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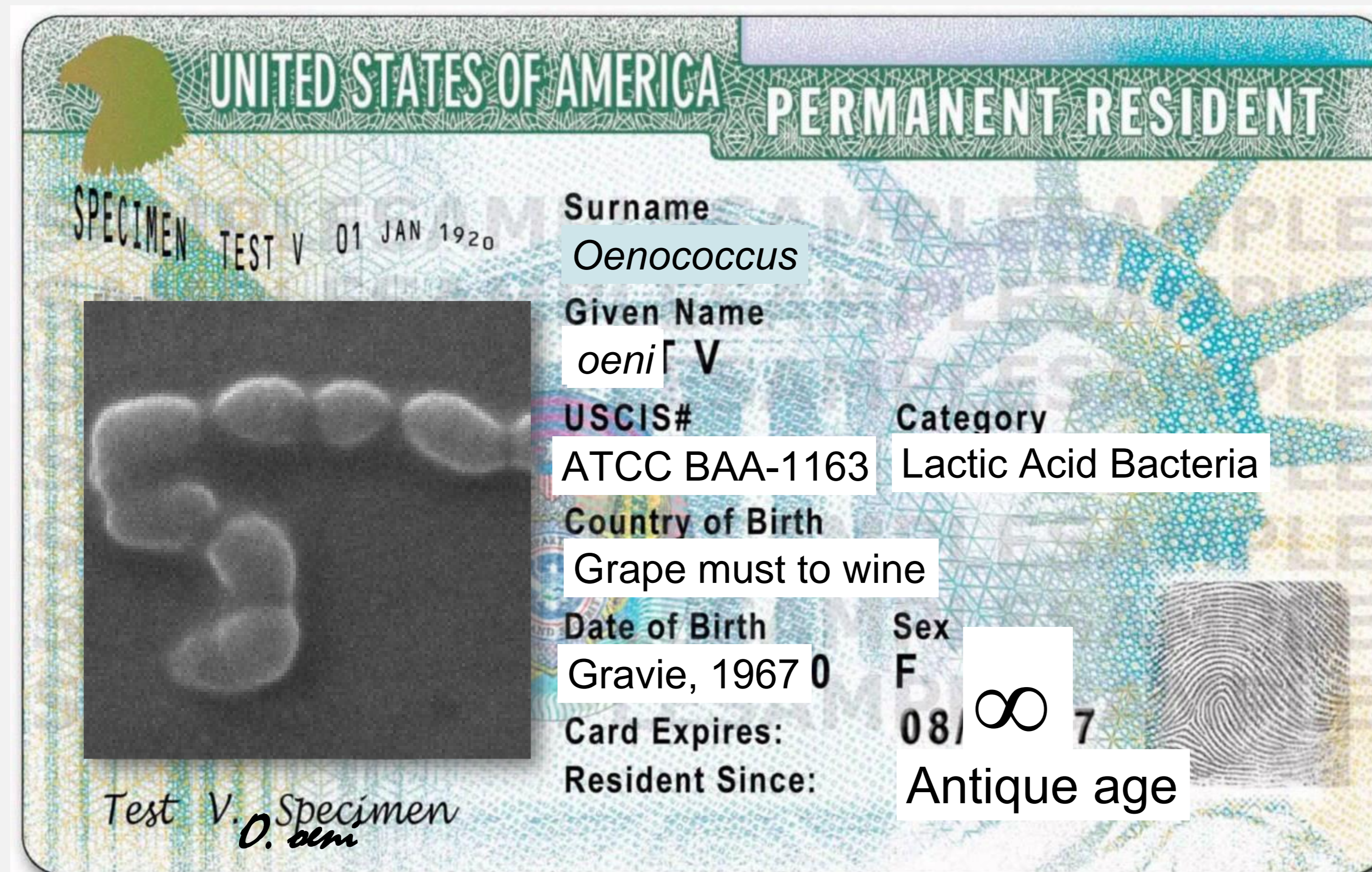
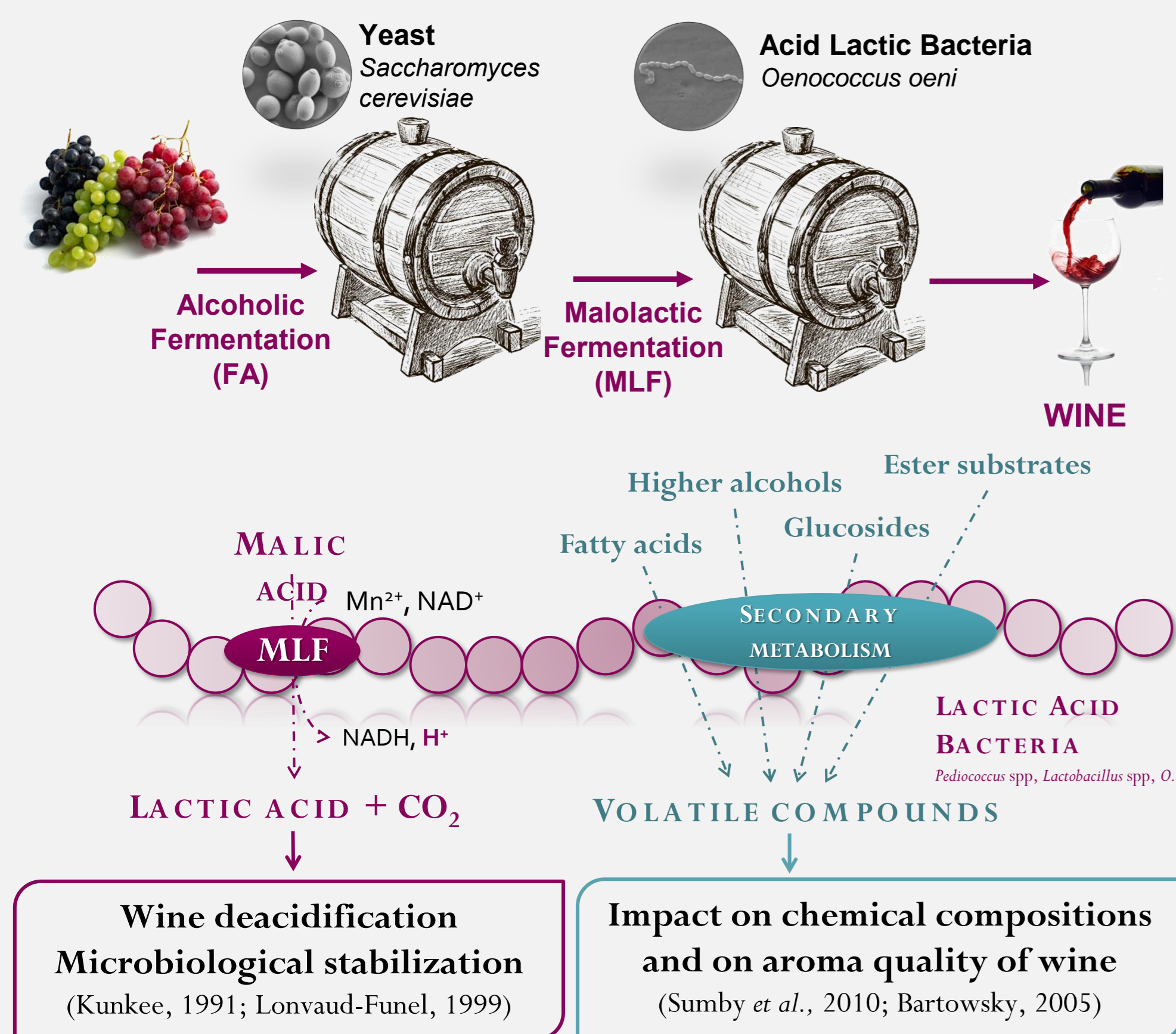
Oenococcus oeni : ADVANCES IN MOLECULAR GENETICS

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WINE MAKING PROCESS



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 transfers Zúñiga et al. 2003

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

An improved protocol for electroporation Assad Garcia et al. 2008

Identification of pOENI-1 plasmids Favier et al. 2012

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

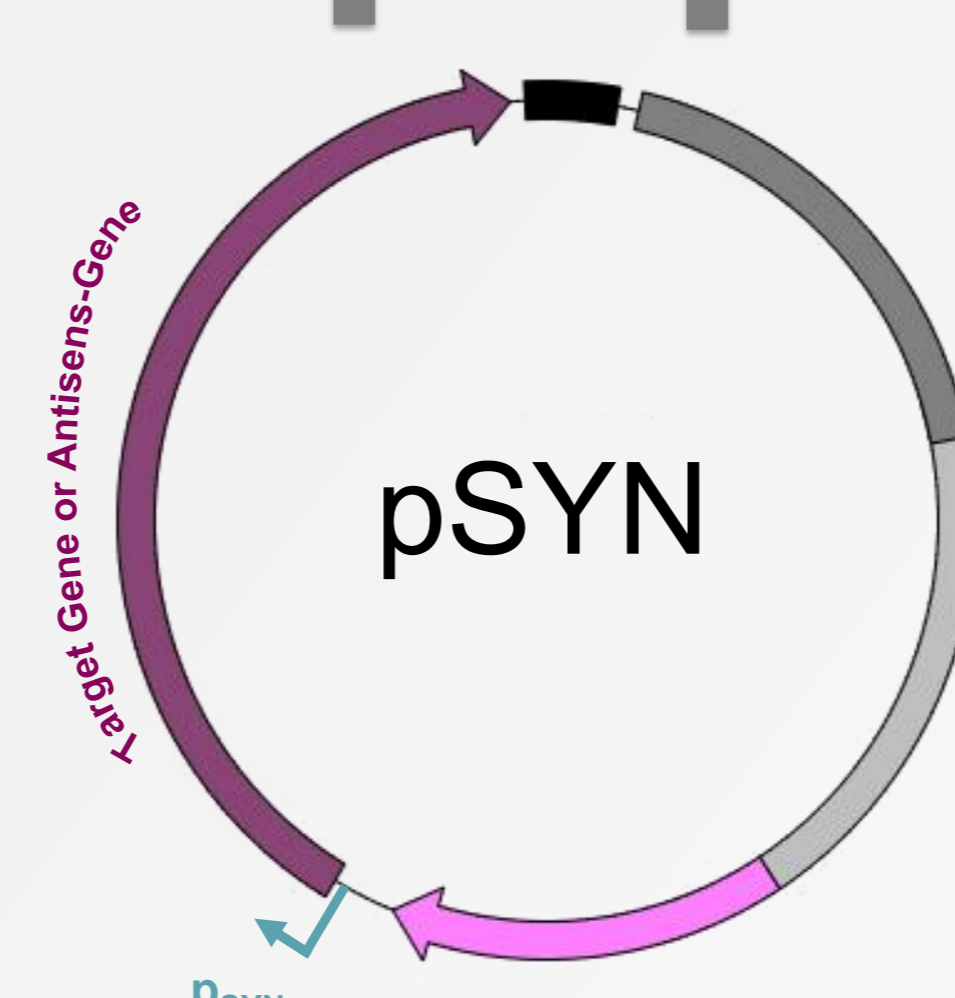
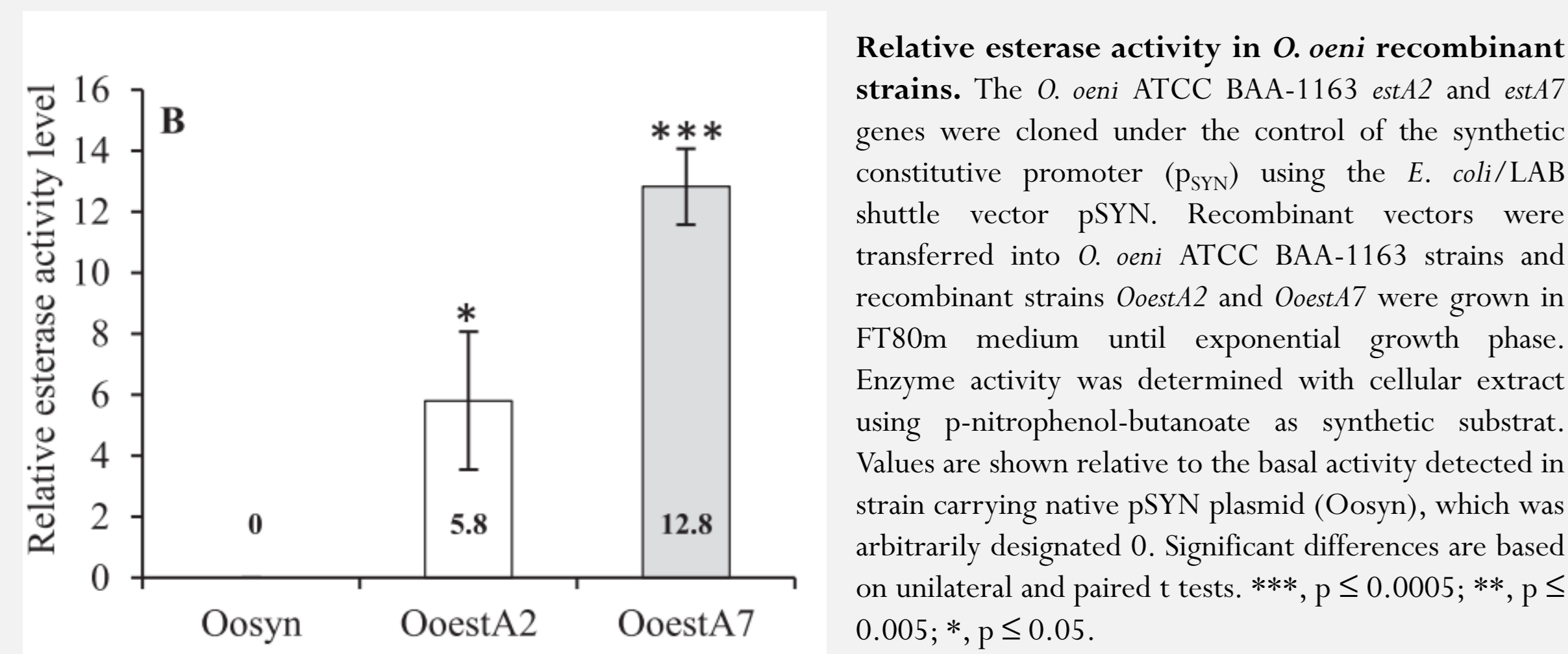
CtsR is the master regulator of stress response Grandvalet et al. 2005

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

WHAT ELSE ?

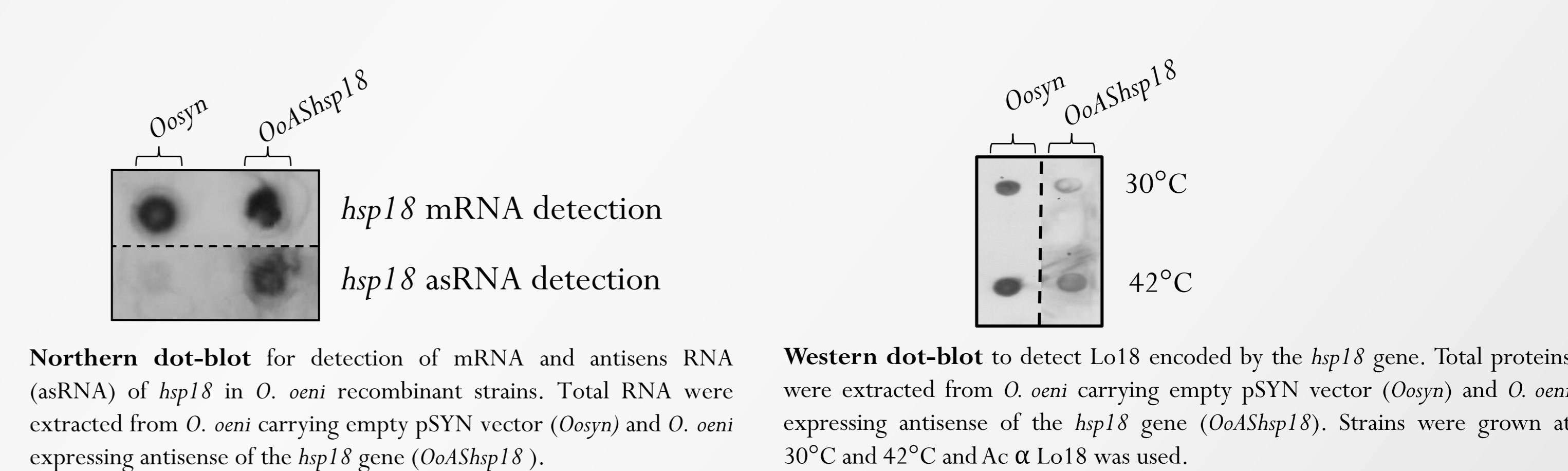
New shuttle vector to express genes of interest

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

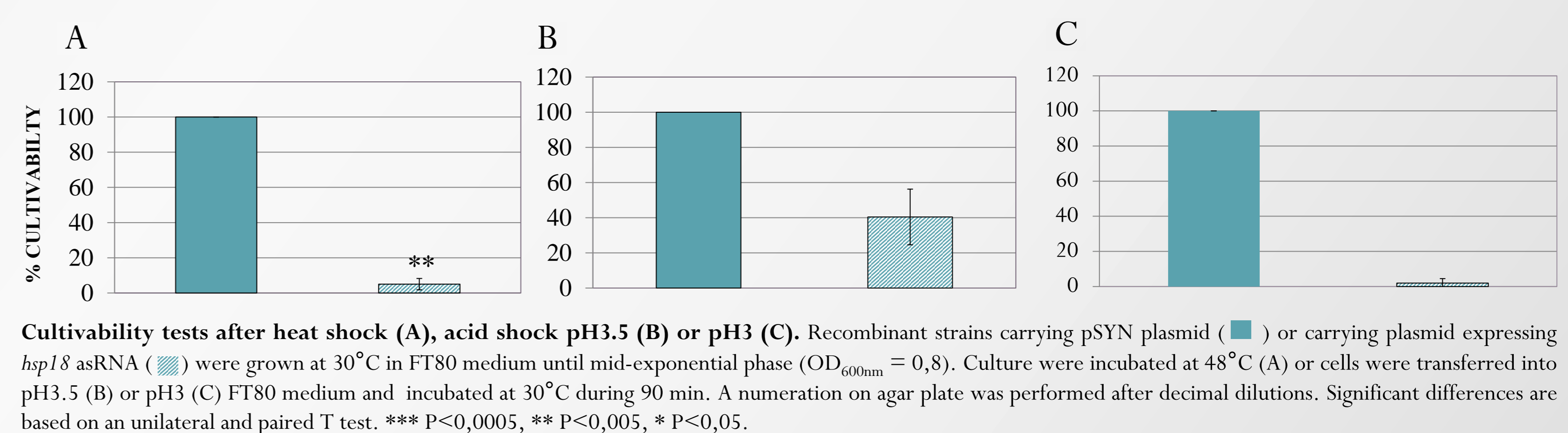


Antisense technology to interfere on gene expression

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

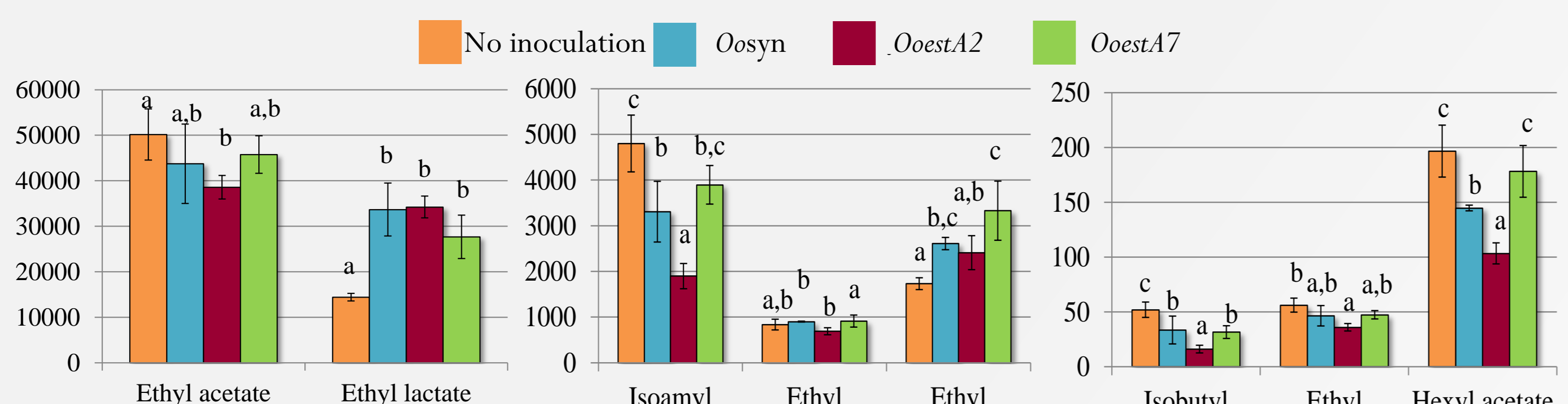


2. Effects of *hsp18* asRNA expression on survival under stress conditions



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



An aligote 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 10⁵ 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 ≤ 0.05).

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