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Oenococcus oeni : ADVANCES IN MOLECULAR GENETICS

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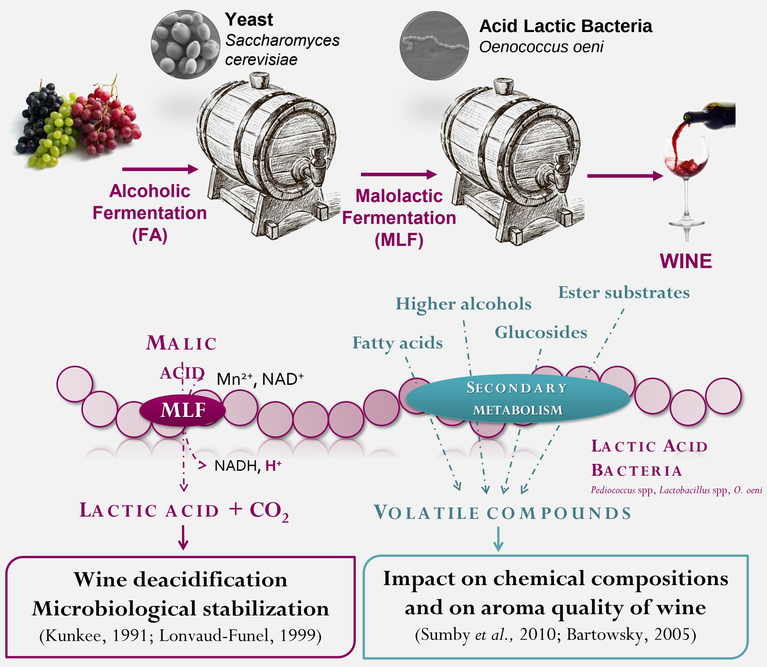
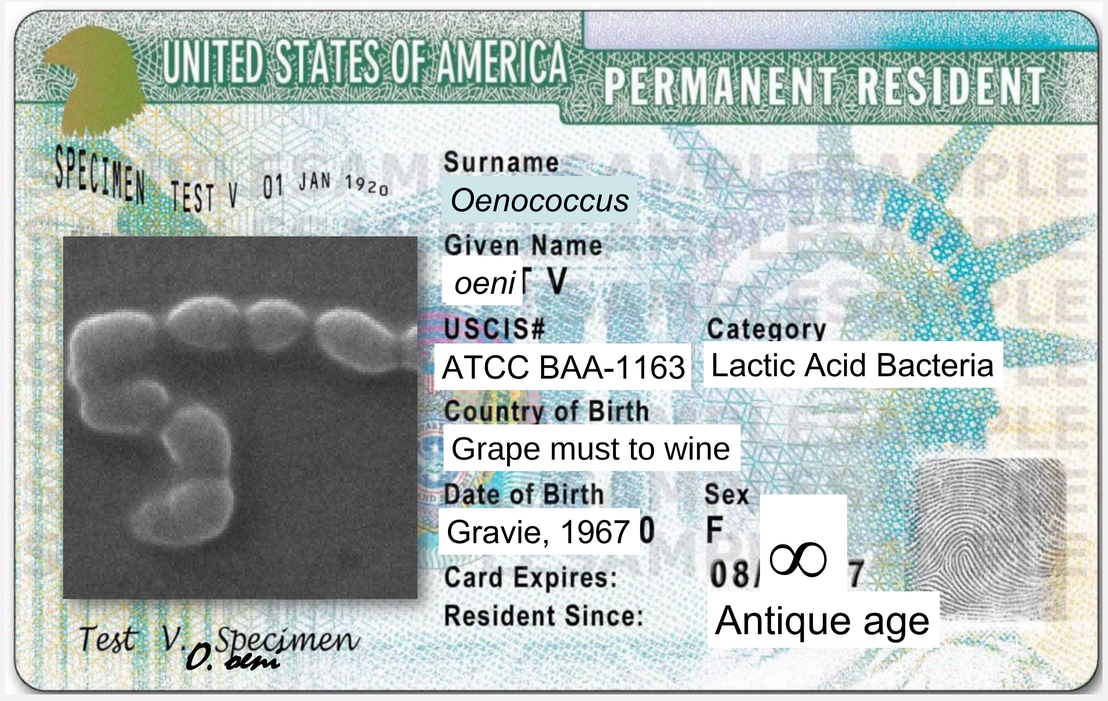
WINE MAKING PROCESS



Wine deacidification
Microbiological stabilization
(Kunkee, 1991; Lonvaud-Funel, 1999)

Impact on chemical compositions and on aroma quality of wine
(Sumby *et al.*, 2010; Bartowsky, 2005)



IMPEDIMENTS

- Not easily manipulable bacterium
- Few reliable tools and weak transformation efficiency
- Mutation not possible to investigate gene function

CHALLENGES

- To overcome the difficulties of manipulation of the genome of *O. oeni* due to the lack of genetic tools for gene replacement.
- To overexpress gene *in vivo*
- To modulate gene expression in *O. oeni* and understand their function *in vivo*.

ADVANCES IN MOLECULAR GENETICS

Leuconostoc oenos described *by* Gravie **1967**

Plasmids in *L. oenos* Janse *et al.* **1987**

Transformation by electroporation Dicks **1994**

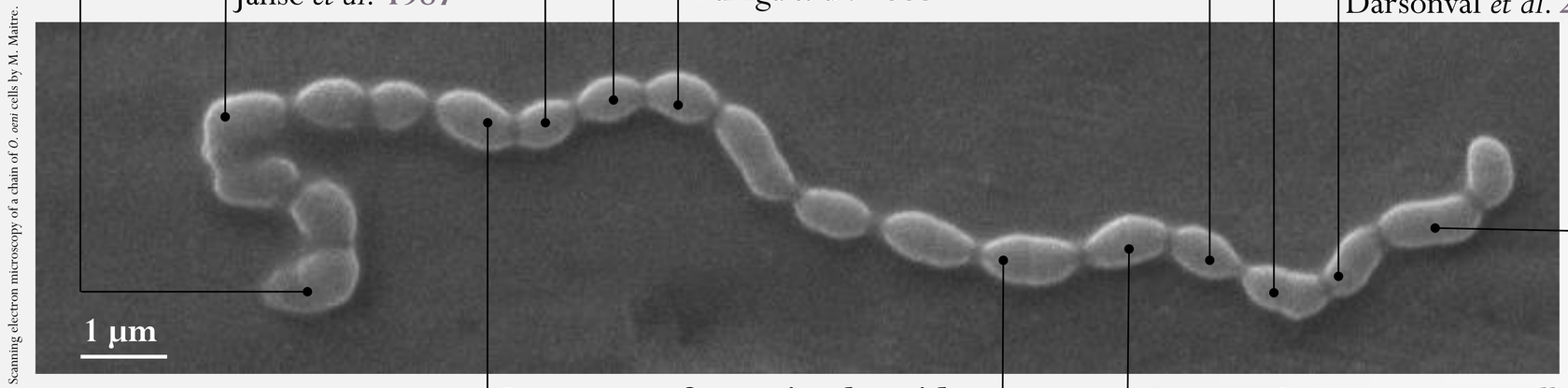
L. oenos* renamed as *Oenococcus oeni Dicks *et al.* **1995**

Conjugative transferts Zúñiga *et al.* **2003**

An improved protocole for electroporation Assad Garcia *et al.* **2008**

Identification of pOENI-1 plasmids Favier *et al.* **2012**

Antisense RNA technology to modulate gene expression. Genetically engineered *O. oeni* strains Darsonval *et al.* **2016** a,b



Scanning electron microscopy of a chain of *O. oeni* cells by M. Maitre.

Sequence of cryptic plasmids Fremaux *et al.* **1993** ; Mesas *et al.* **2001**; Alegre *et al.* **1999** ; Zúñiga *et al.* **1996** ; Brito *et al.* **1999**

***O. oeni* PSU-1 genome.** Mills *et al.* **2005**

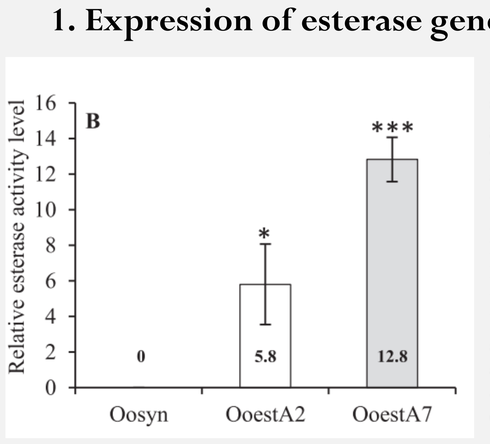
CtsR is the master regulator of stress response Grandvalet *et al.* **2005**

pGID052 vector for genetic transfert in *O. oeni* Beltramo *et al.* **2004**

WHAT ELSE ?

New shuttle vector to express genes of interest

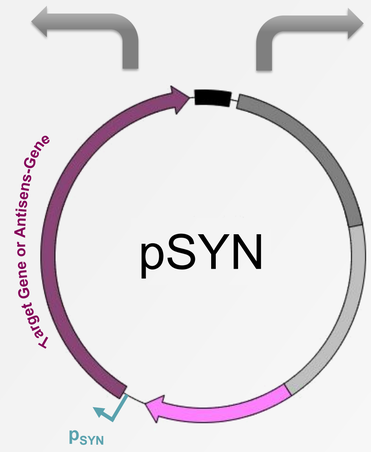
1. Expression of esterase genes (*estA2* and *estA7*) in *O. oeni*

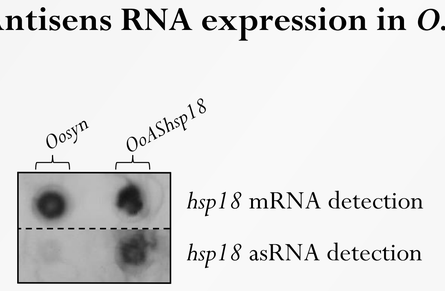


Relative esterase activity in *O. oeni* recombinant strains. The *O. oeni* ATCC BAA-1163 *estA2* and *estA7* genes were cloned under the control of the synthetic constitutive promoter (p_{SYN}) using the *E. coli*/LAB shuttle vector pSYN. Recombinant vectors were transferred into *O. oeni* ATCC BAA-1163 strains and recombinant strains *OoestA2* and *OoestA7* were grown in FT80m medium until exponential growth phase. Enzyme activity was determined with cellular extract using p-nitrophenol-butanoate as synthetic substrat. Values are shown relative to the basal activity detected in strain carrying native pSYN plasmid (Oosyn), which was arbitrarily designated 0. Significant differences are based on unilateral and paired t tests. ***, $p \leq 0.0005$; **, $p \leq 0.005$; *, $p \leq 0.05$.

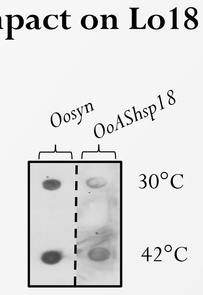
Antisense technology to interfere on gene expression

2. Antisens RNA expression in *O. oeni* and impact on Lo18 protein level

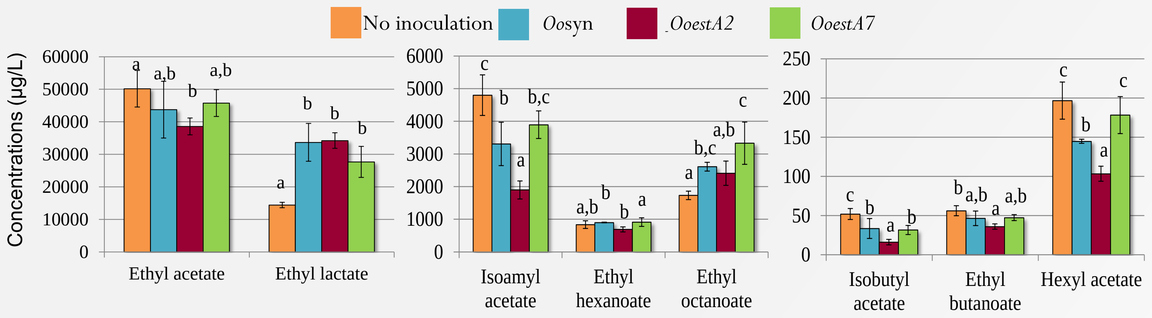




Northern dot-blot for detection of mRNA and antisens RNA (asRNA) of *hsp18* in *O. oeni* recombinant strains. Total RNA were extracted from *O. oeni* carrying empty pSYN vector (*Oosyn)* and *O. oeni* expressing antisense of the *hsp18* gene (*OoASHsp18*).

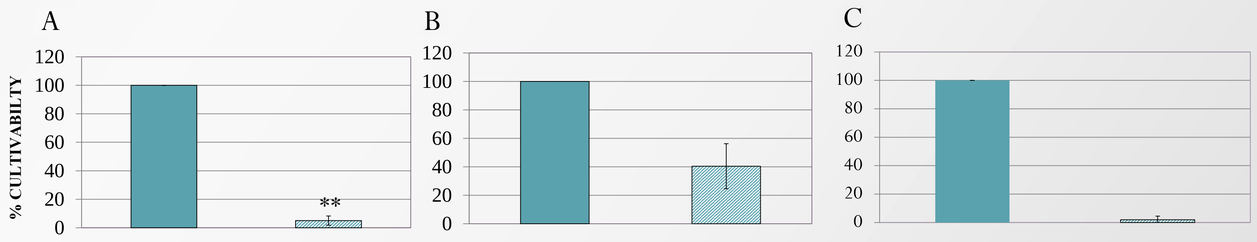


Western dot-blot to detect Lo18 encoded by the *hsp18* gene. Total proteins were extracted from *O. oeni* carrying empty pSYN vector (*Oosyn*) and *O. oeni* expressing antisense of the *hsp18* gene (*OoASHsp18*). Strains were grown at 30°C and 42°C and Ac α Lo18 was used.



Concentrations of selected esters with no inoculation and post MLF performed in Aligote wine with three *O. oeni* recombinant strains. An aligoté wine (pH 3.1, 3.2 g/L malic acid, 11.8% ethanol) sourced from the winery of Burgundy University was collected after AF and was adjusted at pH3.5, then filtered (0.22 µm filter). Wine was inoculated with three recombinant strains, each in triplicate, at 10^5 cells/mL and incubated at 22°C. At the completion of MLF, different ethyl and acetate esters were identified and quantified by SPME-GC-MS. An ANOVA followed by Tukey's multiple comparison test was conducted. Values are the means of triplicate determinations ± standard deviation, of a single wine analyzed. Significant difference between inoculated strains versus control strain O.oeni (pSYN), analyzed using one-way ANOVA of variance Tukey's Multiple Test ($P \leq 0.05$).

2. Effects of *hsp18* asRNA expression on survival under stress conditions



Cultivability tests after heat shock (A), acid shock pH3.5 (B) or pH3 (C). Recombinant strains carrying pSYN plasmid ( ) or carrying plasmid expressing *hsp18* asRNA ( ) were grown at 30°C in FT80 medium until mid-exponential phase (OD_{600nm} = 0,8). Culture were incubated at 48°C (A) or cells were transferred into pH3.5 (B) or pH3 (C) FT80 medium and incubated at 30°C during 90 min. A numeration on agar plate was performed after decimal dilutions. Significant differences are based on an unilateral and paired T test. *** P<0,0005, ** P<0,005, * P<0,05.

Significant loss of cultivability after heat shock (95%) and acid shock (60% at pH3.5 & 98% at pH3)

Lo18 plays a key role in O. oeni stress response

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