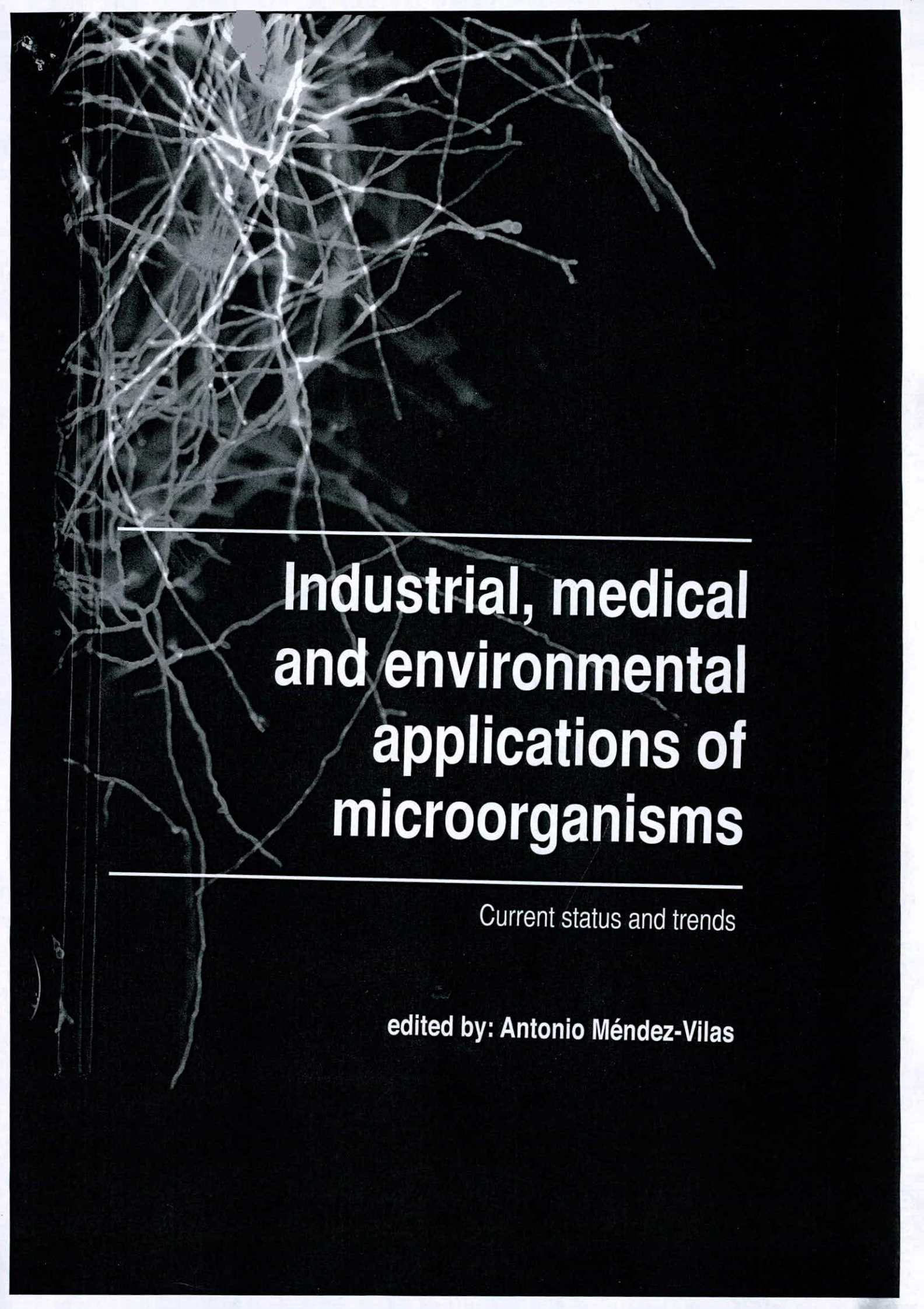

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Current status and trends

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Study of differential gene expression of *Oenococcus oeni* with microarray during the adaptation in different media

A. Costantini[1,2], K. Rantsiou[2], E. Vaudano[1], A. Greppi[2], L. Cocolin[2] and E. Garcia-Moruno[1]
[1]*Consiglio per la Ricerca e la sperimentazione in Agricoltura, Centro di ricerca per l'enologia (CRA-ENO), via P. Micca 35, 14100 Asti, Italy; e.garciamoruno@isenologia.it*
[2]*Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Via Leonardo da Vinci 44, 10095 Grugliasco, Torino Italy*

Abstract

Malolactic fermentation (MLF) is a biochemical transformation conducted by lactic acid bacteria that can take place spontaneously through the action of the autochthonous wine population or by the use of commercial starters, mainly belonging to the *Oenococcus oeni*. A subgenomic array was designed for *O. oeni*, based on the *O. oeni* PSU-1 sequence available on GenBank, in order to consider the main metabolisms of this bacterium and it was applied in the study of the response of the *O. oeni* SB3 commercial starter to the adaptation in different media. This work showed that there is an evident physiological response to the adaptation of *O. oeni* cells. The analyzed strain adapted in MRS was metabolically more active. This global view of the genes expressed when cells are grown in different environmental conditions, could be a tool aimed to increase the functionality of the starter before the inoculation in wine.

Keywords: *Oenococcus oeni* starter; adaptation; microarray; differential gene expression

1. Introduction

Malolactic fermentation (MLF) is a secondary wine fermentation conducted by lactic acid bacteria (LAB). This fermentation is important in winemaking because, in addition to reducing acidity, enables greater microbiological stability to be achieved and multiple transformations occur that make important contributions to the organoleptic qualities of wines due to the production of metabolites. One of the main species identified during spontaneous MLF is *Oenococcus oeni* since it is the most tolerant to adverse wine conditions [1] and it is considered the principal species responsible for this process in wine [2].

Spontaneous MLF can be unpredictable and can start, only after long periods of delay. This delay can entail risks associated to the development of contaminating microorganisms that can generate an abnormal taste and smell.

In order to control the MLF, the use of selected LAB cultures is becoming widely used, to assure a fast development of the process without difficulties [3]. However, inoculation of *O. oeni* starter cultures directly into wine leads to significant cell mortality and, consequently, failure of MLF. Different authors suggested that to overcome this problem, the starter should be submitted to a reactivation phase to adapt it before the inoculation in wine [4-7].

In this study, the effects of the composition of the adaptation medium were investigated, in order to better understand the response of the starter to different media composition by the evaluation of gene expression.

2. Materials and methods

2.1 Array design

A subgenomic array was designed on *O. oeni* genome based on the *O. oeni* PSU-1 sequence available on GenBank (ac. No cp000411.1), in order to consider the main metabolisms of this bacterium. Probes were designed with ArrayDesign with a length of 30 nucleotides. These probes were than spotted on a glass slide by CRIBI (University of Padova, Italy).

This newly designed subgenomic array, consisted of spots for 140 genes. Spots for positive, negative and hybridization controls were also included. Each slide has 2 areas for hybridization of 2 different samples and in each area, the spots are repeated in triplicate.

2.2 Experimental conditions

O. oeni SB3 commercial starter (Laffort, Italy) was used in this study. It was rehydrated in three different media of different composition. Medium 1: MRS supplemented with 3 g/l malic acid; medium 2: MRS diluted ten times and supplemented with malic acid 3 g/l and 8% ethanol; medium 3: MRS diluted and supplemented with malic acid 3 g/l+ 12% ethanol.

The starter was rehydrated in these media at 25 °C for 20 minutes and then adapted in the same media 24 h at 25 °C. Three biological replicates were performed for each experimental condition.

2.3 RNA extraction and Reverse transcription (RT)

After the adaptation phase, 2 ml of sample were centrifuged for 1 minute and 100 µl of RNA*later* (Ambion, Applied Biosystems, Milan, Italy) were immediately added to the pellet.

RNA was extracted using the MasterPure™ Complete DNA and RNA Purification kit (Epicentre, Madison, WI, USA). DNA was eliminated with a treatment with Turbo DNase (Ambion). The RNA was quantified by spectrophotometer and quality checked by Experion (BioRad, Milan, Italy) analysis.

Two µg of RNA were retro-transcribed with M-MLV Reverse Transcriptase (Promega, Milan, Italy) enzyme and random hexamers (Promega), following the instructions of the manufacturer.

The reaction mix contained 150 µM of Biotin-11-dUTP (Fermentas, M-Medical, Milan, Italy), 150 µM of dTTP and 0.7 mM of each of dATP, dCTP and dGTP and RNasin ribonuclease inhibitor (Promega).

The RT reaction was carried out at 42 °C for 1 h and it was stopped by inactivation of the enzyme. The cDNA was precipitated following standard methodology described by Sambrook *et al.* [8]. The pellet was re-suspended directly in 350 µl of the microarray hybridization buffer Q.Hyb (QInstruments, Jena, Germany).

2.4 Microarray hybridization and development

Slides were pretreated with a step of 5 minutes with 0.1% Triton-X100, 10 minutes of KCl 100 mM and 1 h with Blocking Buffer (QInstruments, Jena, Germany).

Hybridization was performed at 30 °C for 24 h in an Eppendorf Thermomixer comfort (Eppendorf). For the development of the slides, the Silverquant detection kit (Eppendorf) was used, according to the manufacturers' instructions. The principle of Silverquant method is shown in Fig. 1.

The slides were then scanned using the Silverquant scanner (Eppendorf). The images obtained were processed and the spots quantified using the Silverquant analysis software (Eppendorf). Afterward, the Micro Array Data Analyser (MADA) program (http://www.mpi-bremen.de), was used for the processing of the spot intensities obtained.

2.5 Statistical analysis

Statistical analysis of the microarray data was performed using SPSS; difference in gene expression was considered statistically significant if their p-value <0.05. Moreover, the software Genesis [9] available at http://genome.tugraz.at/genesisclient/genesisclient_description.shtml, was used for clustering analysis of

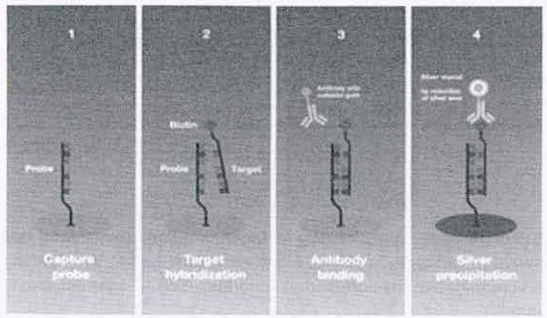

Fig. 1. Schematic representation of the principle of the silverquant kit (Eppendorf) used in this work.

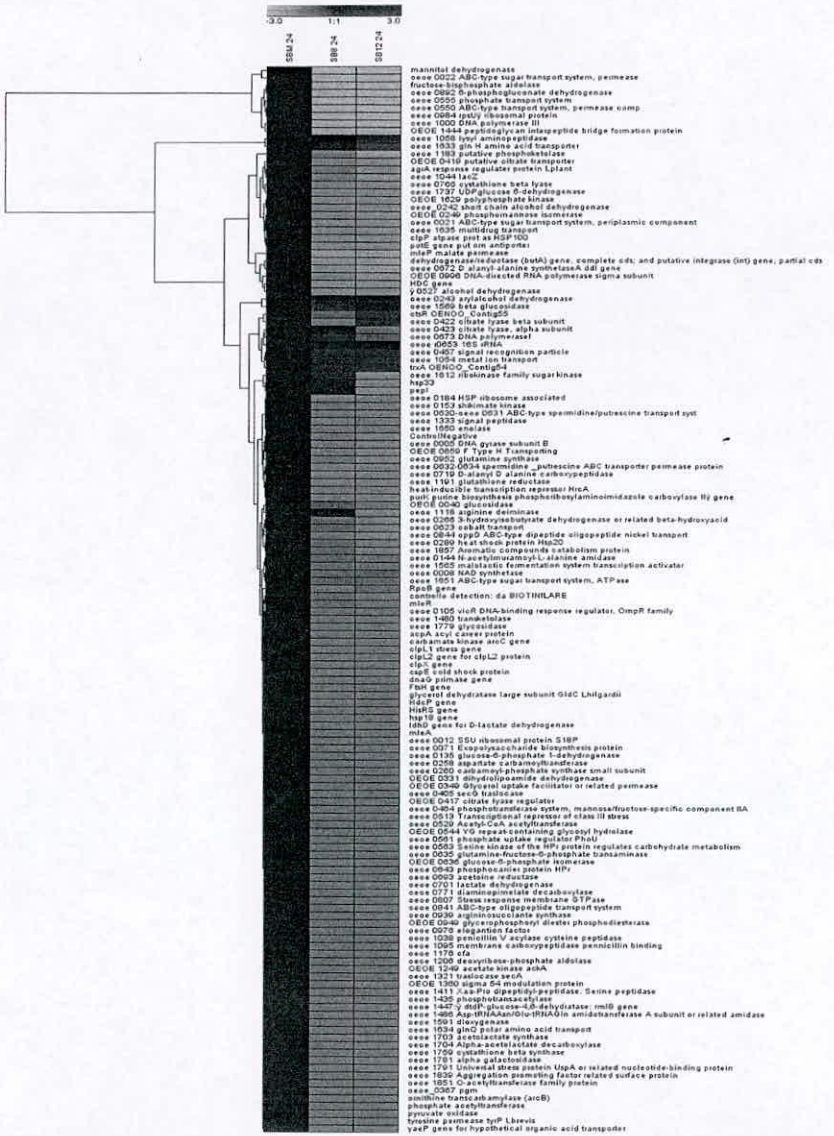

Fig. 2. Genesis analysis of the gene expression data for the strain adapted in different media. Data presented were normalized toward the adaptation in MRS + malic acid (medium 1). Variation in the color indicates level of expression as compared to the normalizing condition. (SB8 24: adaptation in medium 2; SB12 24: adaptation in medium 3).

the gene expression data, employing the Pearson correlation; to this purpose, normalization was performed toward the adaptation of the strain in MRS containing malic acid (medium 1).

3. Results and discussion

Results showed that there are differentially expressed genes in function of the composition of the media. In Fig. 2 the analysis with Genesis of the data normalized against the condition of *O. oeni* adapted in medium 1 is shown. In general, genes are under-expressed in the conditions containing ethanol as shown in Fig. 2.

ANOVA analysis revealed that *O. oeni* adapted in MRS showed a significant increase of the expression of genes involved in the transport systems of sugar, multidrug and phosphate, an increase of the genes related with peptidoglycan formation and malate permease and an increase of the short chain alcohol dehydrogenase.

The strain adapted in the medium 2 and 3, which contained ethanol, showed a significant increase of the gene coding for the arylalcohol dehydrogenase, a significant increase of the gene involved in glycan metabolism, an increase of the genes coding for a signal recognition particle responsible of protein export and secretion system.

This work showed that there is an evident physiological response to the adaptation of *O. oeni* cells. The analyzed strain adapted in MRS was metabolically more active. This global view of the genes expressed when cells are grown in different environmental conditions, could be a tool to employ in the study of starter strain adaptation in different environments, particularly during preparation for inoculation in wine.

Acknowledgements

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