Use of antisense RNA to modulate HSP gene expression in oenococcus oeni

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USE OF ANTISENSE RNA TO MODULATE *HSP* GENE EXPRESSION IN *OENOCOCCUS OENI*

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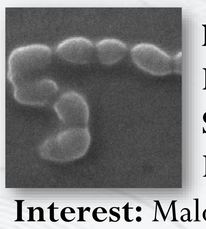
ID *O. oeni*



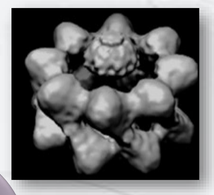
Name: *Oenococcus oeni,* formely *Leuconostoc oenos*
Description : Gram+, cocci in chains
Strain: ATCC BAA-1163
Family: Lactic Acid Bacteria (LAB), Firmicutes
Interest: Malolactic fermentation of wine → aromatic improvement of wine [1]
Environment: wine (low pH, low temperature, nutrient poor, ethanol)
Genome ID and status: AAUV00000000.1, scafold (62 contigs)
Genetic tools: few reliable tools, weak transformation efficiency

Not easily manipulable bacterium,
Mutation not possible to investigate role of genes

Signature : *O. oeni*

&

ID *hsp18*



Gene and GI number: *hsp18,* OENOO_66120 (446 bp)
Protein: Lo18 (148 aa), GI: 118432051
Family: Small Heat Shock Protein (sHSP)
Regulation: Transcriptional repression by **CtsR** under optimal growth conditions [2]
Functions: • Molecular chaperone activity: prevent *in vitro* aggregation of damaged proteins [3]
• Possibly lipochaperone activity: *in vivo* membrane association [4] and *in vitro* regulation of liposome fluidity [5]

***In vivo* function ?**

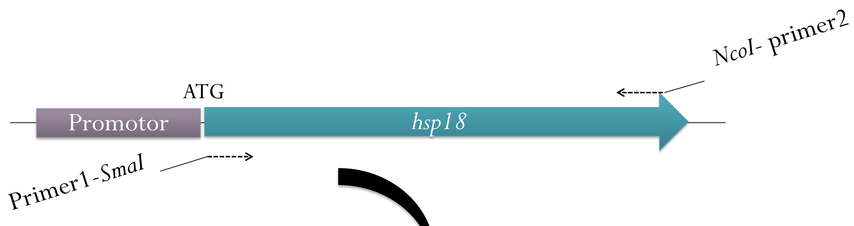
Signature: *Lo18*

OBJECTIVES:

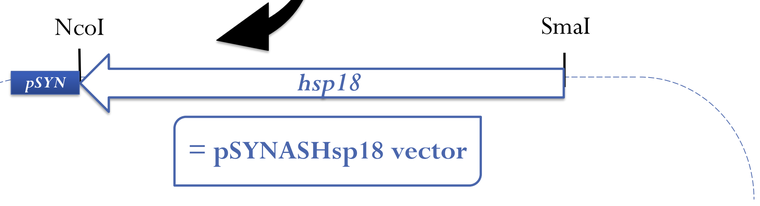
- ☐ To overcome the difficulties of manipulation of the genome of *O. oeni* due to the lack of genetic tools for gene replacement.
- ☐ To develop a gene inactivation method by using antisense RNA approach.
- ☐ To modulate *hsp18* gene expression in *O. oeni* and understand *in vivo* the Lo18 function.

Strategy

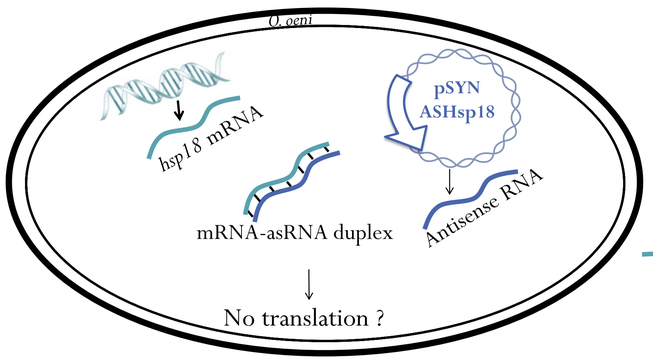
1:Targeting strategy: to target Open reading of the *hsp18* gene frame + PCR amplification



2: Cloning in antisense orientation into *E. coli*/LAB shuttle vector (pSIPSYN)



3:Transfert of pSYNAShsp18 in *O. oeni* ATCC BAA-1163
→ Production of Antisense RNA targeting mRNA of *hsp18* gene in *O. oeni*



- **Antisense RNA detection**: Northen dot-blot revealed with DIG system
- **Protein level detection**: Western dot-blot with Ac α Lo18
- **Phenotypic characterization** by cultivability tests under stress conditions.

Results

1. *Detection of hs18 messengers RNA and antisense RNA.*

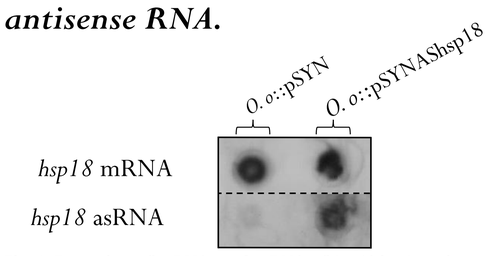


Fig. 1: Detection of asRNA and mRNA of *hsp18* in *O. oeni* recombinant strains by Northern dot-blot with DIG-probes. Total RNA were extracted from O. oeni carrying pSIPSYN (O.o::pSYN) and O.oeni carrying pSIPSYNAShsp18 (O.o::pSYNAhsp18) strains.

2. *Detection of Lo18*

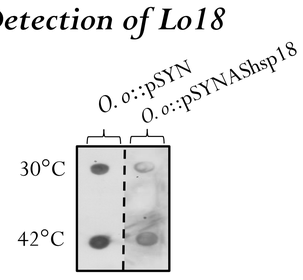


Fig. 2: Detection of Lo18 protein by western dot-blot with Ac α Lo18. O.o::pSYN : O. oeni carrying pSIPSYN (O.o::pSYN) and O.oeni carrying pSIPSYNAShsp18 (O.o::pSYNAhsp18) strains were grown at 30°C or 42°C.

Validation of asRNA EXPRESSION

Validation of asRNA FUNCTION

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

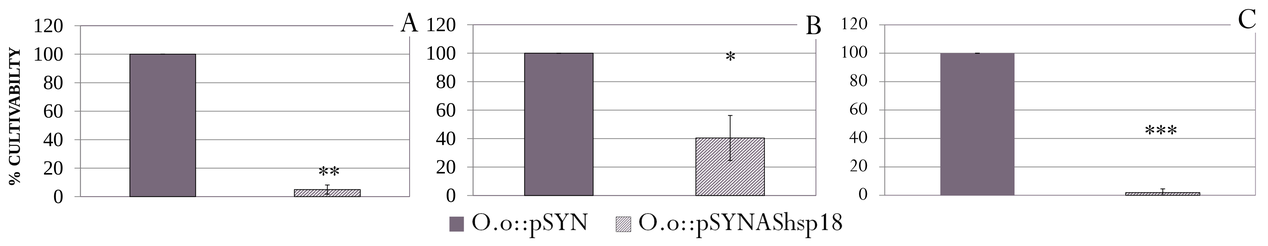


Fig. 3: Cultivability tests after heat shock (A), acid shock pH3.5 (B) or pH3 (C). Recombinant strains carrying native plasmid (O.o::pSYN) or carrying plasmid expressing *hsp18*asRNA (O.o::pSYNAShsp18) were grown et 30°C in FT80 medium until mid-exponential phase (OD_{600} = 0,8). Culture were incubated 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 pairedT 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

Validation of physiological effect of asRNA expression

Conclusions

- ☑ Expression of antisense RNA in *O. oeni via E.* coli/LAB shuttle vector developed in our laboratory.
- ☑ *In vivo* inhibition of *hsp18* gene expression by using antisense RNA approach.
- ☑ Key role of Lo18 in survival of *O. oeni* under stress conditions.

Development of the first efficient genetic tool for *O. oeni* by application of antisense technology to modulate gene expression in *O. oeni*

Perspectives

- ☐ Assess the effect of decreasing Lo18 level on membrane fluidity.
- ☐ Draw up our innovative asRNA method in *O. oeni* by exploring role of *hsp* genes.
- ☐ Draw up our new efficient expression tool to express genes involved in wine aroma and investigate their impact in wine aroma quality.

References :

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3.Maitre, M. *et al*. The oligomer plasticity of the small heat-shock protein Lo18 from *Oenococcus oeni* influences its role in both membrane stabilization and protein protection. *Biochem. J.* **444,** 97–104 (2012).
4.Coucheney, F. *et al*. A small HSP, Lo18, interacts with the cell membrane and modulates lipid physical state under heat shock conditions in a lactic acid bacterium. *Biochim. Biophys. Acta BBA - Biomembr.* **1720,** 92–98 (2005).
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