragments (750 bp and 550 bp), the rest 2 strains had 4 amplified fragments (1700bp, 1100bp, 760bp and 400 bp) and had the same results with S. cerevisiae CBS1171. The results indicated that the isolated S. cerevisiae had different genotype. Finally, 4 S. cerevisiae strains from 4 different clusters and 1 P. anomala strain were identified by 26S rDNA D1/D2 region sequencing. The results indicated that the amplified fragments were 650bp. The sequence similarity of the S. cerevisiae strains from 4 different clusters were 100% with the sequence of S. cerevisiae in genbank, and the sequence similarity of isolated P. anomala were 100% with the sequence of P. anomala in genbank. All the results showed that most of isolated yeasts were S. cerevisiae, but different S. cerevisiae strains may had different phenotype and genotype. Studies on yeast involved in Shanxi aged vinegar fermentation are helpful for starter selection, regulation process parameter, and provide reference for other strain isolation and identification.

Keywords: vinegar, yeast, isolation, identification.

S2-P-05
ISOLATION OF ACETIC ACID BACTERIA SPECIES IN GRAPE MUSTS: “NEW” AND “OLD” SPECIES
D. Navarro,1 E. Mateo,1 A. Mas,1 M.J. Torija1
1Departamento de Bioquímica y Biotecnología. Facultad de Enología. Universitat Rovira i Virgili. C/ Marcel•li Domingo s/n. E: 43007 Tarragona, Spain.
*Corresponding author: albert.mas@urv.cat Phone: +34 977558688.

Acetic Acid Bacteria (AAB) were routinely isolated and identified in grape musts from the Experimental Cellar of the Oenology Faculty in Tarragona (Spain). Different serial dilutions were plated onto glucose solid media and incubated at 28°C. Each bacterial colony was then replicated in glucose with carbonate calcium medium and those that produced a clear halo were considered putative AAB and were typed by (GTG)5-fingerprinting and identified by 16S rRNA gene RFLP-PCR and gene sequencing. Further identification by 16S-23S rRNA ITS sequencing was performed. Consensus sequences from different genotypes were compared with those available in Genbank database. Out of 43 isolates that produced a clear halo around the colonies, only 37 yielded a clear electrophoretic profile and those could be grouped into 21 genotypes. Most of them were identified as Gluconobacter oxydans by RFLP-PCR of 16S rRNA gene. However, sequencing (16S rRNA gene or 16S-23S rRNA ITS) analysis of these genotypes revealed that only one of them was G. oxydans, whereas the other were identified as the close related species G. japonicus, G. albidus, G. cerinus and G. thailandicus. More surprisingly, two of these genotypes were identified as a member of another genus, Kozakia balansiensis, which had never been reported in grape must. The other genotypes were identified as Acetobacter malorum, A. cerevisiae, A. pasteurianus and Gluconacetobacter europaeus, and all of them, needed a further identification by 16S-23S rRNA ITS sequencing. Previous analysis of the AAB presence in grape must provided a main presence of G. oxydans and A. aceti. However, it is most likely that closely related species that have been described lately are also present in relevant numbers in grapes and wines.

Keywords: sequencing, wine, fingerprinting.

S2-P-06
KOMBUCHA TEA: A BIORESOURCE FOR ACETIC ACID BACTERIA
L. Kallel,1* M. Gullo,2 M. Hamdi1
1Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAIT), Centre Urbain Nord, 2 Boulevard de la Terre, B.P. 676, 1080 Tunis, Tunisia; 2Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via G. Amendola 2, Pad. Besta, 42100 Reggio Emilia, Italy.
*Corresponding author: daddalina@yahoo.fr Phone: +21 624330001.

Kombucha or Tea fungus is a traditional fermented beverage obtained during sweetened tea fermentation conducted by acetic acid bacteria (AAB) and yeasts. Historically, Kombucha appeared first in Asia and thereafter its use and consumption spread from Russia, central Europe and then over the world. It is composed of a cellulose floating pellicle and a sour slightly and sparkling broth. However, in spite of its importance, Kombucha remained not well explored mainly as a niche of AAB strains exploitable in biotechnological applications. The aim of this study was to overview the occurrence of AAB in Kombucha samples from liquid and pellicle phases. Kombucha from black and green tea was obtained by inoculating a previously grown culture into a freshly prepared tea infusion containing 10% of sucrose, 3% (v/v) of cellulose pellicle and 10% (v/v) of broth and led to fermentation up to 15 days in a batch system under aerobic condition. Titrable acidity reached a maximum of 12 g/L at the end of fermentation in black kombucha and 6 g/L in green one. pH dropped from approximately 3.7 until 2.75 for both samples as a result of acids formation. Maximum ethanol accumulated at the day 15 did not exceed 0.5% (v/v) for both samples. Cellulose layer become thicker during fermentation, starting from 2-3 mm until 10 mm. AAB strains were isolated from pellicle and liquid phases by sampling each 3 days using GYC and ACBS media. A total of 93 strains were collecting during 15 days of fermentation of which 50 are cellulose producing. The majority of strains were tolerant to 5% (v/v) of ethanol and to 30% (w/v) of glucose. This work opens an issue to explore the diversity of AAB from Kombucha focusing their ability to produce cellulose and to grow in low-acidic and sugary environment.

Keywords: kombucha, fermentation, acetic acid bacteria, cellulose.
The genus *Gluconacetobacter* contains several acetic acid bacteria that are capable of producing extracellular polysaccharides, such as bacterial cellulose and acetic acid. Bacterial cellulose has potential applications in the biomedical field (e.g. artificial skin and vessels, controlled-drug carriers), but is at present mainly applied in food products (e.g. nata, kombucha), paper products, acoustics (e.g. diaphragm for headphones and loudspeakers), electronics and medical devices (e.g. temporary wound coverage). *Gluconacetobacter* species that are known to contain cellulose-producing strains are *G. xylinus, G. obodenensis, G. intermedius, G. hansenii, G. swingsii* and *G. raeticus*, a set of phylogenetic closely related species. Identification of such acetic acid bacteria through phenotypic approaches is not very reliable, partly because these acetic acid bacteria are exposed to a high frequency of spontaneous mutations, possibly due to the presence of many insertion elements present in the genome of these bacteria. Therefore, molecular-biological methods, based on the identification/characterization of specific DNA regions, are regarded to be more reliable for the identification of these bacteria. In this study, two strains of *Gluconacetobacter* have been investigated using different molecular-biological methods. Comparative analysis of nearly complete 16S rRNA gene sequences, and of partial *adhA*, *dnaK*, *groEL* and *rpoB* gene sequences, restriction analysis of the 16S-23S rDNA spacer region, AFLP™ DNA fingerprinting and DNA-DNA hybridizations revealed that these strains belong to a novel species in this genus.

**Keywords:** *Gluconacetobacter*, novel species, extracellular polysaccharides.
Acetic Acid Bacteria (AAB) inhabit niches where sugars are accumulated, such as fruits and flowers. The sugar concentration and thus osmotic status in such environments might be modified in nature drastically by rain and dehydration and modestly by ripeness of fruits. To achieve a model system to comprehend environmental responses of AAB and to acquire new enzymes and metabolic systems practicable in high concentration of sugars, we have started genome-basis analyses using two AAB species, Tanticharoenia sakaeratensis and Asaia bogorensis, which are relatively phylogenetically close to each other and share an interesting features, i.e. the ability of multiplication in media containing glucose up to 30%. First, we have determined draft genome DNA sequences of T. sakaeratensis and A. bogorensis using a next generation sequencer, Illumina HiSeq 2000, and it was shown that their genomes encode 3,155 and 2,787 genes, respectively. Paralog analysis among more than 20 AAB genomic DNA sequences including unpublished draft sequences implicated that 41 paralog groups were specific to only T. sakaeratensis and A. bogorensis, such as monosaccharide-transporting ATPase and oxido-reductase molybdoephrin-binding protein. But functions for approximately half of the genes were unknown. For narrowing down the candidates genes involving in the hyper glucose tolerance, we carried out comparative physiological and omics analyses in both T. sakaeratensis and A. bogorensis grown. Under 30% glucose conditions, T. sakaeratensis and A. bogorensis could utilize glucose and produce acids, however both strains multiplied more slowly than under lower glucose concentration conditions. A. bogorensis, but not T. sakaeratensis, showed drastic morphological changes under high glucose conditions. The proteome analyses were carried out using proteins fractionated into soluble, membrane-bound and SDS-mediated extractants. Comparative analyses illustrated that several proteins were accumulated and decreased in only high glucose condition, for instance, proteins similar to pyruvate decarboxylase and A. pasteurianus OmpA, respectively. In this meeting, we will present current omics data of T. sakaeratensis and A. bogorensis.

Keywords: hyper glucose tolerance, genome, proteome, Tanticharoenia sakaeratensis, Asaia bogorensis.
The strictly aerobic α-proteobacterium *Gluconobacter oxydans* is used for a wide variety of industrial applications such as vitamin C synthesis. A special characteristic is the incomplete oxidation of substrates like sugars or sugar alcohols in the periplasm. Despite its industrial importance, knowledge of the metabolism of *G. oxydans* and its regulation, especially concerning the sugar metabolism, is still very scarce (Hanke et al. 2012). Our *in silico* analysis of the genome sequence of strain 621H identified 117 putative transcriptional regulators (TRs) belonging to 38 different regulator families. None of these has been characterised yet. Based on a number of criteria we preselected a number of TRs which might be of particular interest. Of these, an FNR-like regulator was chosen for detailed studies. FNR (fumarate nitrate reductase regulatory) of *Escherichia coli* is an oxygen sensor which controls the switch from aerobic to anaerobic respiration. As *G. oxydans* is considered to be strictly aerobic and no pathways for anaerobic respiration or fermentation are known, the role of the *G. oxydans* FNR homolog, in particular its target genes, was enigmatic. Therefore its function was studied by chromosomal deletion of the corresponding gene and analysis of the mutant strain with respect to growth properties and global gene expression (using DNA microarrays). In addition, preliminary studies on the biochemical properties of the FNR homolog of *G. oxydans* were performed. The results of these studies will be presented.

**Keywords:** Gluconobacter oxydans, transcriptional regulation, Fnr, GOX0974.

**References:**


**S3A-O-03**

**HIGHLIGHTS ON ACETOBACTER PASTEURIANUS PROTEOME MODIFICATIONS**

C. Andrés-Barrao,1 M.M. Saad,2 M. Chappuis,1 R. Ortega Pérez,1 M. Boffa,1 X. Perret,2 F. Barja1*

1Department of Botany and Plant Biology, Microbiology Unit, University of Geneva, Chemin des Embrouichis 10, CH-1254 Jussy, Switzerland; 2Department of Botany and Plant Biology, Microbiology Unit, University of Geneva, Quai Ernest-Ansermet 30, CH-1211, Geneva, Switzerland; 1Political Economy, University of Geneva, Bd du Pont d’Arve 40, CH-1211, Geneva, Switzerland.

*Corresponding author: francois.barja@unige.ch Phone: +41 22 793750.

Acetic acid bacteria (AAB) show a unique resistance to ethanol and acetic acid. Several members of the *Acetobacter* and *Gluconacetobacter* genera, which are capable to transform ethanol to acetic acid via the action of alcohol dehydrogenase and aldehyde dehydrogenase, are used for the industrial production of vinegar. Known resistance mechanisms of AAB to the moderate to high acetic acid concentrations present in vinegar include (i) the assimilation of acetate through the TCA cycle, (ii) the secretion of acetate by efflux systems, and (iii) modifications in the lipid and polysaccharide composition of the bacterial cell wall. Yet, except for a few acetate-specific proteins identified, little is known about the global responses of AAB to acetic acid at the proteome level. This prompted us to follow the changes that occur in the proteome of *Acetobacter pasteurianus* during acetic acid fermentation. Using two-dimensional differential in-gel electrophoresis (2D-DIGE), the proteome of the strain LMG12625 was examined at different stages of the fermentation process, starting with 4% ethanol until its complete depletion. Using an ANOVA factor <0.05 and 1.5-fold as the minimal level of differential expression as thresholds, at least 325 proteins were shown to be either up- or down-regulated. In addition, scanning electron micrographs confirmed that the median length of AAB cells was reduced by about 30% in length as a consequence of the increasing acidity. A modification in the nature of membrane polysaccharides was also revealed by PATAg specific staining.

**Keywords:** Acetobacter pasteurianus, 2D-DIGE, morphology, acetic acid fermentation.

**S3A-O-04**

**PROMOTERS OF MEMBRANE-BOUND DEHYDROGENASES FROM GLUCONOBACTER OXYDANS 621H AND THEIR ABILITY IN A NEW EXPRESSION SYSTEM**

M. Mientus,1* B. Peters,1 D. Kostner,1 W. Liebl,1 A. Ehrenreich1

1Lehrstuhl für Mikrobiologie Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany.

*Corresponding author: mmientus@tum.de Phone: +49 8161715468.

Acetic acid bacteria are used in biotechnology due to their ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds in a regio- and stereo-selective manner. Most of these reactions are catalyzed by membrane-bound dehydrogenases with a broad substrate range.

As currently very little is known about the promoters of membrane-bound dehydrogenases we investigated the regulation and transcription starting points of different dehydrogenases including one regulated promoter. Furthermore the promoter strength was quantified by a reporter. These promoters are now available tools for the expression of (novel) membrane-bound dehydrogenases using *G. oxydans* 621H as host.

A multi-deletion strain of *G. oxydans* 621H which is lacking its main membrane-bound dehydrogenases was constructed with a clean deletion method (Supp) by our group. In order to complement the deletions of membra-
ne-bound dehydrogenases we constructed a shuttle vector system for their functional expression. This system was successfully used for the complementation of the deleted membrane-bound dehydrogenases in *G. oxydans* 621H and could also be used in other *G. oxydans* strains. Furthermore this vector system is available for the expression and characterization of membrane-bound dehydrogenases from a vinegar metagenome.

**Keywords:** promoters, expression, membrane-bound dehydrogenases, *Gluconobacter oxydans*.

**S3A-O-05**

**TALES OF TWO ENZYMES: MEMBRANE-BOUND ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE OF ACETIC ACID BACTERIA**

T. Yakushi, H. Mukai, T. Kodama, M. Matsutani, G. Theeragool, K. Matsushita

1Faculty of Agriculture, Yamanashi University, Yamanashi, Japan; 2Faculty of Science, Kasetsart University, Bangkok, Thailand.

*Corresponding author: juji@yamaguchi-u.ac.jp Phone: +81 839355857.

Acetic acid fermentation, in which ethanol is oxidized to acetic acid by acetic acid bacteria, is the most characteristic process in vinegar production. The process is catalyzed by two membrane-bound enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). ADH has an ethanal:ubiquinone oxidoreductase activity to work as a primary dehydrogenase in the ethanol-oxidizing respiratory chain. ADHs purified from *Acetobacter* and *Gluconobacter* consist of three subunits, dehydrogenase (sub I encoded by *adhH*), ubiquinone reductase cytochrome c (sub II, *adhB*), and chaperone (sub III, *adhS*) subunits. On the other hand, ADHs purified from *Gluconacetobacter* spp. do not contain sub III. Recently we determined the genome sequence of *Gluconacetobacter xylinus* IFO 32388 and found a homologous gene for sub III. We constructed ΔadhH (ΔGLX_26600) and ΔadhS (ΔGLX_16410) strains of IFO 32388. The ΔadhH cells dramatically lost ethanol oxidase and ethanol dehydrogenase activities. Since a sub I-like band could be detected in heme-catalyzed peroxidase staining of SDS-PAGE analysis, sub I having heme C with very low activity may be produced in the absence of sub II. Ethanol oxidase and ethanol dehydrogenase activities at the wild-type level could be observed in the cell-free extract of the ΔadhS strain. This gene homologous to *adhS* is not required for ADH activity.

ALDH catalyzes oxidation of acetaldehyde produced by the action of ADH to produce acetic acid. Although the genes for ALDH of *Gluconacetobacter europaeus* are cloned and sequenced, their physiological relevance to acetic acid fermentation is not clear yet. Here, we disrupted the *aldH* gene encoding dehydrogenase subunit of ALDH in *Acetobacter pasteurianus* SKU1108, a thermotolerant strain, of which the draft genome sequence is available. The ΔaldH strain failed to do acetic acid fermentation in the medium with 4% ethanol, suggesting that the *aldH* gene is responsible for acetic acid fermentation. However, the ΔaldH strain still showed ALDH activity in the membrane fraction at approx. 30% of the wild-type strain. This remaining ALDH activity is not derived from the ADH molecules, because it was not affected by heat inactivation of ADH. We found another set of genes homologous to *aldFGH* in the genome, which may account for the remaining in vitro ALDH activity.

**Keywords:** *Gluconacetobacter*, alcohol dehydrogenase, PQQ, cytochrome, molecular assembly.

**S3A-O-06**

**A NEW CLEAN DELETION SYSTEM FOR DIFFERENT GLUCONOBACTER OXYDANS STRAINS**

D. Kostner, B. Peters, M. Mientus, W. Liebl, A. Ehrenreich

1Lehrstuhl für Mikrobiologie Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany.

*Corresponding author: david.kostner@tum.de Phone: +49 8161715468.

The genus *Gluconobacter* from the family of Actobacteriaceae is well known for its ability to perform rapid incomplete oxidation of a great variety of carbohydrates, alcohols and related compounds. In a multitude of biotechnological processes for example *G. oxydans* is used because of its regio- and stereo-selective oxidative capabilities. The incomplete oxidation of substrates is catalyzed by various dehydrogenases. For the detailed molecular investigation of *Gluconobacter*, a well-established and easily applicable clean deletion system is essential. A method for markerless clean deletion in *G. oxydans* strain 621H is already available from our group. This method is based on the use of uracilphosphoribosyl transferase (UPRTase) as a counter-selectable marker in the presence of the toxic pyrimidin analogue 5-fluorouracil (5-FU). The bottleneck of the method is the requirement for previously generated mutants of the UPRTase gene (Δupp). The construction of such Δupp mutants is difficult in some strains with effective restriction systems or they might be inconvenient for biotechnological purposes. To allow usage of wild-type strains instead of Δupp-mutants, we developed an improved clean deletion system based on a cytosine-deaminase as the counter-selectable marker in the presence of toxic 5-fluorocytosine (5-FC). This principle is applicable for several other genera of Acetobacteria to establish clean deletion systems.

**Keywords:** *Gluconobacter oxydans*, clean deletion system, fluorocytosin.

**S3A-P-01**

**GENOME SEQUENCE OF GLUCONACETOBACTER EUROPAEUS**

The main acetic acid bacteria (AAB) strains involved in the production of vinegars with high acetic acid concentration belong to the genus *Gluconacetobacter*, and from them, *Gluconacetobacter europaeus* is one of the most prominent species that have been isolated from industrial submerged vinegar fermentors. *Gluconacetobacter* strains are characterised by an extremely high resistance to acetic acid (up to 18-20%).

The genome sequences of other industrially important AAB have been published, such as *Gluconobacter oxydans*, *Acetobacter pasteurianus*, *Gluconacetobacter hansenii* or *Gluconacetobacter xylinus*. We describe here the genome sequence of three strains of *Ga. europaeus* (LMG 18890\textsuperscript{T}, LMG 18494, 5P3), as well as one strain of *Ga. oboediersi* (174Bp2). Strains 5P3 and 174Bp2 were isolated from red wine and spirit vinegar running submerged fermentations that were carried in the laboratory.

Sequences were obtained by using Illumina paired-end (PE insert 500bp, 67bp read length) and mate-paired (MP insert 3Kbp, 80bp read length) libraries. The assembly was carried out with SOAPdenovo, combining PE and MP data. The scaffolds were built with Abacas using *Ga. xylinus* as a reference and the gaps were verified by PCR and sequenced. After remapping of the reads onto the scaffolds, the potential connections between scaffolds were assessed with IGV and a utility for displaying connections graphically in Cytoscape. The final sequence was obtained after treatment with PrInSeS to correct for gaps or misassemblies.

We describe here the main common and different features among the four chromosomes.

**Keywords:** genome sequence, *Gluconacetobacter europaeus*, high-throughput sequencing.
S3B-O-01
SIMULATED COCOA PULP FERMENTATIONS OF COCOA-SPECIFIC ACETIC ACID BACTERIA REVEAL THEIR FUNCTIONAL ROLE AND INTERACTIONS DURING COCOA BEAN FERMENTATIONS
F. Moens,*,† T. Lefeber,1 L. De Vuyst†
†Research Group of Industrial Microbiology and Food Biotechnology, Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
*Corresponding author: ldvuyst@vub.ac.be Phone: +32 26293245.

Cocoa bean fermentation is the first step in chocolate manufacturing. During this fermentation process a microbrial succession occurs in which the initial growth and metabolism of yeast and lactic acid bacteria (LAB) is followed by the growth of acetic acid bacteria (AAB). The present study aimed at investigating the kinetics of substrate consumption and metabolite production by cocosspecific AAB strains to unravel their physiological adaptations to the cocoa pulp ecosystem and their interactions with LAB and yeast to obtain an in-depth view of the functional role of AAB during cocoa bean fermentation. Therefore, four cocoa-specific AAB strains (Acetobacter pasteurianus 386B, A. ghanensis LMG 23848T, A. fabarum LMG 24244T, and A. senegalensis 108B) were analyzed kinetically and metabolically during monoculture fermentations in a cocoa pulp simulation medium (CPSM) for AAB at pH 4.5 under aerobic conditions. CPSM containing ethanol plus lactic acid and/or mannitol was tested. To get a more detailed view of ethanol oxidation by the AAB strains, ethanol was added at a constant flow rate to the medium to compensate for evaporation losses. Acetobacter pasteurianus 386B and A. ghanensis LMG 23848T were analysed during co-culture fermentations with Lactobacillus fermentum 222 in CPSM for LAB, containing glucose, fructose and citric acid, and simulating ethanol, pH, and aeration dynamics of a spontaneous cocoa bean fermentation process. A tri-culture fermentation under similar conditions with Saccharomyces cerevisiae H5S5K23, L. fermentum 222, and A. pasteurianus 386B was also performed. Monoculture fermentations with AAB showed that only A. pasteurianus 386B and A. ghanensis LMG 23848T oxidized mannitol to fructose. With respect to oxidation of ethanol and lactic acid to acetic acid, two groups could be distinguished: A. pasteurianus 386B and A. senegalensis 108B oxidized lactic acid and ethanol consecutively and fast, whereas A. ghanensis LMG 23848T and A. fabarum LMG 24244T did it simultaneously and slow, underlining greater competitiveness of the former species. During co-culture fermentations, A. pasteurianus 386B and A. ghanensis LMG 23848T survived the initial suboptimal pH and non-aeration conditions and oxidized lactic acid, produced by L. fermentum 222, into acetic acid, indicating their further growth when ethanol becomes limiting. During the tri-culture fermentation experiments, ethanol and lactic acid initially produced by S. cerevisiae H5S5K23 and L. fermentum 222, respectively, were oxidized to acetic acid by A. pasteurianus 386B, which accurately reflected the actual cocoa bean fermentation process.

Keywords: acetic acid bacteria, fermentation, oxidation, cocoa pulp.

S3B-O-02
PROTEOMIC STUDY OF ACETOBACTER PASTEURIANUS NBRC3283 AND ANALYSIS OF FACTORS POSSIBLY RELATED TO ACETIC ACID FERMENTATION
A. Okamoto-Kainuma,† M. Ishikawa,† K. Ito,‡ Y. Koizumi†
†Department of Fermentation Science, Tokyo University of Agriculture, Japan; ‡Central Research Institute, Mizkan Group Corporation, Japan.
*Corresponding author: okamoto@nodai.ac.jp Phone: +81 354772384.

Acetic acid bacteria possess various properties that enable acetic acid fermentation. These bacteria are characteristic in acquiring ATPs by oxidative fermentation, although they can also produce ATPs by normal procedures, which is commonly observed in many aerobic microbes. In addition to this energy-generation property, these bacteria possess resistance ability against various stressors that inevitably exist during fermentation. The combination of these properties enables acetic acid bacteria to perform acetic acid fermentation, which is applied to vinegar production. Hence, elucidation of the expression mechanism or combination manner of these facts is important to develop more sophisticated fermentation techniques. Recently, genomic sequences have been determined for several strains of acetic acid bacteria, which have made it possible to investigate the physiology of these bacteria more comprehensively. In this study, we performed proteomic analysis of Acetobacter pasteurianus NBRC3283 and further investigated several factors that have been suggested to be associated with acetic acid fermentation ability.

Agarose-2D electrophoresis was performed on the cell-free extracts of A. pasteurianus NBRC3283, and 97% of the CBB-stained protein spots on the gel were identified by ESI-IT MS/MS analysis. According to the results, we created a protein expression map on a 2D-gel profile. Based on this map, the following proteins showed interesting behavior, possibly reflecting characteristic properties of acetic acid bacteria; 1) enolase and pyruvate phosphate dikinase, enzymes belonging to the glycolytic pathway, 2) AarC, an enzyme that compensates for the lack of succinyl-CoA synthetase in the TCA cycle, and 3) GroEL, DnaK, and ClpB, representative molecular chaperones.

Concerning the above factors, we analyzed the detailed behaviors at the transcription level, and the results suggest that the glycolytic pathway is involved in acetic acid fermentation. In addition, we speculate that AarC possibly has some function other than overoxidation of acetic acid. Furthermore, we constructed an rpoH disruptant, which does not express the molecular chaperones, and
confirmed its importance for resistance to stressors that exist during acetic acid fermentation.

**Keywords:** Acetobacter pasteurianus, proteomic analysis, glycolytic pathway, AarC, molecular chaperone.

**S3B-O-03**
**ELUCIDATING THE METABOLIC PROPERTIES OF GLUCONOBACTER OXYDANS 621H BY 13C-METABOLIC FLUX ANALYSIS**

S. Ostermann,1* W. Wiechert,1 M. Oldiges1

1Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, D-52425 Jülich, Germany.

*Corresponding author: s.ostermann@fz-juelich.de
Phone: +49 02461613374.

The acetic acid bacteria are a very interesting group of organisms, especially for industrial processes. Although they have a great production potential, a lot of their biochemical properties are still unknown. Due to scientific progress in the last years, genomic sequences of first strains are now available. With deeper metabolic insight it will be possible to broaden the range of applications and increase the yield of product by metabolic and bioprocess engineering.

Intracellular metabolic flux rates, the so called Fluxome, can be measured by 13C-metabolic flux analysis (13C-MFA). A labeled 13C-substrate mixture is fed and the resulting labeling pattern is measured via mass spectrometry. In addition all carbon fluxes over the system borders have to be quantified (i.e. substrate uptake rates and product formation rates), and the intracellular isotopic labeling pattern of the metabolites have to be determined. As a result the central carbon metabolism can be visualized in a flux map including absolute values of each reaction with their related standard error. This information about the in vivo situation leads to valuable insight into metabolic network operations and could detect so called bottlenecks in production processes.

As a prerequisite for 13C-MFA, a well-controlled continuous cultivation process is necessary. This mode was established in G. oxydans 621H with 5 g/L yeast extract and 5g/L glucose as substrates. All relevant carbon uptake rates (glucose) and product formation rates (gluconate, 2-keto-gluconate, acetate, biomass, CO2) were successfully quantified. In three biological replicates with μ=0,32±0,02 h⁻¹ a carbon recovery between 90,4±5,76% and 110,4±10,13% could be obtained. To get reliable intracellular flux rates a precise and closed carbon balance is essential. Yeast extract is still used in the cultivation media to gain enough biomass. The addition of this complex component significantly interferes with 13C labeling experiments due to error prone labeling data if 12C moieties from yeast extract are incorporated in addition to 13C glucose. It is possible to detect such incorporation of components or precursors from the yeast extract by finding 12C-contamination in the intracellular metabolites. Thus cultivations with uniformly labeled 13C glucose were performed. It could be shown that 100% of the formed CO2 is 13C-labeled. These data and the closed carbon balance are a hint that no significant amounts of yeast extract are metabolized to CO2. Once the complete workflow of 13C-MFA is validated for the wildtype G. oxydans 621H it will be very easy to adapt it to different production strains and Fluxome data will provide an additional basis for metabolic and bioprocess engineering of acetic acid bacteria.

**Keywords:** metabolic flux analysis, carbon metabolism, defined media.

**S3B-O-04**
**HOW DIFFERENT CARBON SOURCES AND CONDITIONS MAKE AAB “WORKING” STRAINS: ACETOBACTER PASTEURIANUS STRAIN AB0220 IN SUPERFICIAL ACETIFICATION SYSTEM AS CASE STUDY**

M. Gullo1*

1Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via G. Amendola 2, Pad. Besta, 42100 Reggio Emilia, Italy.

*Corresponding author: maria.gullo@unimore.it Phone: +39 0522522057/63.

Metabolic potential of acetic acid bacteria (AAB) is of great interest for several fields of the bio-industry. Acetobacter pasteurianus species accounts strains relevant for the production of both conventional and innovative fermented beverages. Strain AB0220 was isolated in 2002 during a large spectrum of isolation work aimed to build up an AAB collection from superficial vinegar acetification systems. It was preserved for 9 years by short and long time methods. Ethanol oxidation to acetic acid was stable and confirmed, as well as acetate assimilation during preservation. The strain do not produced cellulose. Cultivability checks showed persistence of phenotypic traits over the extended preservation time. Stability of subcultures related to the culture age and subcultures frequency confirmed the suitability of preservation at least over a period of 9 years. Strain performance during superficial acetification, both in laboratory and industrial scale, was assayed. To this aim, the acetification ability was tested on different carbon sources and conditions miming the basic unit operation of superficial acetification technology. The performance of AB0220 during processes was evaluated implementing a molecular and analytical control system. Under the experimental conditions, acetic acid, ethanol and pH were the main parameters dictating the conduction of scaling-up procedure. When fixing ethanol content between 1 and 3% as upper and lower limits and 3% as the lower limit for acetic acid, suitable acidity (6-7%) was reached. The persistence of AB0220 as starter over the time was evaluated after biofilm-enrichment cultures on GYC plates. The biofilm, totally recovered from plates, was processed for genomic DNA extraction. PCR/DGGE and ERIC/PCR were successfully used to assess species and strain persistence respectively, during 178 days of acetification.

**Keywords:** Acetobacter pasteurianus, phenotype, AAB selected starter.

| 18 | Acetic Acid Bacteria 2012; 1 (s1) |
**S3B-O-05**

**ROLE OF THE PENTOSE PHOSPHATE PATHWAY AND THE ENTERN-DOUDOROFF PATHWAY FOR CYTOPLASMIC FRUCTOSE CATABOLISM IN **GLUCONOBACTER OXYDANS** 621H**

J. Richhardt,1 S. Bringer,1 M. Bott1

1Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, D-52425 Jülich, Germany.

*Corresponding author: j.richhardt@fz-juelich.de
Phone: +49 2461613975.

The obligatory aerobic acetic acid bacterium **Glucanobacter oxydans** 621H oxidizes sugars and sugar alcohols primarily in the periplasm, whereas only a small fraction is metabolized in the cytoplasm. The latter can occur either via the Entner-Doudoroff pathway (EDP) or via the pentose phosphate pathway (PPP). The Embden-Meyerhof pathway (EMP) is non-functional due to the lack of a gene for phosphofructokinase and a cyclic operation of the tricarboxylic acid (TCA) cycle is precluded by absence of the genes for succinyl-CoA synthetase and succinate dehydrogenase.

In this study, we tested the relevance of the EDP and the PPP for the cytoplasmic catabolism of the carbon source mannitol. This sugar alcohol is rapidly oxidized to mannitol and caused a strong selection pressure for secondary phosphate dehydrogenase activity and therefore also prevent fructose metabolism via the EDP. This Δmfd zwf mutant was almost totally disabled in fructose catabolism, but still produced about 16% of the carbon dioxide formed by the parental strain. In contrast, deletion of the gnd gene inhibited growth on mannitol. This sugar alcohol is rapidly oxidized to fructose, both in the periplasm and in the cytoplasm.

Two defined mutants of **G. oxydans** were constructed, one defective in the EDP by deletion of the genes **edd** (6-phosphogluconate dehydrogenase) and **eda** (2-keto-3-deoxy-6-phosphogluconate aldolase) and one devoid of the PPP by deletion of the **gnd** gene (6-phosphogluconate dehydrogenase). Based on the growth properties of the two mutants under controlled culture conditions, transcriptome analyses and enzyme activity measurements it was concluded that the PPP is the main route for cytoplasmic fructose degradation in **G. oxydans**, whereas the EDP is dispensable or even unfavorable, as the Δedd-eda mutant showed a significantly increased cell mass compared to the parental strain. In contrast, deletion of the gnd gene inhibited growth on mannitol and caused a strong selection pressure for secondary mutations in the zwf gene that inactivate glucose 6-phosphate dehydrogenase activity and therefore also prevent fructose metabolism via the EDP. This Δgnd zwf mutant was almost totally disabled in fructose catabolism, but still produced about 16% of the carbon dioxide formed by the parental strain, presumably by catabolizing substrates from the yeast extract.

**Keywords:** Gluconobacter oxydans, gnd, edd-eda, zwf, mannitol.

**S3B-O-06**

**DIVERSITY OF THE RESPIRATORY CHAIN OF AAB, ESPECIALLY ACETOBACTER PASTEURIANUS, GLUCONACETOBACTER DIAZOTROPHICUS AND GLUCONOBACTER OXYDANS**

H. Miura,1 S. Nishiyama,1 M. Arimitsu,1 Y. Anami,1 M. Matsutani,1 T. Yakushi,1 K. Matsushita1

1Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan.

*Corresponding author: kazunobu@yamaguchi-u.ac.jp
Phone: + 81 839335858.

Acetic acid bacteria (AAB) are well known for their ability to oxidize various sugars and sugar alcohols into their corresponding acids. The oxidation reactions are carried out by membrane-bound enzymes linked to the aerobic respiratory chain, which consists of the primary dehydrogenases and terminal oxidases via ubiquinone. However, the respiratory components, especially NADH dehydrogenases (NDH) and terminal ubiquinol oxidases (Q-oxidase), are varied depending on the genus of AAB. NDH has been classified as NDH-I and NDH-II, both the genes of which are present in all AAB genomes except for NDH-I in **Gluconobacter oxydans**. Whereas, Q-oxidase is classified as cytochrome ba3, cytochrome bo3, cytochrome bd, and cyanide-insensitive oxidase (CIO). **G. oxydans** genome has genes for cytochrome bo3 and CIO, **Gluconacetobacter diazotrophicus** has those for cytochrome ba3 and CIO, while **Acetobacter pasteurianus** has three genes corresponding to cytochrome ba3, cytochrome bd, and CIO.

As for Q-oxidases, cytochrome bo3 has been isolated long before from **G. oxydans** and **A. aceti** (**A. pasteurianus**), and cytochrome ba3 from **A. pasteurianus** and **G. diazotrophicus**. Recently, CIO was purified from **G. oxydans**, and also partially done from **A. pasteurianus** and **G. diazotrophicus**. Thus, we will present some of the results obtained from the purified CIOs which exhibit rather unique properties such as CN-insensitivity, low O2 affinity, or rather high quinol oxidase activity and its kinetics, compared with cytochrome bd.

Furthermore, based on microarray or RT-qPCR analyses in **A. pasteurianus**, NDH-I was shown to be highly expressed in the over-oxidation phase but not in the ethanol-oxidation phase of acetic acid fermentation. NDH-II expression was rather low compared with NDH-I in any phases. Whereas, cytochrome ba3 was highly expressed in all phases of the fermentation, but both cytochrome bd and CIO were weakly expressed but the most in the ethanol-oxidation phase. We further isolated NDH-I from the membranes of the over-oxidation phase, and found almost all the subunits of NDH-I complex. Thus, NDH-I-dependent NADH oxidase respiratory chain seems to work mainly in the over-oxidation phase, but not in the ethanol-oxidation phase, together with cytochrome ba3 oxidase.

**Keywords:** respiratory chain, cyanide-insensitive oxidase, cytochrome ba3, cytochrome bo3, NADH dehydrogenase.
**S3B-O-07**

**THE QUEST FOR NEW OXIDATIVE CATALYSTS: EXPRESSION OF METAGENOMIC MEMBRANE-BOUND DEHYDROGENASES FROM ACETIC ACID BACTERIA IN GLUCONOBACTER OXYDANS**

B. Peters,⁴ M. Mientus,¹ D. Kostner,¹ W. Liebl,¹ A. Ehrenreich¹

¹Lehrstuhl für Mikrobiologie Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany.

*Corresponding author: bjorn.opeters@wzw.tum.de

Phone: +49 8161715468.

Due to their ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds, acetic acid bacteria are widely used in biotechnology. Many of these oxidations are unachievable using organic chemistry. Because these reactions are mostly catalyzed by membrane-bound dehydrogenases in a rapid, regio- and stereo-selective manner, the substrates do not have to be transported into the cytoplasm. We use a metagenomic approach to indentify new membrane-bound dehydrogenases of potential value for biotechnology from a mother of vinegar, because many acetic acid bacteria can not be cultivated in the laboratory. The membrane-bound dehydrogenases are screened by sequence similarity from the metagenomic sequences and are functionally expressed in specially designed Gluconobacter oxydans strains. In these strains all membrane-bound dehydrogenases were deleted using a clean deletion system developed by our group to avoid overlapping enzymatic specificities. To ensure functional integration in the membrane physiology of these organisms we use specifically designed expression vectors with native promoters. In order to characterize the activity of specific membrane-bound dehydrogenases we set up a high throughput assay, using whole cell catalysis in microtiter-plates. The advantage of this system is a minimized cell preparation together with the ability to compare many stains or substrates in one experiment. We used this approach to determine the in vivo substrate spectrum of several specific membrane-bound dehydrogenases from acetic acid bacteria for the first time.

Keywords: metagenome, membrane-bound dehydrogenases, Gluconobacter oxydans.

**S3B-O-08**

**THE ACTIVE AND INACTIVE FORMS OF THE MEMBRANE-BOUND ALCOHOL DEHYDROGENASE FROM GLUCONACETOBACTER DIAZOTROPHICUS**

J.E. Escamilla,¹* S. Gómez-Manzo²

¹Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad Universitaria 04510, México; ²Laboratorio de Bioquímica-Genética. Torre de Investigación. Instituto Nacional de Pediatría, Mexico.

*Corresponding author: escami@ifc.unam.mx

Phone: +52 5556225627.

The membrane-bound alcohol dehydrogenase of Gluconacetobacter diazotrophicus contain, one pyrroloquinoline quinone moiety (PQQ), one [2Fe-2S] cluster and four c-type cytochromes. Here, we describe a novel and inactive alcohol dehydrogenase (ADHi). ADH, similarly to ADHs is a heterodimer of 72 and 44 kDa subunits and contains the expected prosthetic groups. However, ADHs showed a threefold molecular mass as compared to ADHi. Noteworthy, the PQQ, the [2Fe-2S] and most of the cytochromes in purified ADHi is in the oxidized form, contrasting with ADHa where the PQQ-semiquinone is detected and the [2Fe-2S] cluster as well as the cytochromes c remained fully reduced after purification. Reduction kinetics of the ferricyanide-oxidized enzymes showed that, while ADHa was brought back by ethanol to its full reduction state, in ADHi only one quarter of the total heme c was reduced. The dithionite-reduced ADHi was largely oxidized by ubiquinone-2, thus indicating that intramolecular electron transfer is not impaired in ADHi. The acidic pH of the medium might be deleterious for the membrane-bound ADH by causing conformational changes leading to changes in the relative orientation of heme groups and shift of corresponding redox potential to higher values. This would hamper electron transfer resulting in the low activity observed in ADHi.

Acknowledgements: CONACyT 152779 and PAPIIT-UNAM IN218710-2

Keywords: alcohol dehydrogenase, Gluconacetobacter diazotrophicus, aerobic fermentation, inactive ADH.

**S3B-O-09**

**STRUCTURES AND FUNCTIONS OF ACETOBACTER ACETI COA-TRANSFERASES**

T.J. Kappock,¹* E.A. Mullins¹,²

¹Department of Biochemistry, Purdue University, West Lafayette, Indiana, USA; ²Department of Chemistry, Washington University, Saint Louis, Missouri, USA.

*Corresponding author: kappock@purdue.edu

Phone: +01 7654948383.

Genome sequencing revealed five acyl-CoA transferase genes in the A. acetii strain 1023 genome. One is aarC, an acetic acid resistance factor, and the others were designated actA - uctD. We previously reported that AarC is succinyl-CoA:acetate CoA-transferase (SCACT) and that its resistance function can be attributed to its role as part of a variant citric acid cycle (vCAC) present in A. acetii 1023 [E. A. Mullins, J. A. Francois, and T. J. Kappock (2008) J. Bacteriol. 190, 4933-40]. Subsequent genome sequencing has indicated that some acetic acid bacteria use the vCAC, some use the normal CAC containing succinyl-CoA synthetase, and some may use either one. This implies some degree of metabolic flexibility in a pathway that is relevant to specific acetic acid resistance phenotypes. We used a combined enzymological and crystallographic approach to arrive at a model that explains how AarC recognizes its substrates. Analysis of aarC transcription suggests a combination of inducible and housekeeping expression patterns. In addition, we will
disclose functional assignments for UctB and UctC that expand the scope of likely physiological roles for acyl-CoA: acid interconversions coupled to acetyl-CoA production.

Keywords: acid metabolism, functional annotation, x-ray crystallography.

**S3B-P-01**

**PERFORMING 13C-METABOLIC FLUX ANALYSIS ON ACETIC ACID BACTERIA**

S. Ostermann,1* W. Wiechert,1 M. Oldiges1

1Institute of Bio- and Geosciences IBG1: Biotechnology, Forschungszentrum Jülich, Germany.

*Corresponding author: s.ostermann@fz-juelich.de
Phone: +49 02461613374.

The acetic acid bacteria have a great industrial production potential, which could be exploited if their biochemical properties are better understood. Their metabolic reactions (flux rates) appear as a result of all cellular control hierarchies and determine the material flows. A reliable and predictive biochemical model would enable directed metabolic engineering to increase efficiency and yield of production processes. For determining the intracellular metabolic flux rates, the so called Fluxome, a 13C-metabolic flux analysis (13C-MFA) can be performed. A defined isotopically labeled substrate is fed into a bioreactor and all carbon fluxes over the system borders are quantified (i.e. substrate uptake rates and product formation rates). Moreover a characteristic intracellular isotopic labeling pattern in the metabolites emerges and with bioinformatic tools the flux data can be calculated and displayed. Although 13C-MFA was already performed for i.e. E. coli, S. cerevisiae or C. glutamicum a lot of adaptions to the family of Acetobacteraceae have to be performed.

A biochemical network model of the central metabolic pathways (glycolysis, pentose phosphate pathway, anaerobic reactions, citric cycle) based on the genome sequence of *Glucobacter oxydans* 621H was created. In this model the faith of every single carbon atom from glucose to intermediate and product is determined. To ensure that all relevant reactions are included the carbon flow of the bioprocess is balanced. Therefore a continuous cultivation mode (chemostat) with 13C-glucose as substrate and sufficient biomass for measurements was established. After 4 residence times the substrate uptake rate (glucose) and product formation rates (CO2, biomass, gluconate, 2-keto-gluconate, acetate) were measured with a carbon recovery between 90.4 ± 5.76% and 110.4 ± 10.13%. To receive the intracellular labeling information all cellular reactions were stopped instantly by cold methanol quenching and the metabolites were extracted followed by LC-MS/MS analytics for measurement of the 13C labeling distribution. For every sample 48 metabolites with 448 mass traces are considered in total. These raw data were combined with organism specific biochemical assumptions, e.g. fixation of known reaction directionals or drain of carbon into biomass. Thereof the software 13CFLUX2 calculates a flux distribution and labeling pattern *in silico*. This pattern is iteratively fitted to the measured labeling pattern to approximate to the *in vivo* metabolic flux distribution.

Keywords: metabolic flux analysis, carbon metabolism, isotopic labeling.

**S3B-O-10**

**SCREENING OF QUORUM SENSING AND QUORUM QUenchING ACTIVITIES IN THE GENOME OF A GLUCONACETOBACTER XYLINUS STRAIN**

M.J. Valera,1 W. Streit,2 M.J. Torija,1 A. Mas,1 E. Mateo1*

1Departamento de Biosinctima y Biotecnología. Facultad de Enología. Universitat Rovira i Virgili. C/ Marcel·lí Domingo s/n. E-43007 Tarragona, Spain; 2Abteilung für Mikrobiologie und Biotechnologie, Biozentrum Klein Flottbek, Universität Hamburg, Germany.

*Corresponding author: estibaliz.mateo@urv.cat
Phone: +34 977558688.

Quorum sensing (QS) is a cell density dependent system, which is used to regulate diverse physiological functions, as biofilm formation. N-Acylhomoserine lactones (N-AHLs) are autoinducers involved in many QS mechanisms to regulate gene expression in gram-negative bacteria. On the other hand, proteins interfering with these bacterial QS molecules have been found. They can stop the activity of this signalization and cut the communication between cells. It is the Quorum Quenching (QQ) signalization, which has been described, in different microorganisms and their diverse mechanisms involved are being studied. Some acetic acid bacteria are able to produce cellulose as secondary metabolite and form biofilms. Previously in some species of *Glucanacetobacter* a Quorum Sensing N-AHLs-dependent system (GinI/GinR proteins homologous to LuxI/LuxR described in *Vibrio fischeri*), was reported; however, the opposite process, a Quorum Quenching, has never been studied in this group of bacteria.

The aim of this study was to screen for QS and QQ activities in the genome of one *Gluconacetobacter xylinus* strain, cellulose overproducer and biofilm-former, in order to better understand the mechanisms involved. A fosmid library of *G. xylinus* genome was constructed in *Escherichia coli*. The strain *Agrobacterium tumefa- ciens* NTL4, carrying a *traI-lacZ* promoter fusion, was used as reporter to make the suitable screening in order to find activities related with the autoinducer AHL. We have obtained 2 clones with putative Quorum Sensing activities and 13 with Quorum Quenching activities. Further studies are underway to characterize the genes involved in production of AHL or anti-AHL molecules.

Keywords: cell-cell communication, homoserine lactone, fosmid library, biofilm.
OVER-EXPRESSED TRUNCATED C-TERMINAL OF ASPARTYL-tRNA SYNTHETASE FROM ACETOBACTER PASTEURIANUS SKU1108 INHIBITS CELL DIVISION OF ESCHERICHA COLI AT HIGH TEMPERATURE

G. Theeragool, K. Tasanapak, W. Yongmanitchai, K. Matsushita

1Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; 2Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan.

*Corresponding author: fscignt@ku.ac.th Phone: +66 818017710.

Thermotolerant acetic acid bacteria play important role in high temperature vinegar fermentation due to their ability to grow and produce acetic acid at high temperature. To clarify the molecular mechanism of thermostolerance, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) was used to investigate the genetic variations between thermotolerant and thermostensitive strains of acetic acid bacteria. Three typical RAPD-PCR amplified DNA fragments of 0.6, 1.0 and 1.3 kb could be clearly observed in 13 different thermotolerant but not in thermostensitive strains. The 1.3 kb DNA fragment carries partial aspS gene encoding truncated 138 amino acid residues of C-terminal aspartyl-tRNA synthetase (AspRS). The entire aspS gene from a thermotolerant acetic acid bacterium, Acetobacter pasteurianus SKU1108, has been further cloned and characterized. The cloned aspS gene consists of 1,788 bp coding for 595 amino acid residues. The deduced amino acid sequences showed 99 and 82% identity with the same protein from A. pasteurianus NBRC 3283 and Gluconacetobacter diazotrophicus, respectively. The strong tetrapeptide Gly-Leu-Asp-Arg motif (motif 3) which acts as ATP binding domain is located at the position 537th-540th amino acid of C-terminal AspRS. Investigation of regulatory region of aspS gene by deletion analysis suggested that the tentative promoter is located at around 173 bp upstream from ATG initiation codon. The plasmid pGEM-TAspRS138 or pUCAspRS138 carrying partial aspS gene encoding 138 amino acids of truncated C-terminal AspRS (motif 3) caused cell morphological change in E. coli from short rod to long filament at 37 and 42°C but not at 30°C. This morphological change was also observed in E. coli harboring pGEM-TAspRS316 or pUCAspRS316 which synthesize 316 amino acids of C-terminal AspRS. However, the normal cell shapes were observed in E. coli harboring pUCAspRS595 synthesizing 595 amino acids and pGEM-TAspRS138 carrying disrupted aspS gene. The results revealed that the over-expressed truncated C-terminal AspRS may cause the titration effect of ATP inside the cell and therefore less amount of ATP is available for cell division. This cell division inhibition effect is more critical at high temperature so more ATP is required for growth at high temperature.

Keywords: aspartyl t-RNA synthetase, aspS gene, truncated C-terminal, ATP-binding, cell division.
are used additionally. One example is the installation of inhomogeneous mixing zones in mini plant bioreactors. Experiments with non ideal mixed vessels are helpful to anticipate the performance of large scale systems. Tools for final design like CFD, cell models or dimension analysis based on the determined critical mixing parameters and the necessary mass transfer coefficient $k_{G}$ are used subsequently. Other methods will be presented to decide about the process design and the use of batch, fed-batch and repeated-(fed)-batch processes depending on the above criteria.

Keywords: acetic acid bacteria, technology, industrial processes, scale up.

S4-O-01

4-PENTULOSONATES, NEW PRODUCTS WITH ACETIC ACID BACTERIA

O. Adachi,¹ R.A. Hours,² E. Shinagawa,³ Y. Akakabe,¹ T. Yakushi,¹ K. Matsushita¹


*Corresponding author: osao@yamaguchi-u.ac.jp Phone: +81 893935857.

4-Keto-D-aldopentoses and 4-pentulosonates (4-keto-D-pentonates) have been identified for the first time. These are entirely new sugars and sugar acids involving a keto group at the C4 position in their molecules, while keto acids from D-glucose via 2,5-diketo-D-gluconate decarboxylase, 4-keto-D-aldopentose 1-dehydrogenase, 2,5-diketo-D-gluconate dehydrogenase, 4-keto-D-aldopentose, 4-keto-D-pentonate, D-aldopentose 4-dehydrogenase has been confirmed as the direct precursor of 4KAB and that D-ribose was oxidized to 4-keto-D-ribose and it was confirmed in the membrane fraction of acetic acid bacteria. In this study, four different novel compounds and four different new membrane-bound enzymes have been identified. Among the membrane-bound enzymes characterized, D-aldopentose 4-dehydrogenase has been confirmed as a new quinoprotein, PQQ-dependent enzyme.

Keywords: D-aldopentose 4-dehydrogenase, 4-keto-D-aldopentose 1-dehydrogenase, 2,5-diketo-D-gluconate decarboxylase, 4-keto-D-aldopentose, 4-keto-D-pentonate.

S4-O-02

COMPARISON OF NOVEL GH 68 LEVANSUCRASES OF LEVAN- OVERPRODUCING GLUCONOBACTER SPECIES

F. Jakob,¹ D. Meißner,¹ R.F. Vogel¹*

¹Lehrstuhl für Mikrobiologie Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany.

*Corresponding author: rudi.vogel@wzw.tum.de Phone: +49 8161715169.

Strains of different Gluconobacter species are capable of incompletely oxidizing a wide range of carbohydrates, alcohols and sugars, whose end products can be used for various (food) biotechnological applications. We recently demonstrated that also levan isolated from exopolysaccharide (EPS)-overproducing Gluconobacter species are promising functional compounds for food applications, e.g. to improve volume, texture and shelf-life of breads. Fructan production strongly depends on the corresponding fructosyltransferases (Fts), which catalyze the formation of these polymers from sucrose. Therefore, we characterized novel, highly active Fts from three EPS-overproducing food-grade strains, i.e. Gluconobacter sp. TMW 2.767 and Gluconobacter sp. TMW 2.1191 isolated from water kefir, and Gluconobacter cerinus DSM 9533T isolated from cherries.

Several PCR techniques including degenerate gradient temperature PCR, modified and standard inverse PCR, modified site-finding PCR and modified single primer PCR, were used to finally detect complete open reading frames coding for Fts. The prospective ftf-gene sequences were heterologously expressed in Escherichia coli Top 10 cells, which were cultivated on solid media supplemented with sucrose (substrate) and arabinose (inducer of gene expression). E. coli transformants harbouring one of the three different ftf-genes produced polysaccharides from sucrose in contrast to the E. coli wildtype.

Each of the heterologously expressed proteins encoded a levansucrase, catalyzing the formation of β-(2→6)-linked fructose polymers, which corresponded to our previous analyses of the chemical nature of the isolated polymers formed by the Gluconobacter strains. This suggests that the expressed genes are responsible for the production of these fructans. Structurally, these enzymes belong to the glycoside hydrolase 68 family (GH 68), sharing the typical modular topology of gram-negative levansucrases (no predictable signal peptides, conserved motifs involved in fructan polymerization). Based on similarity calculations, levansucrases of G. sp. TMW 2.767 and G. cerinus DSM 9533T (91% positives in 445 amino acids) as well as levansucrases of G. sp. TMW 2.1191 and G. oxydans 621
Acetic acid bacteria (AAB) are microorganisms naturally present on grapes and therefore, in wine. Even though studies on AAB have focused on ethanol to acetic acid transformation in wine and vinegar, AAB have other physiological characteristics that make them biotechnologically important. For instance, they can perform incomplete oxidation of sugars, alcohols and acids accumulating the corresponding products in the medium. One product that can be obtained by the incomplete oxidation of glucose is gluconic acid. This acid can destabilize wines during aging and storage, increasing the risk of final spoilage. However, in other beverages the presence of gluconic acid enhances natural sweetness and acts as a buffer for other acids.

Gluconic acid production can be carried out by some AAB species belonging to Acetobacter and Gluconobacter genera, more specifically by the latter genus since glucose is its preferred carbon source. The aim of this study was to carry out the gluconic acid production from glucose in strawberry pulp concentrate without fructose consumption to preserve the natural sweetness of the fruit. For this reason, different strains of Gluconobacter genus, as well as the strain SS8 of Acetobacter malorum, the unique AAB strain isolated from strawberry pulp, were tested in order to choose the best for gluconic acid production. Furthermore, in order to find out the best conditions, two different pHs were tested: 3.3, the natural pH of strawberry pulp concentrate and 4.8, the optimal pH for gluconic acid according to the literature.

The results showed that all the strains were capable of producing gluconic acid but some of them also consumed the fructose. Furthermore, at the strawberry pH, although not being the optimal pH, strains produced an important amount of gluconic acid.

**Keywords**: Gluconobacter, Acetobacter malorum, glucose, fructose.
higher concentration of acetic acid readily, this biomass has a potential to be used as a starter.

**Keywords:** starter, 2-DiGE, respiration, membrane integrity, viability; Acetobacter senegalensis.

### S4-O-05

**CHEESE WHEY RECYCLING IN DAIRY FOOD CHAIN: EFFECTS OF VINEGAR FROM WHEY IN DAIRY COW NUTRITION**

C. Belli,1 E. Salimei,2 G. Alfano,1 G. Lustrato,1 C. Maglieri,2 F. Fantuz,3 L. Grazia,4 G. Ranalli1*

1DiSTAT, University of Molise, 86900 Pesche (IS) Italy; 2DiSTAAM, University of Molise, 86100 Campobasso, Italy; 3Dip. Scienze Ambientali, University of Camerino, 62024 Matelica, Italy; 4DiPROVAL, University of Bologna, 40127 Bologna, Italy.

*Corresponding author: ranalli@unimol.it
Phone: +39 874404155.

Selected yeast (Klyveromyces marxianus Y102 strain) and an acetic acid bacterium (Acetobacter aceti, DSM G3508 strain) were used as inocula respectively in cheese whey for alcoholic and acetic fermentations. The experimental tests were carried out at both laboratory and pilot plant (20 l and 2,000 l) levels. The data from the trials (working period 28 days) showed increased ethanol production, increased acetic acid yield (whey vinegar = wheynegar), and greater fermentation stability with biomass recycling (18.6 g/l). Batch and feed-batch fermentation tests resulted in increased and standardized alcoholic fermentation, and allowed acetic acid recovery (average lactose consumption 56%, ethanol 6.7 g/l per day and acetic acid production 4.35 g/l per day). The effects of wheynegar administration were then investigated on milk yield and composition, nutritional status of dairy cows and physical characteristics of total mixed ration (TMR). Twenty Holstein cows were divided into two groups; group C, receiving the traditional TMR, and group W, receiving the TMR plus 10 l wheynegar. The dietary treatment, lasted 35 days, did not affect milk yield and composition except for the urea content, significantly lowered in group W. The selection of coarse (<19 mm), medium (8-19 mm) and fine (< 8 mm) dietary particles was not influenced by the wheynegar administration however a tendential lower selection against coarse particles was noted in W group. The results clearly highlight that microbial biotechnologies may significantly contribute to both the reduction of the pollutants load of whey and the development of a stable nutrient recycling system within the dairy food chain.

**Keywords:** whey, microbial fermentation, wheynegar, dairy cow nutrition.

### S4-O-06

**INFLUENCE OF DIFFERENT HIGH-MOLECULAR WEIGHT LEVANS PRODUCED BY SELECTED ACETIC ACID BACTERIA ON THE VOLUME AND TEXTURE OF BREADS**

F. Jakob,1 H. Rübsam,2 T. Becker,2 A. Pfaff,3 R. Novoa-Carballal,3 A.H.E. Müller,3 R.F. Vogel1*

1Lehrstuhl für Mikrobiologie Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany; 2Technische Universität München, Lehrstuhl für Brauereitechnologie, Germany; 3Universität Bayreuth, Lehrstuhl für Makromolekulare Chemie II, Germany.

*Corresponding author: rudi.vogel@wzw.tum.de
Phone: + 49 816175169.

Acetic acid bacteria (AAB) are applied in several food fermentations like vinegar or kombucha mainly for their ability to synthesize flavouring acids. Some AAB strains are also known to produce exopolysaccharides (EPS) like gluconacetan or levan. Hydrocolloids such as polysaccharides can be used to improve the textural properties of bakery products acting as thickening and gelling agents. However, the potential of positive functional effects of novel EPS types produced by AAB strains for such applications has not been explored yet.

Therefore, we screened 21 AAB strains for their ability to produce homopolysaccharides from sucrose. *Glucobacter sp.* TMW 2.767, *Glucobacter cerinus* DSM 9533T, *Neosasaia chiangmaiensis* NBRC 101099 and *Kozakia baliensis* DSM 14400 synthesized high EPS yields ranging from 6-12 g/l in liquid glucose media supplemented with 8 % (w/v) sucrose. The isolated, lyophilized EPS of each strain were characterized as β-(2→6)-linked fructofuranosyl polymers (levans) using HPLC and NMR (1H, 13C, HMQC, COSY) analysis and subsequently used as baking ingredients for wheat breads. The addition of each tested fructan in the lowest tested dosage (1% w/w flour) caused an increased volume and a softer crumb of fresh wheat breads. Furthermore, a retarded staling of the EPS breads was observed during 1-week storage. Since these positive functional effects were comparatively higher upon addition of EPS from *N. chiangmaiensis* and *K. baliensis*, we furthermore investigated the isolated polymer fractions of each strain in terms of their molecular weight distribution using asymmetric field flow fractionation (AF4). Whereas the isolated levan polymers of G. *fraterrir* and G. *cerinus* had an average molecular mass (Mₐ) in the range of 10⁷ Dalton (Da), fructans from N. *chiangmaiensis* and K. *baliensis* mainly consisted of fractions with a Mₐ in the range of 10⁸ and 10⁹ Da, respectively. Conformation plots of all investigated levans revealed highly similar distributions of branching. Combined with data of NMR analyses (no detectable signals indicating branching) we suggest that these fructans are linear. Consequently, linear levans synthesized by the selected AAB strains caused increased volume, softer crumb and antistaling of wheat breads more effectively with increasing molecular weight. In conclusion,
we could demonstrate for the first time a novel application and structure/function relation of AAB EPSs in baking.

*Corresponding author: wendu.tesfaye@upm.es

S4-O-07
BREWEAGE OF NOVEL MULTIFUNCTION CHINESE QUINCE VINEGAR
B. Gao,1 L.P. He2*
1School of Biological and Pharmaceutical Engineering, Wuhun Polytechnic University, No.1 West Zhonghuang Road, Changqing Garden, Wuhun 430023, Hubei Province, P.R. China; 2School of Life Sciences of Guizhou University, Huaxi, Guyang 550025, Guizhou Province, P.R. China.

The demand for multifunctional vinegar is increasing nowadays. Based on the demand, in this study, a kind of novel multifunction vineger was prepared rich in γ-aminobutyric acid and amino acids using Chinese quince and germinated rice as main raw materials through a series of processes of liquefaction, saccharification, alcohol fermentation and acetic acid fermentation. Pre-treatment of Chinese quince was conducted by pectinase and cellulose under the condition of time 8 h and temperature 55 °C. The liquefaction process of germinated rice was catalyzed by amylase at pH 7.5 and 92 °C for 30 min, followed by the saccharification 8h at pH 5.5 and 55 °C by addition of glucoamylase and pre-treatment of Chinese quince. Subsequently, alcohol fermentation was conducted by addition of active dry yeast at pH 4.5 to 5.5 and 38 °C for 60 h. Then, acetic acid fermentation was carried out by cycle liquid-solid process using its solution as reflux for 96 h. Thus, novel multifunction Chinese quince vinegar was obtained with the multifunctional ingredients of 3.7% acetic acid, 7 mg/100 mL γ-aminobutyric acid and 340 mg/100 mL amino acids through the series of processes above.

Keywords: vinegar, Chinese quince, germinated rice, γ-aminobutyric acid, cycle liquid-solid fermentation.

S4-P-02
COINOCULATION OF ACETIC ACID BACTERIA IN MICROACETIFICATIONS OF STRAWBERRY WINE
D. García,1 E. Mateo,1 A. Mas,1 M.J. Torija1*
1Departamento de Bioquímica y Biotecnología, Facultad de Enología, Universitat Rovira i Virgili, C/ Marcel·lí Domingo s/n. E- 43007 Tarragona, Spain.

*Corresponding author: mjesus.torija@urv.cat Phone: +34 977558442.

Traditional vinegar is a process usually initiated by a vinegar mother, an undefined starter culture obtained from previous vinegar. But, this practice does not ensure a total control of the aceticification or the quality of the product. For this reason, some studies have been carried out to test the possibility of using pure strains of acetic acid bacteria (AAB) as starters for the production of vinegar by traditional methods. These few studies have demonstrated the difficulty for a unique AAB strain to start and finish the process.

Vinegar may be defined as a condiment made from different sugary and starchy raw materials by alcoholic and subsequent acetic fermentation. Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation in acetators. Actually the Honey market in Spain and Europe is loss making because of the cheapest imported honeys from the third countries. One of the main reasons for making honey vinegar is to provide an outlet for honey which would otherwise not profitable to the bee keepers. In many industries the margin of profit is realized by transforming such a material into sub products in this case to vinegar. The balsamic honey vinegar samples are withdrawn periodically after submitting the cooked mead to alcoholic and acetic fermentation in an oak laboratory scale barrel. A blend of oak, chestnut, cherry and ash chips filled in a plastic strainer, and was immersed periodically during the surface culture aceticification process. The evolutions of volatile compounds are monitored during 124 days. The analysis of variance pointed to a clear relationship (p < 0.05) between type of vinegar and volatile compounds content. During the aceticification process methanol, diacetyl, acetoin, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-3, butanediol, hexanol and 2-Phenylethyl acetate increased their concentration. Meanwhile, ethanol, 1-propanolethyl acetate, isobutanol, ethyl lactate and isovalyl acetate decreased their concentrations. The level of furfural, furfuryl alcohol, 5-hydroxymethyl furfural are higher in the Balsamic honey vinegar compared to vinegars elaborated without cooking the mead as it was expected. Indeed, whiskylactone, syringaldehyde and vainillin increase their concentration during aging either with chips or in barrel. The sensory analysis data revealed that the overall aroma and flavour intensity of this balsamic honey vinegar is higher than the rest of honey vinegars without the chips or those with only oak chips.

Keywords: balsamic honey vinegar, surface culture aceticification, volatile compounds, sensory analysis.

S4-P-01
BALSAMIC HONEY VINEGAR ELABORATION USING OAK, CHESTNUT, MULBERRY, CHERRY AND ASH CHIPS
I. Anton,1 W. Tesfaye,1* A. Morata,1 F. Palomero,1 S. Benito,1 J.A. Suárez-Lepe1
1Dept. Tecnología de Alimentos, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria S/N, 28040 Madrid, Spain.

*Corresponding author: wendu.tesfaye@upm.es Phone: +34 913363987.
In previous studies, only one strain (SS8) of *Acetobacter malorum* was isolated during strawberry spontaneous acetifications and the use of this strain as pure starter showed a lack of imposition at the end of the process. Furthermore, a succession of species or genera has been reported in vinegar production, suggesting that a mixed inoculum of *Acetobacter* and *Gluconacetobacter* species could be used as a good starter culture. Therefore, the aim of this study was to analyse the effect of coinoculation of two strains of different AAB species to produce strawberry vinegar. Different mixed cultures were tested by using the strain SS8 of *A. malorum* and another strain from *Gluconacetobacter europaeus*. These processes were compared with acetifications carried out with these strains inoculated separately. Microacetifications were performed in 250 mL-erlenmeyers with 100 mL of strawberry wine (6.5 % (v/v) Ethanol) and were considered finished when the acidity achieved 5.5-6% (w/v). Microacetifications with mixed cultures finished in 12 days while, none of the microacetifications inoculated with only one strain achieved the expected acidity. Furthermore, important differences in the aceticification behavior were observed depending on the strain tested. Although more assays should be done, AAB coinoculation seems to be a good solution to improve the production of vinegars by traditional methods.

**Keywords:** mixed culture, *Acetobacter malorum*, *Gluconacetobacter europaeus*, surface aceticification.

**S4-P-03**

**DEPENDENCE OF VIABLE CELL CONCENTRATION ON OPERATIONAL VARIABLES DURING AN ACETIFICATION PROCESS IN A FED-BATCH PILOT FERMENTER**

I.M. Santos-Dueñas,1* J.E. Jiménez-Hornero,2 A.M. Cañete-Rodríguez,1 J.C. Mauricio,3 T. García-Martínez,2 S. Baena-Ruano,1 I. García-García1

1Department of Chemical Engineering, Marie Curie Building, University of Córdoba. Spain; 2Department of Computing and Numerical Analysis, Leonardo da Vinci Building, University of Córdoba, Spain; 3Department of Microbiology, Severo Ochoa Building, University of Córdoba, Spain.

*Corresponding author: ines santos@uco.es
Phone: +34 957218586.

The operational variables in a fed-batch aceticification fermenter can affect significantly the mean reaction rate as well as the final productivity. As it is known, different values in such variables can affect the mean values for ethanol and acetic acid concentrations. Considering the bacterial dependence on these compounds, it would be interesting to know how the bacterial cell concentration changes throughout the cycles. The comparison of this information with the bio-reaction rates could throw light on the complex influence of ethanol and acetic acid on both cell concentration and their activity. In this work, in the scope of a study aimed to maximize the productivity of the acetic acid wine fermentation in a fed-batch pilot fermenter, the evolution of viable and total cell concentration have been found. The influence of three operational variables has been studied: ethanol concentration at unloading (E), unloaded volume (V) and loading flow rate (R). The variables ranged between 0.5-3.5% v/v, 25-75% of total volume and 0.01-0.06 mL min⁻¹ for E, V and R respectively.

The results show that mean values for bacterial cell concentrations in the cycles are not depending on the studied operational variables, nevertheless, instantaneous cell concentration profiles throughout the cycles could be quite dependent on the working conditions, particularly when different values for V are used. So, when V has a low value, a flat profile is obtained; on the other side, the unloading of 75% of total volume results in an important variation on the instantaneous cell concentration throughout the cycle. Also, it is interesting to find that neither E nor R have significant influence on the instantaneous cell concentration profiles.

On the other hand, taking into account that the highest and the lowest bio-reaction rates are obtained when a low value of V is used, as well as high and low values of E respectively, it is clear that cells activity is quite dependent on the mean ethanol and acetic acid concentrations during the cycles. Finally, the variation on the cells activity does not mean a similar change in cell viability, in fact, the percentage of cell viability has been found constant (roughly 92% of total cell concentration) and independent of the operating conditions.

**Keywords:** wine vinegar, acetic acid bacteria, viability, submerged culture, operational variables.

**S4-P-04**

**EFFECT OF YEAST AND ACETIC ACID BACTERIA INOCULATION IN STRAWBERRY VINEGAR PRODUCTION BY TRADITIONAL METHODS**

C. Hidalgo,1 M.J. Torija,1* A. Mas,1 E. Mateo1


*Corresponding author: mjesus.torija@urv.cat
Phone: +34 977558688.

Strawberry (*Fragaria ananassa*) vinegar production is a good alternative to take advantage of this seasonal fruit with important health benefits. The aim of this study was to analyze the effect of the inoculation on the production of strawberry vinegar using pure cultures of yeast and acetic acid bacteria (AAB) strains in order to improve the vinegar elaboration process. These strains were previously isolated from strawberry. Fruit vinegar was produced by a two-step bioprocess: alcoholic fermentation and aceticification. The alcoholic fermentations were inoculated with the strain RP1 of *Saccharomyces cerevisiae* in 100 L stainless vats. Sugars (120 g/L) from strawberry pulp concentrate yielded about 6.5% (v/v) of ethanol in 3 days. The inoculated strain took over the fermentation, being the only strain recovered and identified by molecular methods.
On the other hand, the acetification was conducted by traditional method in 60 L cherry barrels designed to increase the air-contact surface. A pure “vinegar mother” was produced using the AAB strain, SS8 of Acetobacter malorum. This vinegar mother was used to carry out the acetification process, which was performed in different steps, increasing the volume by strawberry wine addition when the titratable acidity reached 3% (w/v), till a final volume of 45 L. The final yield of acetic acid was 6.3% (w/v) after 44 days. The inoculated AAB strain was molecularly identified at the beginning of the process; however, two different genotypes (Ap1 and Ap2) of Acetobacter pasteurianus appeared in the middle of the process and one of them (Ap2) took over the acetification. Results showed that the use of selected microbiota from strawberry allowed a successful process. Despite the AAB inoculation was not as expected, it allowed to shorten the length of the process.

Keywords: wine, Saccharomyces cerevisiae, Acetobacter malorum, starter cultures.

S4-P-05
KINETIC MODELLING OF ACETIC FERMENTATION OF ONION JUICE BY GENETIC ALGORITHMS
J.M. González-Sáiz,1 C. Pizarro,1 I. Esteban-Diez,1 N. Pérez del Notario,1 A. Sáenz-Mateo1
1Department of Chemistry, University of La Rioja, C/Madre de Dios 51, 26006 Logroño (La Rioja), Spain.
*Corresponding author: josemaria.gonzalez@unirioja.es Phone: +34 941299634.

Wastes and by-products of the onion-processing industry pose an increasing disposal and environmental problem and represent a loss of valuable sources of nutrients. Recycling, reprocessing and alternative utilization of onion processing residues offer potential of converting the waste materials into useful products of added value. The overall purpose of the study was to examine the feasibility of onion waste as a support-substrate for the profitable production of vinegar. The basic tool to simulate the evolution of a bioprocess is the kinetic model. The model must be simple and have a high predictive ability to give results capable of explaining the real behaviour. The difficulties in the kinetic modelling of biological processes are mainly related to the description of the bacterial growth. In this work, a genetic algorithm is designed to obtain a set of kinetic parameters for the specific growth rate that enables the model to explain the industrial fermentation. Only acetic concentration data from pilot fermentator are required. The codification of the different kinetic parameters that form a chromosome in a binary one, the response chosen and the out-of-range parameters generated during the execution of the GA are critical points in the algorithm. A four-composed desirability function was developed as the evaluation function to minimized.

Keywords: acetic fermentation, onion juice, kinetic model, genetic algorithm.

S4-P-06
OXIDATION OF SUGARS AND POLYALCOHOLS BY ACETIC ACID BACTERIA DURING SURFACE CULTURE FERMENTATIONS
P. Giudici,1* M. Gullo,1 L. De Vero1
1Department of Agricultural and Food Sciences (DipSAA), University of Modena and Reggio Emilia, via Amendola 2 (Padiglione Besta), 42122 Reggio Emilia, Italy.
*Corresponding author: paolo.giudici@unimore.it Phone: +39 0522522034.

Acetic Acid Bacteria (AAB) are well known for their ability to oxidize alcohols, aldehydes, sugars, polyalcohols and others molecules with ketonic or aldehydic functional groups. Species of Acetobacter and Gluconobacter lack a functional Embden-Meyerhof-Parnas pathway and are unable to metabolize hexose sugars by this route. Hexose and pentose sugars are oxidatively metabolized by the hexose monophosphate pathway to acetic and lactic acids. In some cases, hexose sugars may be directly oxidized to gluconate and ketoglucuronates without further catabolism, leading to an accumulation of these end products in the culture medium. Furthermore, Gluconobacter oxydans has been used to oxidize various sugars and sugar alcohols to substances of industrial significance, such as sorbose, gluconic acid and ketoglucuronic acids. Dihydroxyacetone, 2,3-butanediol, and acetoin are also significant products of carbohydrate metabolism evolved by AAB.

Our trials of surface static fermentation was carried out on complex media such as base wine (BW) for Traditional Balsamic Vinegar, where sugars and alcohols are present in different amount. In this conditions AAB sequentially oxidized the several carbon sources, first ethanol, then glucose and glycerol. In particular the oxidation of glucose to gluconate occurred when the ethanol was exhausted, then high amount of gluconate was accumulated in the medium. BW had an initial composition of around 7% (v/v) of ethanol and 25% of sugars with glucose and fructose in the ratio 1/1. At the end of the fermentation the glucose/fructose ratio was in favour of fructose, titratable acidity was very high due to the gluconate formed, while volatile acidity decreased. In summary, when surface fermentation is extended afterward the ethanol run out, other substrates are oxidized and give origin to a vinegar with a completely different composition. The extension of the oxidative step is a tool for increasing vinegar’s sweetness and acidity without the acetic acid sensorial pungency.

Keywords: gluconic acid, sugar oxidation, surface static acetification.
**S4-P-07**

**POLYNOMIAL MODELLING FOR THE OPTIMIZATION OF ACETIC ACID WINE FERMENTATION IN A FED-BATCH PILOT FERMENTER**


1Department of Chemical Engineering, Marie Curie Building, University of Córdoba, Spain; 2Department of Computing and Numerical Analysis, Leonardo da Vinci Building, University of Córdoba, Spain; 3Department of Chemical Engineering, University of Cádiz, Spain.

*Corresponding author: ines.santos@uco.es
Phone: +34 957218586.

The operational complexity in biological industries demands for models which facilitate the optimization and control of their processes. Throughout the years many models, particularly deterministic semi-structured ones, have been proposed to describe experimental evidence on the influence of diverse variables of the process. However, such a modelling approach makes difficult to succeed because of the complex interrelations between the different variables as well as the arisen mathematical problems. Indeed, this type of biotechnological models are difficult to solve because of the high-order non-linear multi-parametric equations systems as well as the variation in experimental data, both hinder the estimation of the system parameters. When this model-based approach is followed, one of the first problems to solve is then ascertain whether the parameters concerned can be unambiguously determined; this is a very complex analysis known as study of “identifiability” that rarely gives a positive answer for biological models. In this way, no meaningful physical conclusions can be drawn from the parameters – main advantage for these models. For these reasons, different alternatives are considered in order to modelling the process at the same time that the minimization of the costly experimental work is looked for. Among these methods, stands out the response surface methodology by quadratic models. This alternative has been successfully used for the optimization of fermentation processes.

In this work, a 8 L Frings pilot fermenter working in fed-batch mode has been used for studying the influence of the main operational variables: ethanol concentration at unloading volume (E), ranging between 0.5-3.5% v/v, unloaded volume (V), ranging between 25-75% of total volume and loading flow rate (R), ranging between 0.01-0.06 mL·min⁻¹. As objective functions, mean acetification rate (A) and productivity (P) have been evaluated. After using a second order polynomial and a Doehlert design for three variables, next conclusions can be drawn: both acetification rate and productivity are mainly depending on E and V, their maximum values are reached for low values of V and high ones for E.

**Keywords:** wine vinegar, acetification.

**S4-P-08**

**PRELIMINARY EVALUATION OF HONEY VINEGAR PRODUCTION IN A SUBMERGED CULTURE FED-BATCH PILOT FERMENTER**


1Department of Chemical Engineering, Marie Curie Building, University of Córdoba, Spain; 2Department of Biochemistry, Severo Ochoa Building, University of Córdoba, Spain; 3Department of Zoology, Charles Darwin Building, University of Córdoba, Spain.

*Corresponding author: b42carom@uco.es
Phone: +34 957218586.

The honey is an important product for the European Union (EU) from both the consumption and production points of view. Approximately, EU consumes 22% of the world’s annual production and its own production accounts for 13% of the global one, but in any case, only produces around half the honey it consumes. Inside EU the leading markets are: Germany, Spain, UK, France, Italy and The Netherlands, but the main producer is Spain. Among the world’s leading producers, Spain is in the thirteenth place. Though with large fluctuations, Spanish honey production has increased in roughly a 30% since 1990, amounting to 32 thousand tonnes in 2009 (FAOSTAT). Then, the previous figures, beside the fact that honey is a natural product which complies with many food trends such as health, taste and convenience, make honey and its by-products an important issue for this country.

Though an estimated 85% of all honey is for direct consumption, other uses for the rest, such as raw material for mead and subsequent biotransformation, could be profitable alternatives for using the honey which does not comply with the legislative requirements for a direct use. For instant, honey containing a Hydroxymethylfurfural (HMF) concentration higher than 40 mg/kg cannot be marketed for direct consumption, only industrial use is allowed. At this point, a study on different options to appreciate as much as possible this “residual” honey seems to be quite interesting. Honey vinegar might stand out among the possible alternatives. This condiment could have a high added value beside many of the desired features found in the original honey. In this work the behaviour of previously produced mead [9.4±0.2%(v/v) ethanol concentration] has been studied as a raw medium for vinegar elaboration; a fed-batch process in submerged culture similar to that used in other industrial types of vinegars has been followed: 31°C, aeration rate of 7.5 L·L⁻¹·h⁻¹, unloading 50% of total volume at the end of each cycle when ethanol concentration reached a value of 1%(v/v) and a loading stage to the final working volume (8 L) without exceeding a preset ethanol concentration [4% (v/v)] at any time. The low nitrogen content in honey entails the addition of some nitrogen sources previously the alcoholic fermentation stage, apart from that the mead is easily produced. The acetification results show that an stable process can be obtained with an acetification rate of 0.15±0.01%(v/v)·h⁻¹ and a productivity of 10.3±0.2 g acetic acid·h⁻¹, further optimization could improve these figures.

**Keywords:** honey, mead, acetification, honey vinegar.

*Acetic Acid Bacteria 2012; 1 (s1) | 29 |
**S4-P-09**

**PRODUCTION OF STRAWBERRY VINEGAR AS A BASE PRODUCT IN THE DEVELOPMENT OF NEW HEALTHY BEVERAGES**

A.M. Cañete-Rodríguez,1 I.M. Santos-Dueñas,1 J.L. Bonilla-Venceslada,1 J.E. Jiménez-Hornero,2 I. García-García1*

1Department of Chemical Engineering, Marie Curie Building, University of Córdoba, Spain; 2Department of Computing and Numerical Analysis, Leonardo da Vinci Building, University of Córdoba, Spain.

*Corresponding author: isidoro.garcia@uco.es
Phone: +34 957218589.

Keywords: strawberry, vinegar, submerged culture, new beverages.

Modern societies are not only concerned with the production of high quality traditional foods but with the development of new healthy products. On the other hand, many developed countries have important levels of different foods productions as well as very restrictive regulations for obtaining prime commercial quality. Then, important amounts of many products can be qualified as lower quality category, increasing in this way, the risk of becoming wastes, an example is strawberry. Spain is the third main strawberry producer in the world after United States and Turkey (FAO-STAT, 2009); an estimation of 20% of the whole production can be lost unless used as raw material for other uses despite of being a percentage of healthy and nutritionally valuable fruit. The development of strawberry based new fermented beverages is an interesting alternative for using these leftovers with benefits from the commercial and nutritional points of view. The oxidation capacity of acetic acid bacteria on different type of substrates make them key microorganisms for taking advantage of this surplus fruit. In this work, some basic aspects regarding the production in submerged culture of strawberry vinegar are considered; this vinegar would be mixed with other strawberry fermented products for obtaining innovative beverages.

The main aim is evaluate, at bench scale, the possibilities of developing the two staged bio-transformation process: alcoholic fermentation followed of aceticification. Different concentrated strawberry pulps with total sugars concentrations ranging between 30 - 100 g L⁻¹ are used. Preliminary studies show that a concentrated pulp with a sugar concentration around 55 g L⁻¹ may yields a final acetic acid concentration (25 g L⁻¹) suitable for the subsequent mixtures to prepare; additionally, its viscosity and emulsifying features can be properly dealt with inside the bioreactor. The alcoholic fermentation by *Saccharomyces cerevisiae* yields 25 g of ethanol L⁻¹ approximately in less than 24 hours. Once finished the alcoholic fermentation, the conversion of ethanol into acetic acid was carried out in the same bioreactor; an inoculum from a Frings acetaor producing alcohol vinegar supplied a fully active mixture of acetic acid bacteria for the process. Though a lag phase is observed, this procedure guarantees that in less than 70 hours all the ethanol is eliminated. In collaboration with other research groups, the behaviour of inoculating specific acetic acid bacteria strains is being studied.

**Keywords**: strawberry, vinegar, submerged culture, new beverages.

---

**S4-P-10**

**SMALL SCALE CULTIVATION OF ACETIC ACID BACTERIA FOR VINEGAR PRODUCTION**

T. Schlepütz,1 J. Büchs1

1Aachener Verfahrenstechnik - Biochemical Engineering, RWTH Aachen University, Germany.

*Corresponding author: tino.schlepuetz@avt.rwth-aachen.de
Phone: +49 02418027179.

Fermentations for the production of vinegar by acetic acid bacteria are typically initiated with inoculation vinegar - undefined residues of former fermentations. These bacteria are obligatory aerobic and have to survive at high concentrations of ethanol and acetic acid in the vinegar production. Already short interruptions of the oxygen supply lead to severe harm of the bacteria in submerged fermentation. Hence, typical inoculation cascades are not possible with these bacteria, so that vinegar is industrially produced in repeated batch processes. Furthermore, isolation and preservation of acetic acid bacteria from vinegar is only possible with much effort in diluted media. Accordingly, studies on the production of vinegar are generally done in fully instrumented lab scale bioreactors. This hampers efficiency and throughput of investigations on that process.

In this contribution we present the scale down of the fermentation for spirit vinegar production into milli- and microliter scale shaken cultures. Realizing a high degree of parallelization, less workload and high throughput are achieved. Typical inoculation procedures of shake flask cultures by pipetting result in lack of reproducibility of the bacterial activities. This was demonstrated by respiration measurements in a RAMOS® device. Good reproducibility of respiration curves was achieved with an aerated transport of the inoculum by a bubble column during the inoculation of the shaken cultures.

Vinegar fermentation was examined in microtiter plates. The extraordinary low aeration rates of conventional bioreactors for vinegar production were mimicked by a customized lid. The design of that lid was guided by a diffusion model. The fermentations were then monitored using microtiter plates with optodes for dissolved oxygen tension and the BioLector® device.

Finally, a method for the repeated batch operation in special shake flasks of the COSBIOS® device was developed. These flasks are characterized by a precise outlet at the reactor wall so that in shaken operation culture broth can leave the reactor if fresh medium is supplied. A flushing method was applied which matched the draining and filling phase of repeated batch fermentations in conventional bioreactors. With the help of semiconductor ethanol sensors in the head space of the COSBIOS® reactors to monitor the fermentation and to initiate the medium exchange parallel repeated batch fermentation for the production of vinegar could be established.

**Keywords**: small scale cultivation, microtiter plate, shake flask, vinegar, parallelization, repeated batch.
S4-P-11
STUDY OF THE VOLATILE AND POLYPHENOLIC CONTENT OF A SHERRY WINE VINEGAR ENRICHED WITH DIETARY FIBRE
A. Marrufo,1 E. Durán,1 M.J. Cejudo,1 R. Castro,1* R. Natera,1 C.G. Barroso1
1Analytical Chemistry Department, Faculty of Sciences-CAIV, University of Cádiz, Agrifood Campus of International Excellence, Spain.
*Corresponding author: remedios.castro@uca.es Phone: +34 956016456.

Nowadays, the beverage market is turning towards diversification of traditional beverage categories, looking for different and improved sensory properties in relation to the conventional products. Moreover, the development of new products with functional properties is becoming an incentive to raise consumers’ consciousness about their consumption. In this context, several experiences of enrichment of a Sherry wine vinegar with dietary fibre from orange and lemon have been carried out. Different doses of lemon and orange fibre have been studied (0-10 g/L). Two storage conditions were studied: room temperature (25°C) and 4°C. Polyphenols were analysed by UPLC-DAD whereas volatile compounds were determined by SBSE-GC-MS. Both methodologies had been previously optimized and validated. Enriched vinegars exhibited higher polyphenolic contents. Naringin, narirutin and neohesperidin were only found in the enriched vinegars. For volatile compounds, some new compounds were also found in the enriched samples, standing out valencene and limonene for vinegars enriched with orange and lemon fibres, respectively. Both facts influenced positively on the final quality of new products.

Keywords: sherry vinegar, dietary fibre, polyphenols, volatile compounds, new products.

S4-P-12
SUBMERGED CULTURES OF GLUCONOBACTER GENUS IN STRAWBERRY PULP FOR MAKING A GLUCONIC ACID BASED PRODUCT. AN INGREDIENT FOR NEW HEALTHY BEVERAGES
A.M. Cañete-Rodríguez,1 I.M. Santos-Dueñas,1 J.C. Mauricio,2 C. Martínez-Pedrajas,1 I. García-García1*
1Department of Chemical Engineering, Marie Curie Building, University of Córdoba, Spain; 2Department of Microbiology, Severo Ochoa Building, University of Córdoba, Spain.
*Corresponding author: isidoro.garcia@uco.es Phone: +34 957218589.

In the scope of the development of new healthy foods at the same time that a proper use of the overproduction of some fruits is made, in this work, a study about the production of gluconic acid from concentrated strawberry pulp in submerged culture is carried out.

The resulting product would be used as a base product for mixing with other fermented products into new potentially healthy beverages. In other communication (Production of strawberry vinegar as a base product...) authors refer to the production of strawberry vinegar with the same aim. The whole project is the resulting effort with two additional complementary research groups (Microbiology: Group Oenological Biotechnology, University Rovira i Virgili - URV; and Chemical and Sensory Analysis: Group AGR-167, University of Sevilla; both in Spain). The project looks for a new beverage with all the original health properties from the strawberry and a complex equilibrium between acetic and gluconic acids as well as a natural sweetness from residual fructose during the gluconic acid fermentation. Then, the biotransformation by a microorganism that uses mainly glucose and no fructose is a key issue.

In this work, the behaviour in a 5 L fermenter (Sartorious) of two Gluconobacter japonicus strains (F9 and E1) selected by the URV Group, is studied. Taking into account the final intended use as well as that the strawberry pulp was pasteurised during the production process, no sterilization operation was carried out; then, contamination by other microorganisms, particularly yeast, must be checked. An adequate pulp concentrate must be used in order to avoid emulsion problems if no pectolytic enzyme preparation is added. So, the proper strawberry pulp has a total sugars content of 55 g L-1 approximately. Previous results show that both strains produce a gluconic acid concentration of roughly 20 g L-1 in 26 hours. During this period also fructose consumption is observed but this is lower for the strain E1, additionally, no yeast growth was observed until the glucose and most of the fructose are exhausted. For the strain F9, final ethanol concentrations around 1% (v/v) were founds, this suggests some yeast activity which is confirmed by the increasing in yeast cell concentration.

The obtained results for strain E1 suggest that this microorganism might be able to use only glucose. The effect of several operational variables must be studied in order to find the optima conditions for a selective use of glucose.

Keywords: strawberry, gluconic acid, submerged culture, new beverages.

S4-P-13
USING A SERIES OF TWO FED-BATCH PILOT FERMENTERS FOR OPTIMIZING THE WINE VINEGAR PRODUCTION PROCESS
C. Álvarez-Cáliz,1 I.M. Santos-Dueñas,1 A.M. Cañete-Rodríguez,1 M.C. Millán-Perez,2 J.L. Bonilla-Venceslada,1 I. García-García1
1Department of Chemical Engineering, Marie Curie Building, University of Córdoba, Spain; 2Department of Microbiology, Severo Ochoa Building, University of Córdoba, Spain.
*Corresponding author: q42alcac@uco.es Phone: +34 957218586.

In the scope of the optimization of the wine vinegar pro-
duction, the authors have carried out a vast experimental work previously to several modelling approaches aimed to maximise acetification rate as well as productivity. If just one fermenter is used, simulations by different models coincide in the impossibility of getting high rates and productivities values when a maximum use of substrate is also looked for. Since, for many industries, the proper use of the substrate as well as obtaining high productivities are issues of great importance from an economical point of view, it would be desirable to find an operational mode able to achieve both objectives simultaneously. Among the possible alternatives, stands out the use of a series of two fermenters by which the second one could ensures the ethanol exhaustion without hindering the possibility of reaching a global high productivity. Indeed, meanwhile the operational variables in the first fermenter would offer a proper environment for bacteria to work in optimal conditions, the unloaded medium - containing an important ethanol concentration, will be depleted in the second one.

The independent operational variables to be considered in the first fermenter are: maximum ethanol concentration to be reached at any time (Ec1), ethanol concentration at unloading time (Ed1), unloaded volume (Vd1) and working temperature (T1). On the other side, for the second fermenter the variables are: maximum ethanol concentration to be reached at any time (Ec2) and temperature (T2).

The number of operational variables involved in each fermenter as well as the relationship among some of them because of the physical connection between both bioreactors, entail a complex modelling approach for allowing the optima conditions to be found. In this case, a response surface methodology by quadratic models and a fractional Doehlert design for six variables have been used. Setting the ranges of variation for Ec1, Ed1, Vd1 and Ec2 involve a previous complex analysis the result of which is: Ec1 [6-4%(v/v)], Ed1 [2-4%(v/v)], Vd1 [4.25-5.75 L] and Ec2 [2.5-4.5%(v/v)]. Additionally, T1 and T2 ranged between 28-32°C.

The model predictions suggest that maximum productivity is obtained when low and high temperatures are set in the first and second fermenter respectively; additionally, low values of Vd1 and high ones for Ed1, Ec1 and Ec2 must simultaneously be used.

Keywords: wine vinegar, acetification.

### SESSION V. How do we know what they are up to? Measurement techniques. Biosensors. Quality control. Other aspects.

#### S5-KN

**CHEMICAL CHARACTERIZATION AND HEALTH CLAIMS AND AAB FERMENTED PRODUCTS**

M.C. García-Parrilla,¹ A.B. Cerezo,¹ W.M. Tesfaye,² A.M. Troncoso-González³

¹Nutrition and Food Science, Faculty of Pharmacy, University de Sevilla, Spain; ²Food Technology, Politechnical University of Madrid, Spain.

*Corresponding author: mcparrilla@us.es
Phone: +34 954556760.

The diversity of fermented products is increasing to satisfy the requirements in the market. The innovations focus on the development of vinegars with high sensorial quality. Additionally to those vinegars protected by a Denomination of Origin, the challenge is to offer original vinegars elaborated from uncommon substrates as honey, strawberries or other fruits.

Chemical characterization is a powerful tool to establish quality control. It permits to discriminate vinegars for authentication purposes, useful for the Denominations of Origin. The elaboration processes (fermentation or aging) play a crucial role on chemical composition. New strategies of production must consider these aspects to ensure high quality products. More recently, health claims of food or food ingredients is an outstanding topic in the EU. Therefore, the assessment of bioactive compounds is a new feature required.

This communication presents more relevant results of the research group in the authentication of vinegars differentiating products by the substrate, elaboration method involved or aging. Special attention was devoted to Sherry vinegars. Additionally more recent work aims to study innovative vinegars elaborated in acacia, oak, cherry or chestnut woods thanks to the Winegar European Project. Polyphenolic compounds are a useful tool to characterize vinegars. Criteria to discriminate these products according to their production area, fermentation process or aging period were achieved. The identification of polyphenolic compounds with antioxidant properties as (+)-taxifolin or dihydrorobinetin is also presented.

Keywords: vinegar, polyphenol, aging, wood, authentication.

#### S5-O-01

**USE OF DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR RAPID DETECTION AND IDENTIFICATION OF ACETIC ACID BACTERIA**

N. Sagarzazu,¹ M. Martinez,¹ R. Virto,¹ C. Gonzalez¹

| 32 | Acetic Acid Bacteria 2012; 1 (s1) |
Modern vinegar production is carried out using a standard and automated process technology. However, the microbiology of vinegar fermentation is not adequately developed with regard to a thorough knowledge of the taxonomic-systematic position of the organisms involved, their response to ecological factors, their physiology and genetics. The lack of defined pure cultures is due to problems in strain isolation, cultivation and preservation of the vinegar bacteria. Numerous strategies have been used for microbial community analysis, including traditional culture methods, microscopic techniques and nucleic-acid based methods. The identification methods based on analysis of the phenotypic characteristics of the acetic acid bacteria, are not only inaccurate, but also very time-consuming. Therefore, the application of molecular methods, based on the identification/characterization of specific DNA segments, could be a proper solution for the quick and accurate identification of these microorganisms. Denaturing high-performance liquid chromatography (DHPLC) is a new and promising approach for bacterial community analysis, monitoring and identification. This technique has primarily used to detect DNA sequence variations such as insertions, deletions and SNPs in various applications such as clinical diagnostics, gene cloning and microbial investigations. The major advantages of the DHPLC method for community analysis compared with other molecular-based techniques include the use of automated instrumentation, high sensitivity and less laborious and time-consuming operations. DHPLC is used in this study to discriminate genetic differences in the 16S rRNA and Adh (Alcohol dehydrogenase) genes among bacterial species based on its efficiency and sensitivity to enable the detection and discrimination of different genetic sequences. To optimize DHPLC protocols for the analysis of 16S rRNA gene fragments amplified we analyzed DNA isolated from 10 different reference strains representing main bacterial groups of interest (pathogens and spoilage microorganisms, lactic and acetic acid bacteria). Variations of technical DHPLC parameters (acetonitrile gradient, column temperature and flow rate) resulted in a protocol that allowed effective and reproducible separation. For the discrimination of different strains of Acetobacter malorum (DSM 14337 as reference strain and two wild strains) the Adh gene were used.

Keywords: denaturing high performance liquid chromatography (DHPLC), acetic acid bacteria identification, vinegar fermentation.

**S5-O-02**

**SENSORY ANALYSIS OF SHERRY VINEGARS ENRICHED WITH DIETARY FIBRE**

A. Marrufo, M.C. Rodríguez, E. Durán, M.J. Cejudo, R. Castro, R. Natera, C.G. Barroso

Abstracts

Nowadays, oenological industry is turning to the development of new products with healthy benefits due to the increasing consumers’ demand. In addition, these new products should present positive organoleptic properties in order to assure the acceptance from consumers. Therefore sensory analysis of new products is an essential step prior to their possible commercialisation. In this research, a new type of Sherry vinegar enriched with different dietary fibres (orange and lemon) has been developed and sensory analysed. Different amounts of fibre (0-10 g/L) and storage temperatures (25°C and 4°C) have been studied, and an overall olfactory and gustatory analysis of the vinegars enriched with fibre has been carried out. Moreover, the effect of storage temperature, type of added fibre and amount of fibre on sensory characteristics of the developed vinegars has been studied. The addition of fibre to the vinegars increased both olfactory and gustatory impressions, so enriched vinegars presented better sensory characteristics. No preference between both types of fibres was observed. Neither storage temperature was a significant parameter on sensory preference, so the cheapest storage should be preferred, i.e. storage at room temperature. In relation to amount of fibre, 0.5 g/L for lemon and 5 g/L for orange fibre were the optimal values taking into account olfactory and gustatory impression.

Keywords: sherry vinegar, dietary fibre, sensory analysis, new products.

**S5-O-03**

**CHANGES IN AMINO ACIDS CONTENT DURING THE PRODUCTION OF STRAWBERRY AND PERSIMMON VINEGARS**


Abstracts

The major amino acids found in strawberry puree were bamate (AQC) as precolumn derivatization reagent. high-performance liquid chromatography (HPLC), employment 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) as precolumn derivatization reagent. The major amino acids found in strawberry puree were
asparagine, glutamine and proline, while in persimmon were -aminobutyric acid, glutamine, threonine and tyrosine. Although some amino acids increased during the acetifications, many of them were consumed by acetic bacteria. Some differences between inoculated and spontaneous acetifications were observed with respect to the most consumed amino acids, which were, in most cases, the most abundant in wine substrate.

GABA and Pro were the major amino acids in final persimmon vinegars. However, there were differences regarding the major ones in the strawberry vinegars. Hence, GABA and Pro were the most abundant compounds in 2008 strawberry vinegars obtained from inoculated wines, Cys in those from spontaneous wines, and Arg in strawberry vinegars from 2009 and 2010 harvests. The Principal Component Analysis reveals that samples of the same harvest can be separated in substrates, wines and final vinegars by using amino acid compounds as variables. In addition, this statistic analysis allowed us to separate the final vinegars according to the type of fruit, year of harvest and fresh fruit or commercial puree.

Keywords: amino acid, 6-aminooquinolyl-N-hydroxysuccinimidyl carbamate, strawberry vinegar, persimmon vinegar, acetification.

S5-P-02
DETERMINATION OF BIOGENIC AMINES IN VINEGARS USING SOLID-PHASE EXTRACTION AND 6-AMINOQUINOLYL-N-HYDROXYSUCCINIMIDYL CARBAMATE AS DERIVATIZING AGENT
J.L. Ordoñez,1* R.M. Callejón,1 M.L. Morales,1 M.C. García-Parrilla1
*Corresponding author: josordducia@alum.us.es Phone: +34 954556761.

Biogenic amines ingested in high amounts can have toxicological effects and can cause problems in consumers. These have been reported in a wide range of food products including wines, but the determination of biogenic amines has not been deeply studied in vinegars. In this study, main amines were determined by high-performance liquid chromatography (HPLC) after derivatization with 6-aminooquinolyl-N-hydroxysuccinimidyl carbamate (AQC) in different types of vinegars: balsamic, apple, and red, white, and Sherry wine vinegars. A solid-phase extraction (SPE) with mixed-mode resins method was used before analysis. The total content of biogenic amines in vinegars ranged from 0.023 mg/L to 1.445 mg/L, being lower than those found in wines. Although Cadaverine was present in a higher number of samples, Putrescine followed by Histamine were the amines that showed highest concentrations, reaching values up to 0.525 and 0.309 mg/L, respectively. However, methylamine and phenylethylamine were not determined in any vinegar. Balsamic and “Pedro Ximenez” Sherry vinegars reached the highest amounts of biogenic amines. On the other hand, apple, white and Sherry wine vinegars had the lowest concentrations. Principal component analysis using the biogenic amines as variables, allowed to separate the different kind of vinegars, excepting red vinegars.

Keywords: HPLC, AQC, solid-phase extraction, biogenic amines, vinegar.

S5-P-03
EFFECTS OF SACCHARIFICATION METHODS ON THE ORGANOLEPTIC PROPERTIES OF RICE VINEGAR
A. Mori,1* S. Shiratori,2 N. Nakamura3

Biogenic amines ingestion in high amounts can have toxicological effects and can cause problems in consumers. These have been reported in a wide range of food products including wines, but the determination of biogenic amines has not been deeply studied in vinegars. In this study, main amines were determined by high-performance liquid chromatography (HPLC) after derivatization with 6-aminooquinolyl-N-hydroxysuccinimidyl carbamate (AQC) in different types of vinegars: balsamic, apple, and red, white, and Sherry wine vinegars. A solid-phase extraction (SPE) with mixed-mode resins method was used before analysis. The total content of biogenic amines in vinegars ranged from 0.023 mg/L to 1.445 mg/L, being lower than those found in wines. Although Cadaverine was present in a higher number of samples, Putrescine followed by Histamine were the amines that showed highest concentrations, reaching values up to 0.525 and 0.309 mg/L, respectively. However, methylamine and phenylethylamine were not determined in any vinegar. Balsamic and “Pedro Ximenez” Sherry vinegars reached the highest amounts of biogenic amines. On the other hand, apple, white and Sherry wine vinegars had the lowest concentrations. Principal component analysis using the biogenic amines as variables, allowed to separate the different kind of vinegars, excepting red vinegars.

Keywords: HPLC, AQC, solid-phase extraction, biogenic amines, vinegar.
Vinegar is one of oldest brewed seasonings from racial favorite drinks. Rice vinegar is made from Japanese sake in Japan and its saccharification is used koji mold or enzyme (amylase). We wished to find out difference of taste among the effects of the saccharification methods, by chemical analyses and organoleptic tests.

Rice vinegar used in these tests were acetified with Acetobacter aceti IFO 3283 strain. Analytical methods used were chemical analyses [total acid, nonvolatile acid, ethyl alcohol, direct producing sugar, soluble solid, protein, folmol nitrogen, total nitrogen, pH, optical density, common salt, total phosphorus], organic acid (by HPLC) flavoring substance (by GC), and organoleptic tests namely pungent, thickness (strong or weak), refreshing, sweetness, sourness, synthetic favorite.

Multiple regression equation was gained. Level of significance was less than 1%, R^2 was 93%, analysis of variance was less than 1%. Results of factor analysis among difference of saccharifying material gave little effects. As the results, difference of saccharification method gave significant effects.

Keywords: Acetobacter aceti, organoleptic test, rice vinegar, saccharification.

**S5-P-04**

**EVALUATION OF BIOGENIC AMINES IN PRODUCTS OBTAINED BY ALCOHOLIC AND GLUCONIC FERMENTATION OF STRAWBERRIES**

J.L. Ordóñez,1* M.A. Álvarez-Fernández,1 R.M. Callejón,1 M.L. Morales,1 M.C. García-Parrilla1


*Corresponding author: josoraldia@alum.us.es Phone: +34 954556761.

Biogenic amines were determined in different alcoholic and gluconic fermentations of strawberries purees by high-performance liquid chromatography (HPLC) using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) as derivatization agent. A solid-phase extraction (SPE) with mixed-mode resins method was used before analysis. This resin is composed of reverse-phase (C18) and ion exchange sorbents (sulfonic groups). The method was successfully validated obtaining adequate values of selectivity, response linearity, precision, accuracy, and low detection and quantification limits. The total content of biogenic amines in samples ranged from 0.115 mg/L to 0.237 mg/L, being lower than those found in others fermentation products. Most of biogenic amines such as histamine, agmatine, methyamine, tyramine, phenylethylamine and spermidine were not detected in any alcoholic and gluconic products. Only spermine was present in all samples reaching the highest concentrations. Some differences were observed in the gluconic products. Hence cadaverine was only present in those samples fermented by aceto bacter strains.

Keywords: HPLC, AQC, solid-phase extraction, biogenic amines, strawberry.

**S5-P-05**

**STUDY OF THE VOLATILE PROFILE OF HIGH QUALITY VINEGARS BY STIR BAR SORPTIVE EXTRACTION**

A. Marrufo,1 M.J. Cejudo,1 E. Durán,1 R. Castro,1 R. Natera,1* F. Chimici,2 C.G. Barroso1

1Analytical Chemistry Department, Faculty of Sciences-CAIV. University of Cádiz, Agrifood Campus of International Excellence, Post Office Box 40, Pol. Río San Pedro. Puerto Real 11510, Cádiz, Spain; 2Food Science Department, University of Bologna, Viale Fanin 40, Bologna 40127, Italy.

*Corresponding author: ramon.natera@uca.es Phone: +34 686121125.

A stir bar sorptive extraction gas chromatography-mass spectrometry (SBSE-GC-MS) method has been used for the characterization of the volatile composition of 26 high quality vinegars of three different protected geographical indications (traditional balsamic vinegar of Modena, balsamic vinegar of Modena, and Sherry vinegar).

In this research work, a total of 113 individual volatile compounds have been identified in different vinegars by SBSE-GC-MS. Some of the studied compounds have been previously identified in wine but 39 tentatively identified volatile compounds have not been previously reported in vinegars from this origin.

From the results obtained, it can be seen that the numerous volatile compounds identified in vinegar samples have allowed an optimal differentiation of the vinegars studied on the basis of their raw material, aging time and aging type. So, a close relationship can be established between these last ones and the volatile content of vinegar, which undergoes significant changes during the aging in wood. The analytical parameters selected are suitable descriptors to differentiate vinegar samples according to the raw material employed and the aging in wood. Furanes and terpenes together with acids and alkanes were the most discriminant variables for raw material and aging time, respectively; whereas for aging type, these ones were acetates and esters.

Keywords: traditional balsamic vinegar of Modena, balsamic vinegar of Modena, sherry vinegar, volatile compounds, SBSE.

**S5-P-06**

**VINEGAR CLASSIFICATION BASED ON UV-VIS AND NIR DATA FUSION AND VARIABLE SELECTION**

J.M. González-Sáiz,1* I. Esteban-Díez,1 A. Sáenz-Mateo,1 C. Pizarro1

Acetic Acid Bacteria 2012; 1 (s1) | 35 |
Quality control and food authentication are tasks of great importance to increase consumer awareness of food safety issues, to protect genuine food processors and to fight frauds and unfair competition. In particular, considering the great diversity of vinegars available nowadays on the market and the differences in prices depending on the declared quality of the products, fast and reliable methods of quality assurance are needed. In this context, the use of spectroscopic fingerprints has gained attention in last years as an effective approach for authentication purposes. However, the discrimination of different types of vinegar is still a complex problem and data fusion from different spectroscopic techniques could help to improve classification performance.

The aim of this study was to develop a class model to discriminate between vinegar samples (n=31) belonging to 4 categories (cider, Sherry, red wine and white wine vinegar), using chemometrics to combine the information from multivariate and unspecific data obtained by UV-Visible and NIR spectroscopy. The wavelength ranges 400-700 nm, and 1100-1880 and 2080-2300 nm were used as the working regions for UV-Visible and NIR spectra, respectively. Several pre-processing methods were applied to data to try to minimise physical effects on spectra. The first derivatives of the UV-Vis spectra and the SNV NIR spectra were selected for use in the subsequent analyses.

As a first step, the SIMCA method was applied to develop a model for each data set separately, using 10 cross-validation groups for internal prediction, 2 PCs for the inner space of each class and separate category centring as scaling option (full spectrum approaches). Next, the two data sets were joined (low level data fusion approach) and SELECT, as feature selection technique, was applied to extract a subset of discriminant variables. To ensure a suitable ratio of samples to variables and to obtain a reliable model, only 5 variables were retained. Finally, SIMCA was performed using the 5 selected and autoscaled variables. The results obtained by data fusion were compared with those obtained using the two data sets separately. The adoption of a data fusion approach together with the application of a variable selection method provided improved results, particularly in terms of prediction ability and model sensitivity.

<table>
<thead>
<tr>
<th>Data set</th>
<th>Variables</th>
<th>% Class. (10 CV)</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNV NIR</td>
<td>502</td>
<td>100</td>
<td>80.7</td>
<td>67.7</td>
</tr>
<tr>
<td>1st Der. UV-Vis</td>
<td>301</td>
<td>100</td>
<td>93.6</td>
<td>61.3</td>
</tr>
<tr>
<td>Fused dataset</td>
<td>5</td>
<td>100</td>
<td>93.6</td>
<td>100</td>
</tr>
</tbody>
</table>

Keywords: chemometrics, data fusion, UV-Visible, NIR, vinegar classification.

Acetic acid bacteria (AAB) inhabit sugar-rich niche, especially fruits and flowers, and thus to have an ability to utilize sugars or sugar alcohols for their energy sources. The strategy of sugar utilization is rather unique compared with others; they use such the sugars or sugar alcohols mainly by “oxidative fermentation”, which is carried out by the respiratory chain comprising (primary) quinoprotein dehydrogenases and (terminal) ubiquinol oxidases, both located on the cytoplasmic membrane. The unique respiratory chain seems to be acquired by adaptive evolution of AAB to such the sugar-rich niche by interacting with other microbes living at the same habitat. Genetic instability of AAB, which is an ability well known for a long time, and recently has been confirmed by the genome study of *Acetobacter pasteurianus*, may contribute the prompt adaption of AAB.

Since the fermentation with AAB is usually done below or around 30°C, the optimum growth temperature, a large cooling expense is required in the industrial fermentation processes, especially nowadays by global climate warming. Thus, the development of thermotolerant AAB is expected to release us from such a strict temperature control, and thus to enable us to perform stable fermentation without serious care. We have isolated many thermotolerant AAB adapted to tropical habitat from nature, and without serious care. We have isolated many thermotolerant AAB adapted to tropical habitat from nature, and how the adaptability of AAB and comparative genomics remain to be clarified for understanding AAB physiology and how the adaptability of AAB and comparative genomics are useful for AAB research and development.

This study is partly supported by ACP program (JSPS, NRCT), PROBRAIN (BTRAI), and ALCA (JST).

**Keywords:** adaptive evolution, adaptive breeding, thermotolerance, comparative genomics, *Acetobacter pasteurianus*.
# Index of Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adachi O.</td>
<td>23</td>
</tr>
<tr>
<td>Akakabe Y.</td>
<td>23</td>
</tr>
<tr>
<td>Alfano G.</td>
<td>25</td>
</tr>
<tr>
<td>Álvarez-Cálix C.</td>
<td>31</td>
</tr>
<tr>
<td>Álvarez-Fernández M.A.</td>
<td>35</td>
</tr>
<tr>
<td>Amano S.</td>
<td>4</td>
</tr>
<tr>
<td>Anami Y.</td>
<td>19</td>
</tr>
<tr>
<td>Andrés-Barrao C.</td>
<td>7, 9, 12, 14, 15</td>
</tr>
<tr>
<td>Anton I.</td>
<td>26</td>
</tr>
<tr>
<td>Arimitsu M.</td>
<td>19</td>
</tr>
<tr>
<td>Azuma Y.</td>
<td>13</td>
</tr>
<tr>
<td>Baena-Ruano S.</td>
<td>27, 29</td>
</tr>
<tr>
<td>Barja F.</td>
<td>7, 9, 12, 14, 15</td>
</tr>
<tr>
<td>Barroso C.G.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Bartowsky E.</td>
<td>8</td>
</tr>
<tr>
<td>Becker T.</td>
<td>25</td>
</tr>
<tr>
<td>Beli C.</td>
<td>25</td>
</tr>
<tr>
<td>Benagí C.</td>
<td>7</td>
</tr>
<tr>
<td>Benito S.</td>
<td>26</td>
</tr>
<tr>
<td>Boffa M.</td>
<td>14</td>
</tr>
<tr>
<td>Bonilla-Venceslada J.L.</td>
<td>30, 31</td>
</tr>
<tr>
<td>Bott M.</td>
<td>13, 19</td>
</tr>
<tr>
<td>Bourgeois J.F.</td>
<td>2</td>
</tr>
<tr>
<td>Bravo D.</td>
<td>9</td>
</tr>
<tr>
<td>Bringer S.</td>
<td>13, 19</td>
</tr>
<tr>
<td>Büchs J.</td>
<td>30</td>
</tr>
<tr>
<td>Calderon-Copete S.P.</td>
<td>15</td>
</tr>
<tr>
<td>Callejón R.M.</td>
<td>33, 34, 35</td>
</tr>
<tr>
<td>Calo N.</td>
<td>12</td>
</tr>
<tr>
<td>Cañete-Rodríguez A.M.</td>
<td>27, 29, 30, 31</td>
</tr>
<tr>
<td>Cantero-Moreno D.</td>
<td>29</td>
</tr>
<tr>
<td>Castro R.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Cejudo M.J.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Cerezo A.B.</td>
<td>32</td>
</tr>
<tr>
<td>Chappuis M.</td>
<td>7, 9, 12, 14</td>
</tr>
<tr>
<td>Chen C.X.</td>
<td>2</td>
</tr>
<tr>
<td>Chen F.S.</td>
<td>2, 4, 10</td>
</tr>
<tr>
<td>Chen T.</td>
<td>4</td>
</tr>
<tr>
<td>Cleenwerck I.</td>
<td>6, 12</td>
</tr>
<tr>
<td>De Vero L.</td>
<td>9, 28</td>
</tr>
<tr>
<td>De Vos P.</td>
<td>6</td>
</tr>
<tr>
<td>De Vuyst L.</td>
<td>7, 17</td>
</tr>
<tr>
<td>Deppenmeier U.</td>
<td>16</td>
</tr>
<tr>
<td>Descombes P.</td>
<td>15</td>
</tr>
<tr>
<td>Doccquier M.</td>
<td>15</td>
</tr>
<tr>
<td>Durán E.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Ehrenreich A.</td>
<td>14, 15, 20</td>
</tr>
<tr>
<td>Eklu-Natey R.</td>
<td>12</td>
</tr>
<tr>
<td>Emde F.</td>
<td>22</td>
</tr>
<tr>
<td>Escamilla J.E.</td>
<td>20</td>
</tr>
<tr>
<td>Esteban-Díez I.</td>
<td>28, 35</td>
</tr>
<tr>
<td>Falquet L.</td>
<td>15</td>
</tr>
<tr>
<td>Fantuz F.</td>
<td>25</td>
</tr>
<tr>
<td>Flores-Serrano J.M.</td>
<td>29</td>
</tr>
<tr>
<td>Gao B.</td>
<td>26</td>
</tr>
<tr>
<td>García D.</td>
<td>26</td>
</tr>
<tr>
<td>García-García I.</td>
<td>2, 27, 29, 30, 31</td>
</tr>
<tr>
<td>García-Martínez T.</td>
<td>27</td>
</tr>
<tr>
<td>García-Parrilla M.C.</td>
<td>32, 34, 35</td>
</tr>
<tr>
<td>Giudici P.</td>
<td>1, 3, 5, 28</td>
</tr>
<tr>
<td>Gómez-Manzo S.</td>
<td>20</td>
</tr>
<tr>
<td>Gonzalez C.</td>
<td>32</td>
</tr>
<tr>
<td>González V.</td>
<td>3, 4</td>
</tr>
<tr>
<td>González-Sáiz J.M.</td>
<td>28, 35</td>
</tr>
<tr>
<td>Grazia L.</td>
<td>25</td>
</tr>
<tr>
<td>Guillaumón J.M.</td>
<td>13</td>
</tr>
<tr>
<td>Gullo M.</td>
<td>8, 10, 11, 18, 28</td>
</tr>
<tr>
<td>Hadano H.</td>
<td>13</td>
</tr>
<tr>
<td>Hamdi M.</td>
<td>11</td>
</tr>
<tr>
<td>He L.P.</td>
<td>26</td>
</tr>
<tr>
<td>Heredia F.</td>
<td>3</td>
</tr>
<tr>
<td>Hidalgo C.</td>
<td>27, 33</td>
</tr>
<tr>
<td>Hirakawa H.</td>
<td>13</td>
</tr>
<tr>
<td>Hours R.A.</td>
<td>23</td>
</tr>
<tr>
<td>Illeghems K.</td>
<td>7</td>
</tr>
<tr>
<td>Inagawa H.</td>
<td>4</td>
</tr>
<tr>
<td>Ishikawa M.</td>
<td>17</td>
</tr>
<tr>
<td>Ito K.</td>
<td>17</td>
</tr>
<tr>
<td>Jakob F.</td>
<td>23, 25</td>
</tr>
<tr>
<td>Jiménez-Hornero J.E.</td>
<td>27, 29, 30</td>
</tr>
<tr>
<td>Junier P.</td>
<td>9</td>
</tr>
<tr>
<td>Kallel L.</td>
<td>10, 11</td>
</tr>
<tr>
<td>Kappock T.J.</td>
<td>20</td>
</tr>
<tr>
<td>Kodama T.</td>
<td>15</td>
</tr>
<tr>
<td>Kohchi C.</td>
<td>4</td>
</tr>
<tr>
<td>Koizumi Y.</td>
<td>17</td>
</tr>
<tr>
<td>Kostner D.</td>
<td>14, 15, 20</td>
</tr>
<tr>
<td>Labbé M.</td>
<td>34</td>
</tr>
<tr>
<td>Lascheras J.</td>
<td>3, 4</td>
</tr>
<tr>
<td>Latos A.I.</td>
<td>8</td>
</tr>
<tr>
<td>Lefeber T.</td>
<td>17</td>
</tr>
<tr>
<td>Lemmetti F.</td>
<td>3, 5</td>
</tr>
<tr>
<td>León J.M.</td>
<td>3, 4</td>
</tr>
<tr>
<td>Li L.</td>
<td>2</td>
</tr>
<tr>
<td>Liebl W.</td>
<td>14, 15, 20</td>
</tr>
<tr>
<td>López F.</td>
<td>34</td>
</tr>
<tr>
<td>López I.</td>
<td>3, 4</td>
</tr>
<tr>
<td>López-Colom P.</td>
<td>34</td>
</tr>
<tr>
<td>Lustrato G.</td>
<td>25</td>
</tr>
<tr>
<td>Magliieri C.</td>
<td>25</td>
</tr>
<tr>
<td>Mamlouk D.</td>
<td>8, 1</td>
</tr>
<tr>
<td>Marrufo A.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Martínez M.</td>
<td>32</td>
</tr>
<tr>
<td>Martínez-Pedrajas C.</td>
<td>31</td>
</tr>
<tr>
<td>Mas A.</td>
<td>8, 11, 13, 21, 24, 26, 27</td>
</tr>
<tr>
<td>Mateo E.</td>
<td>8, 11, 13, 21, 24, 26, 27, 33</td>
</tr>
<tr>
<td>Giudici P.</td>
<td>12, 13, 15, 19, 22, 23, 26, 27</td>
</tr>
<tr>
<td>Matsushita K.</td>
<td>15</td>
</tr>
<tr>
<td>Matsutani M.</td>
<td>19</td>
</tr>
<tr>
<td>Mauricio J.C.</td>
<td>27, 31</td>
</tr>
<tr>
<td>Meißner D.</td>
<td>23</td>
</tr>
<tr>
<td>Mientus M.</td>
<td>14, 15, 20</td>
</tr>
<tr>
<td>Millán-Perez M.C.</td>
<td>31</td>
</tr>
<tr>
<td>Miura H.</td>
<td>19</td>
</tr>
<tr>
<td>Moens F.</td>
<td>17</td>
</tr>
<tr>
<td>Morales M.L.</td>
<td>33, 34, 35</td>
</tr>
<tr>
<td>Mori A.</td>
<td>34</td>
</tr>
<tr>
<td>Mukai H.</td>
<td>15</td>
</tr>
<tr>
<td>Müller A.H.E.</td>
<td>25</td>
</tr>
<tr>
<td>Mullins E.A.</td>
<td>20</td>
</tr>
<tr>
<td>Nagashima T.</td>
<td>4</td>
</tr>
<tr>
<td>Nakamura N.</td>
<td>34</td>
</tr>
<tr>
<td>Natera R.</td>
<td>31, 33, 35</td>
</tr>
<tr>
<td>Navarro D.</td>
<td>11</td>
</tr>
<tr>
<td>Nishiyama S.</td>
<td>19</td>
</tr>
<tr>
<td>Novoa-Carballal R.</td>
<td>25</td>
</tr>
<tr>
<td>Ojha M.</td>
<td>9</td>
</tr>
<tr>
<td>Oldiges M.</td>
<td>18, 21</td>
</tr>
<tr>
<td>Omura K.</td>
<td>13</td>
</tr>
<tr>
<td>Ordoñez J.L.</td>
<td>34, 35</td>
</tr>
<tr>
<td>Ostermann S.</td>
<td>18, 21</td>
</tr>
<tr>
<td>Padilla-Álvarez F.</td>
<td>29</td>
</tr>
<tr>
<td>Palomero F.</td>
<td>26</td>
</tr>
<tr>
<td>Papalexandratou Z.</td>
<td>7</td>
</tr>
<tr>
<td>Peters B.</td>
<td>14, 15, 20</td>
</tr>
<tr>
<td>Perret X.</td>
<td>14</td>
</tr>
<tr>
<td>Pfaff A.</td>
<td>25</td>
</tr>
<tr>
<td>Pizarro C.</td>
<td>28, 35</td>
</tr>
<tr>
<td>Qn J.</td>
<td>2</td>
</tr>
<tr>
<td>Qureshi O.</td>
<td>8</td>
</tr>
<tr>
<td>Ramírez P.</td>
<td>3, 4</td>
</tr>
<tr>
<td>Ranalli G.</td>
<td>25</td>
</tr>
<tr>
<td>Revillo C.</td>
<td>12</td>
</tr>
<tr>
<td>Richhardt J.</td>
<td>19</td>
</tr>
<tr>
<td>Rodríguez C.</td>
<td>33</td>
</tr>
<tr>
<td>Santos-Dueñas J.M.</td>
<td>27, 29, 30, 31</td>
</tr>
<tr>
<td>Schweikerk S.</td>
<td>13</td>
</tr>
<tr>
<td>Shi J.J.</td>
<td>4</td>
</tr>
<tr>
<td>Shiratori S.</td>
<td>34</td>
</tr>
<tr>
<td>Sohail H.</td>
<td>8</td>
</tr>
</tbody>
</table>

| 38 | *Acetic Acid Bacteria* 2012; 1 (s1) |