



HAL
open science

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

Maud Darsonval, Tarek Msadek, Herve Alexandre, Cosette Grandvalet

► To cite this version:

Maud Darsonval, Tarek Msadek, Herve Alexandre, Cosette Grandvalet. Use of antisense RNA to modulate HSP gene expression in oenococcus oeni. FEMS 6th congress of european Microbiologists, Jun 2015, Maastricht, Netherlands. hal-03150745

HAL Id: hal-03150745

<https://institut-agro-dijon.hal.science/hal-03150745>

Submitted on 24 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

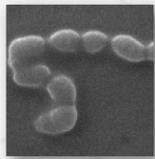
¹Darsonval M., ³Msadek T., ¹Alexandre H., ^{1,2}Grandvalet C.

¹ Equipe VALMiS (Vin Aliment Microbiologie et Stress), Institut Universitaire de la Vigne et du Vin (IUVV) – Université de Bourgogne, Dijon - FRANCE

² AgroSup Dijon, Institut national supérieur des sciences agronomiques, de l'alimentation et de l'environnement, Dijon – France

³ Unité de biologie des bactéries pathogènes à Gram positif, Institut Pasteur, Paris– France

ID *O. oeni*



Name: *Oenococcus oeni*, formerly *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, scaffold (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:

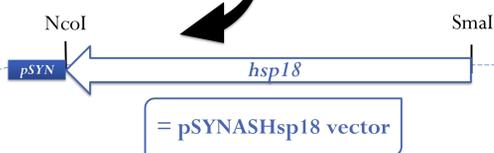
- To overcome the difficulties of manipulation of the genome of *O. oeni* due to the lack of genetic tools for gene replacement.
- To develop a gene inactivation method by using antisense RNA approach.
- To modulate *hsp18* gene expression in *O. oeni* and understand *in vivo* the Lo18 function.

Strategy

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

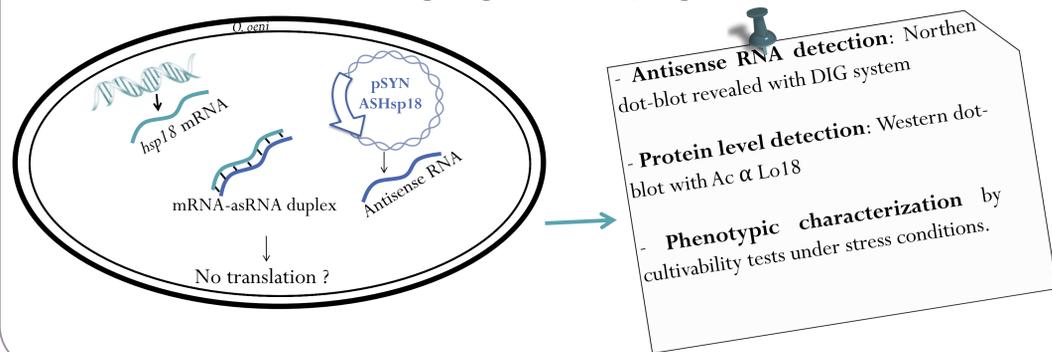


2: Cloning in antisense orientation into *E. coli*/LAB shuttle vector (pSIPSYN)



3: Transfert of pSYNASHsp18 in *O. oeni* ATCC BAA-1163

➔ Production of Antisense RNA targeting mRNA of *hsp18* gene in *O. oeni*



Results

1. Detection of *hsp18* messengers RNA and antisense RNA.

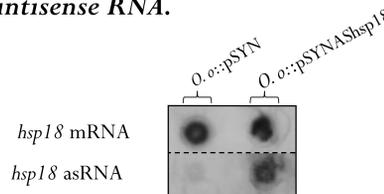


Fig. 1: Detection of asRNA and mRNA of *hsp18* in *O. oeni* recombinant strains by Northern dot-blot with DIG-probes. Total RNA were extracted from *O. oeni* carrying pSIPSYN (O.o.:pSYN) and *O. oeni* carrying pSIPSYNASHsp18 (O.o.:pSYNASHsp18) strains.

2. Detection of Lo18

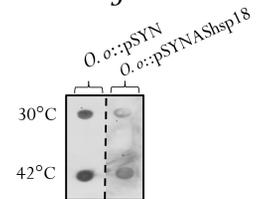


Fig. 2: Detection of Lo18 protein by western dot-blot with Ac α Lo18. O.o.:pSYN : *O. oeni* carrying pSIPSYN (O.o.:pSYN) and *O. oeni* carrying pSIPSYNASHsp18 (O.o.:pSYNASHsp18) strains were grown at 30°C or 42°C.

Validation of asRNA EXPRESSION

Validation of asRNA FUNCTION

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

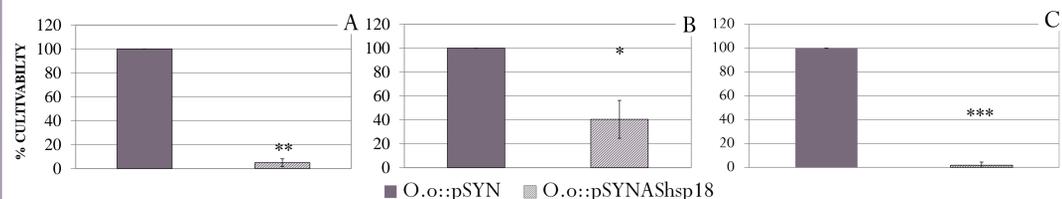


Fig. 3: Cultivability tests after heat shock (A), acid shock pH3.5 (B) or pH3 (C). Recombinant strains carrying native plasmid (O.o.:pSYN) or carrying plasmid expressing *hsp18* asRNA (O.o.:pSYNASHsp18) were grown at 30°C in FT80 medium until mid-exponential phase ($OD_{600} = 0.8$). Culture were incubated 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 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

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:

1. Lonvaud-Funel, A. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie Van Leeuwenhoek* **76**, 317–331 (1999).
2. Grandvalet, C *et al.* CtsR is the master regulator of stress response gene expression in *Oenococcus oeni*. *J. Bacteriol.* **187**, 5614–5623 (2005).
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. Coucheny, 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).

5. Maitre, M. *et al.* Adaptation of the Wine Bacterium *Oenococcus oeni* to Ethanol Stress: Role of the Small Heat Shock Protein Lo18 in Membrane Integrity. *Appl. Environ. Microbiol.* **80**, 2973–2980 (2014).