Foods 2024 Conference

The 5th International Electronic Conference on Foods

28–30 October 2024 | Online

MDPI

# Proteomic and transcriptomic analyses revealed cell changes and physiological adaptations in ethanol-stressed *Oenococcus oeni* strain

Maria Grazia Bonomo [1], Rocco Rossano [2], Giovanni Salzano [1]

[1] Department of Health Sciences, [2] Department of Basic and Applied Sciences, University of Basilicata

## INTRODUCTION & AIM

*Oenococcus oeni* is involved in the malolactic fermentation and its metabolic activities can modify taste, aromatic properties and microbial stability of wine. For this reason, there is a growing interest in formulate starter cultures from it, as the resistance to the harsh environment of wine is strictly strain-dependent.

## METHOD

To investigate the effect of ethanol stress on cell physiology, we characterized the proteome and phosphoproteome of *O. oeni* DSPZS12, from Aglianico wine produced in Vulture zone (Basilicata region, Southern Italy) and stressed with different ethanol concentrations (7, 12, 13 and 15%). Total proteins were separated by two-dimensional gel electrophoresis and identified by MALDI-TOF mass spectrometry and ElectroSpray Ionization-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-ICR/FT-MS). Proteins exhibiting Post-Translation Modification (PTM), especially phosphorilation on Ser, Thr or Tyr, were also investigated. For protein identification, we chose a bottom-up approach and we performed Peptide Mass Fingerprinting (PMF) and tandem MS analyses.
Moreover, RT-qPCR was introduced to validate the protein identification in the same stress conditions. Some genes were selected due to their involvement in stress response as described by the protein analyses and others were selected to obtain a better comprehension of stress response and to explore metabolic pathways and mechanisms activated to deal with changes due to ethanol stress. Three genes (*rrs*, *pta*, *rpoB*) were evaluated as internal controls for RT-qPCR.

## RESULTS & DISCUSSION

The changes in protein patterns obtained under different ethanol stress conditions were analyzed by 2-DE electrophoresis. 2-D gels of control (bacterial cells incubated at 30°C for 1 h in MRS-TJ pH 4.8 with no ethanol addition) and ethanol stress responses (bacterial cells incubated in MRS-TJ containing ethanol 7, 12 and 15%) are shown in fig. 1.
Each gel revealed a high-resolution 2-DE map of *O. oeni* S12 strain with approximately 440±10 spots.

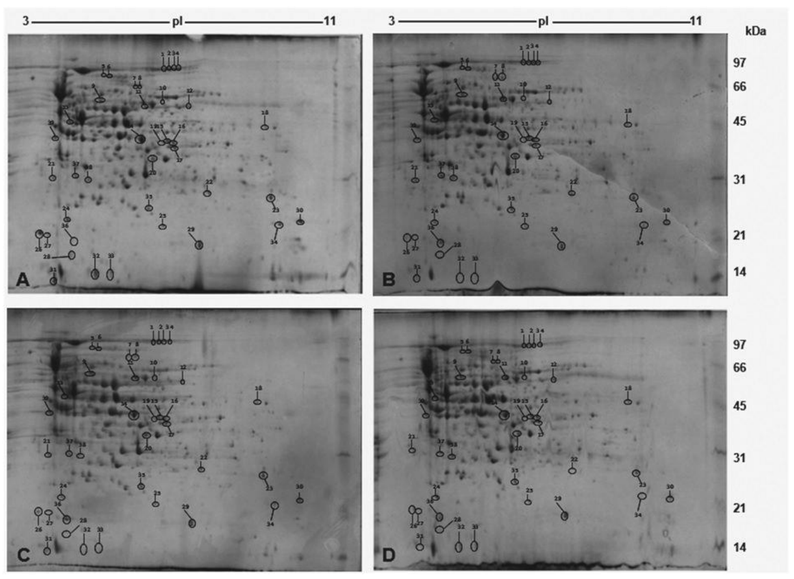


Figure 1 Bidimensional electrophoresis (2-DE) of *O. oeni* total protein extracts

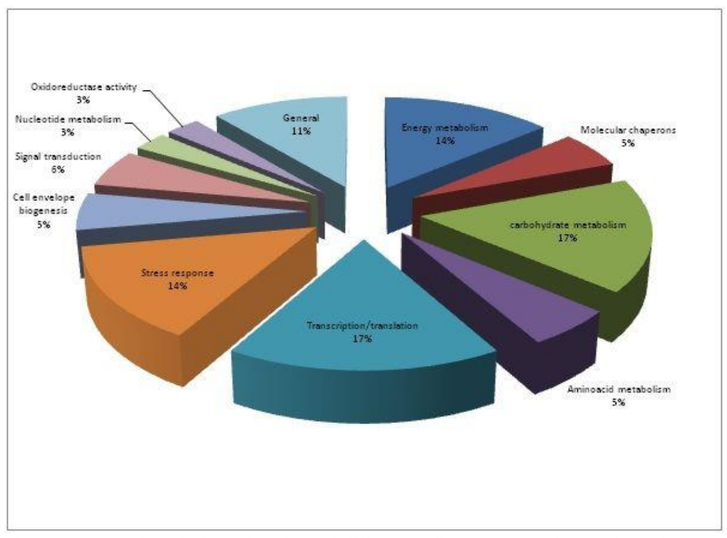


Figure 2 Functional classification of differentially expressed proteins of *O. oeni*

A total of 133 peptide and 99 proteins were identified; besides, MS/MS data processing leads to the identification of 78 phosphorylated peptides from 50 spots and 39 proteins. The presence of ethanol promoted a shutdown of several proteins involved in energy/carbohydrate metabolism, protein synthesis and stress response.
The identified proteins were then classified in 11 groups on the basis of their metabolic function, as shown in Fig. 2.

In Figure 3 was showed the expression profile of some representative protein spots in the control and in the ethanol treated samples (left panel), and the corresponding abundance pattern (right panel).

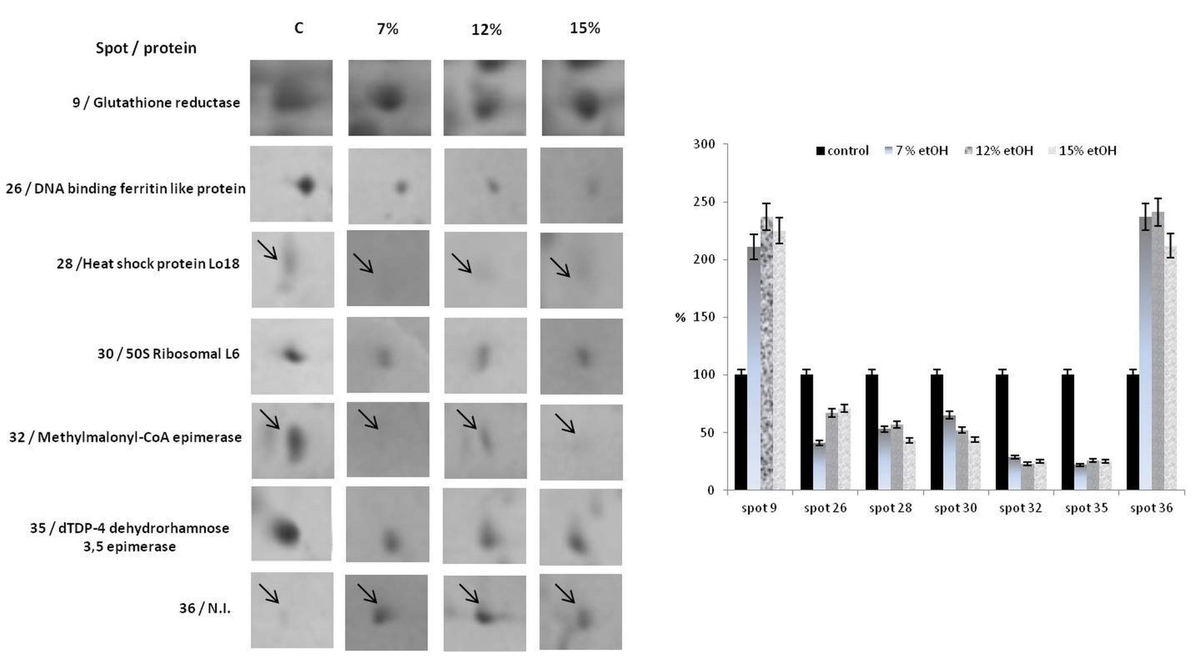


Figure 3 Expression profile of some representative protein spots

Moreover, changes in cell physiology are often accompanied by the modulation of gene expression profiles to ensure cell vitality and proliferation. So, we investigated, also, the transcriptome expression profile of *O. oeni* DSPZS12 strain by quantitative Real Time PCR (qPCR) that allowed to identify and characterize the differentially expressed genes and the pathways most influenced by stress conditions tested, such as the regeneration of NADPH and maintenance of redox balance and the cell morphology, involving peptidoglycan biosynthesis and cell wall components.
The figure 4 shows the Bar Plot showing the Fold Change (FC) ± Standard Error of Mean (SEM).

| Genes of interest |
|---|
| MREB |
| MREC |
| MRED |
| PENICILLIN BINDING PROTEIN (PBP) |
| D-ALANINE POLYPHOSPHORIBITOL LIGASE |
| D-ALANYL-D-ALANINE CARBOXYPEPTIDASE |
| N-ACETYLMURAMOYL-L-ALANINE AMIDASE |
| ACYL-ACP THIOESTERASE |
| 6-PHOSPHOGLUCONATE DEHYDROGENASE |
| SHORT-CHAIN ALCOHOL DEHYDROGENASE |
| LACTATE DEHYDROGENASE |
| PYRUVATE OXIDASE |
| ACETOLACTATE SYNTHASE |
| NADPH OXIDOREDUCTASE |
| SERINE DEHYDROGENASE |
| FAD (FATTY ACID DEGRADATION) |
| FAB (FATTY ACID SYNTHESIS) |
| **Internal Control Genes** |
| RRS (16S) |
| PTA |
| RPOB |

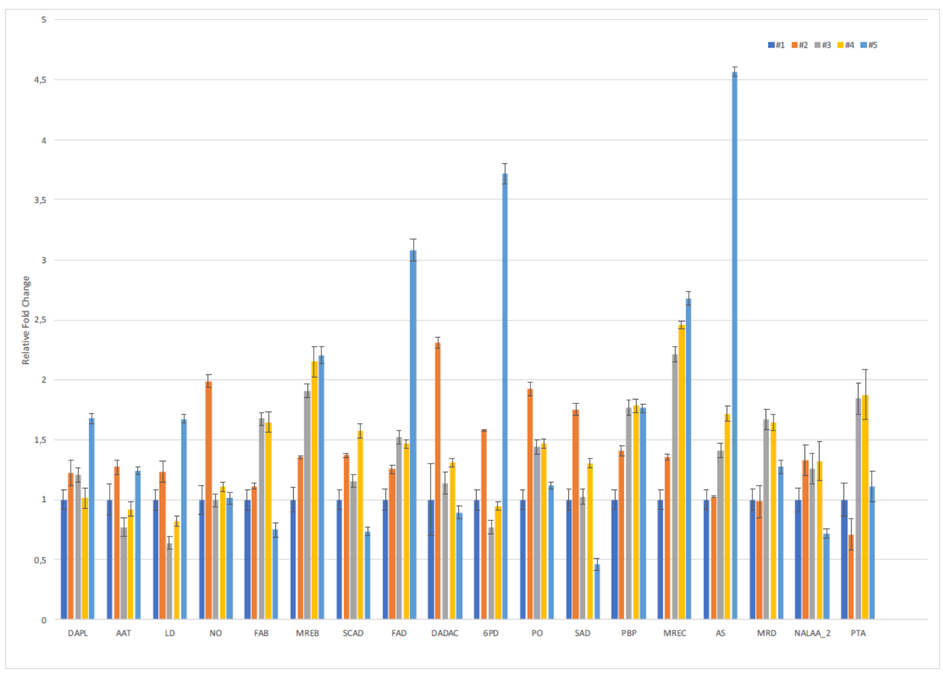


Figure 4 Bar Plot showing the FC ± SEM

## CONCLUSION

*O. oeni* is able to respond to environmental changes by varying its gene expression and implementing a series of mechanisms that ensure its survival and the performance of its vital functions.
Our results represent an important advance to clarify and understand the bacterial defense mechanisms as well as the changes in gene expression in *O. oeni* influenced by ethanol stress show a response of defense and adaptation to survival in hostile conditions. Further studies are needed to determine the structure of cell wall of this bacterium but also to evaluate as ethanol influence on the membrane fluidity and permeability and, thus, the role of membrane fluidity as factor in ethanol stress tolerance of *O. oeni*.

## REFERENCES

Bonomo M.G., Cafaro C., Guerrieri G., Crispo F., Milella L., Calabrone L., Salzano G. (2017). Flow cytometry and capillary electrophoresis analyses in ethanol-stressed *Oenococcus oeni* strains and changes assessment of membrane fatty acids composition. Journal of Applied Microbiology, 122 (6), 1615-1626.
Bonomo M.G., Di Tomaso K., Calabrone L., Salzano G. (2018). Ethanol stress in *Oenococcus oeni*: transcriptional response and complex physiological mechanisms. Journal of Applied Microbiology, 125, 2-15.
Sico, M.A., Bonomo, M.G., Salzano, G., 2008. Isolation and characterization of *Oenococcus oeni* from Aglianico wines. World J. Microbiol. Biotechnol. 24, 1829-1835.
Silveira, M.G., Baumgartner, M., Rombouts, F.M., Abee, T. 2004. Effect of Adaptation to Ethanol on Cytoplasmic and Membrane Protein Profiles of *Oenococcus oeni*. Appl. Environ. Microbiol. 70, 2748-2755.