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**Oenococcus oeni: ADVANCES IN MOLECULAR GENETICS**

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**ADVANCES IN MOLECULAR GENETICS**

### Transformation by electroporation

**Dicks 1994**

**L. oenos renamed as Oenococcus oeni**

**Dicks et al. 1995**

**Conjugative transfers**

**Zúñiga et al. 2003**

### 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**

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**WHAT ELSE?**

### New shuttle vector to express genes of interest

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

![Graph showing esterase activity in O. oeni recombinant strains](image)

Concentrations of selected esterases in non-inoculated and post-MID performed in Aligot wine with three O. oeni recombinant strains. An aligot wine (pH 3.1, 13° alcoholic acid, and 11.5% ethanol) partially inoculated from the vineyard of Burgundy. University was collected after 48 h and was adjusted to pH 3.5, then filtered (0.22 µm). Wine was inoculated with three recombinant strains of O. oeni, estA2 and estA7, at 30°C. At the completion of 5 days, each strain was identified and quantified by HPGE-EC-MS. An ANOVA followed by Tukey’s multiple comparison test was conducted. On this data, the mean of triplicate determinations ± standard deviation, ± a unique sample analyzed. Significant differences between inoculated strains were noted (O. oeni estA2, p<0.05), analyzed using one-way ANOVA of three replicates. Tukey’s Multiple Test (p<0.05).

### Antisense technology to interfere on gene expression

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

- **pGID052 vector for genetic transfer in O. oeni**

- **Beltramo et al. 2004**

![Graph showing antisense RNA expression on survival under stress conditions](image)

Cultivability tests after heat shock (A), acid shock (pH 5.1) (B) or pH (C). Recombinant strains carrying pGID052 plasmid (--) or carrying plasmid expressing hsp18 antisense (--) were grown at 30°C in M9/PB medium and and exponential phase (O.D. 0.5). Cultures were incubated at 35°C in a water or at 47°C in a water bath. Aeration on agar plates was performed after 24 h of incubation. Significant differences are based on standard error and paired T test. p<0.05, **p<0.001, ***p<0.0001.

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**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.